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Study of Bacterial Protein

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ABSTRACT

The land and water requirements of H_2 -oxidizing bacteria-based MP via the carbon capture and renewable energy had not been previously studied. **Protein** is the basic component of living cells and is made of carbon, hydrogen, oxygen, nitrogen and one or more chains of amino acids. The three types of **proteins** are fibrous, globular, and membrane. The global food demand is projected to significantly increase. To maintain global food security in the future, protein production needs to become more efficient regarding the use of limited land and water resources. Protein-rich biomass can be produced via direct air capture of CO_2 with the help of H_2-oxidizing bacteria and renewable electricity in a closed, climate-independent system. This quantitative literature review conservatively estimated the direct land and water use of bacterial protein production relying on secondary data for the components of the technology and for the reference protein sources

Keywords: Carbon dioxide, Hydrogen-oxidizing bacteria**,** Microbial protein, Microbial biomass**,** Bio-electrochemical system

Abbreviations: MP: Microbial protein

INTRODUCTION

Protein extraction study from tissues with tough extracellular matrices (e.g., biopsy samples, venous tissues, cartilage, skin) is often achieved in a laboratory setting by impact pulverization in liquid nitrogen. The growing human population and the projected decline of agricultural yields due to climate change challenge global food security. To provide the increasing population with a sufficient amount of protein, approximately 110% more crop protein will have to be produced in 2050 than in 2005, according to the estimate of while the availability of additional arable land and water for agricultural use is limited. There are also competing claims for land and freshwater use from urbanization, the forest industry, biofuel production and carbon stock conservation via forests. In addition, agriculture, forestry and other land uses cause approximately one quarter of anthropogenic greenhouse gas emissions and most nutrient emissions. The production of animal proteins accounts for the major part of the use of these resources, even though their share of the global protein consumption is approximately one third only. Consequently, there is a need for novel approaches to realize environmentally sustainable, climate-resilient protein production that is less dependent on land and water and has the ability to adapt to climate change.

Proteins are one of the main components in food and their availability, quality and environmental impacts are good indicators by which to measure food security. The lack of access to high-quality protein due to unequal distribution in the world causes malnutrition, especially in developing countries. For example, millions of children subsist on poor sources of essential amino acids [1]. In the future, the access to high-quality protein will become even more challenging due to the increase in protein demand along with population growth and increase in wealth under climate change. To produce high-quality proteins to maintain food security with minimal environmental impact, microbial proteins (MPs), especially bacteria-based MPs, are seen as promising alternatives compared to traditional animal-and plant-based proteins. For example, in animal feed, MPs from gram-negative soil bacterium *Curpiavidus necator* have been found to be comparable with those from basic protein sources, such as soymeal and fishmeal, and include all essential amino acids required for growth.

There are several beneficial characteristics that bacterial proteins have over other protein sources. Bacteria-based

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MPs have high protein contents ranging from 50% to 83% of the dry biomass, and the bacteria have high growth rates relative to other protein sources. The bacterial conversion efficiency from substrates to protein-rich biomass is high compared to that of other MP sources, such as yeasts and fungi, and to that of traditional protein sources. MP production is usually based on inexpensive carbon substrates, including waste, which are available in large quantities. In addition, the production process for MPs can be designed as a closed system highly independent of seasonal changes, making efficient nutrient use without runoff to the surrounding environment possible, and the system does not require herbicides or pesticides. MP production applications can have higher solar-to-biomass efficiencies than those of crops, but efficiencies among different applications vary greatly. Regarding the biomass of crops that can be harvested annually, the yearly average solar-to-biomass efficiency does not typically exceed 1%. During the growing season, the energy use efficiency may reach 3.5% for C3 plants, such as most staple cereals and vegetables, and 4.3% for C4 plants, such as maize (corn), sorghum and sugarcane, and even the theoretical efficiency is limited to 6% .Using the cyanobacterium *Arthrospira plantensis,* an efficiency of 2% has been reached while significantly higher solar-to-biomass efficiencies of up to 10% have been achieved using H_2-oxidizing bacteria [2].

The possibility of producing MP using H_2-oxidizing bacteria has long been known. H_2-oxidizing bacteria are able to utilize H_2 as an energy source, with the help of CO_2 and O_2, for microbial growth [3]. H_2 and O_2 can be produced by electrolyzing water inside a bioreactor and CO_2 can be separated from the atmospheric air by adsorbents). Although CO_2 separation and water electrolysis are energy-intensive processes, there exists a growing interest in opportunities for MP production, because renewable energy sources can be used.

The growing capacity of wind and solar power increases utility fluctuation, leading to occasional oversupplying of power. In particular, the availability of solar energy is increasing due to the rapid decline in its production costs. Food-insecure regions with high amounts of non-arable land area could be used for producing solar or wind energy, which could be directed to MP production upon oversupply or when required. For instance, the International Renewable Energy Agency (2014) has estimated for Africa annual theoretical energy production potentials of 660 PWh and 460 PWh for photovoltaics (PVs) and wind energies, respectively. The energy production potentials exclude the areas used for agriculture and exceed the global annual electricity demand of 25 570 TWh in 2017.

Despite the growing interest in MP production, the environmental sustainability of MP production has been quantitatively evaluated only in a few studies. In a comparison of different MP production technologies, production via water electrolysis requires the least land and causes the lowest greenhouse gas emissions. However, to our knowledge, no studies have evaluated the land and water needs of MP production by H_2-oxidizing bacteria, taking into account the energy requirements of state-of-the-art technology using direct air capture (DAC), bioreactors with an in-situ water electrolyser and separation of the bacterial biomass from the cultivation medium. Separating CO_2 from ambient air to produce MP by binding CO_2 to the biomass without increasing atmospheric CO_2 makes the application less dependent on location compared solutions with point sources of CO_2 [3]. The greatest potential of the approach is to decouple protein production from the use of agricultural land and minimal water use, in comparison with traditional protein sources, whereas the climate impact crucially depends on the used energy source. Here we have assumed a renewable energy source, which increases the land use, and thus provide a conservative estimate of the potential to reduce land and water use.

The aim of this study is to quantify the potential reduction in land and water use from bacterial MP production via the use of direct air capture of CO_2, a water electrolyser and renewables in comparison with soybean production and other MPs available on markets having studied requirement of land and water. Soybeans are a widely used plant-based protein source with a high protein content and high yields, making soybean protein a well-suited plant-based protein for comparison. The land and freshwater use were assessed based on the energy and raw material requirements of the system components reported in the literature.

MATERIALS AND METHODS

System boundary and methods

The studied system consists of the determined energy and material consumption of the components of technologies of MP production. The performance data from the best electricity-to-biomass efficiency of a lab-scale bioreactor found in literature was used for energy consumption calculations. For other processes, the electricity consumptions of commercially available products and estimates from literature were used. The energy requirements for producing nutrients for microbial growth were neglected. The theoretical energy and material requirements of the application were determined by calculating the stoichiometry of the substance requirements for biomass growth and by combining experimental data from system components found in the literature. The production process was assumed to be a closed system. Thermal energy was assumed to be taken from waste heat sources. The technologies selected consisted of direct electrolysis in the bioreactor, post-processing to dry the biomass, and DAC to provide the source of CO_2, and the bacterial species used was *C. necator*. The simplistic scheme of the application can be seen in **Figure 1**.

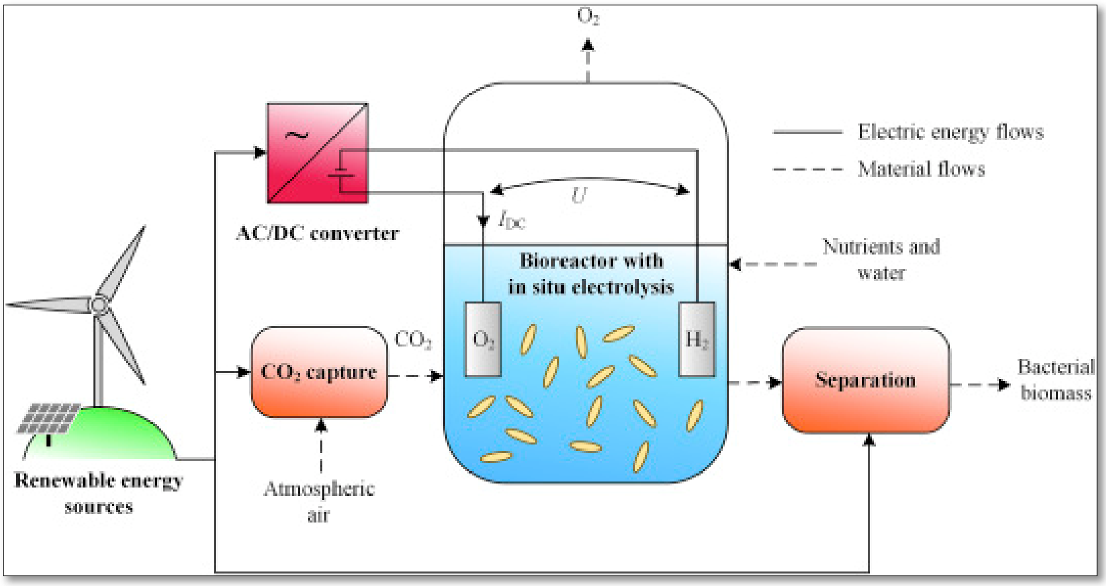

Figure 1. The scheme of MP production by the selected technology. *U* refers to voltage and *I* refers to electric current.

The conservative land and water requirements of MP production were compared to the requirements of soybean and FeedKind® production using a quantitative literature review. FeedKind® is a bacterial MP used for feed purposes in markets [4]. The land use comparison between MP and soybean production is based on direct land use values in the U.S, which is one of the major soybean producers in the world. The production facilities for MP have only a minor impact on land use, and they can be located on non-arable land or even under the ground; thus, their impact is excluded from the analysis. MP production is energy-intensive and uses renewables as energy sources. The land area required by energy production and the related infrastructure was included in the calculation. The land occupation of energy production required for MP production was calculated by multiplying the energy consumption per produced biomass and land occupation per energy generation and time:

$$Land\ use_{MP} = \frac{Land\ occupation_{kWh}}{Energy\ generation_{kWh} \times time} \times \Sigma\left(\frac{Energy\ consumption_{MP}}{Mass_{biomass}}\right) \quad (1)$$

For the water requirement of soybean production, values including green and blue water requirements for soybean production was used for comparison. Green water measures the volume of rainwater consumed, and blue water measures the ground and surface water consumed during the growth period. The water consumption of MP was calculated based on the stoichiometry of the production process:

$$Water\ consumption_{MP} = \Sigma\left(\frac{Water\ consumption_{MP}}{Mass_{biomass}}\right) \quad (2)$$

THE DESCRIPTION OF THE CASE TECHNOLOGY

Hydrogen-oxidizing bacteria cultivation in bioreactors

Microorganisms can be cultivated in bioreactors, where they can be grown on a wide variety of substrates. H_2-oxidizing bacteria, also called Knallgas bacteria, are able to use H_2 as an electron donor and O_2 as an electron acceptor to fix CO_2 to build up their biomass. In addition, some nutrients are required to increase the biomass in the cultivation medium. There exist different species of H_2-oxidizing bacteria, such as *C. necator* (also called *Ralstoniaeutropha* and *Alcaligeneseutrophus*), *Rhodococcusopacus* and *Hydrogenobacter thermophiles*. H_2-oxidizing bacteria are mostly aerobic and facultative chemolithoautotrophs using Calving cycle for carbon fixation. They can derive their energy through chemosynthesis or grow heterotrophically using organic substrates.

Microorganisms can be cultivated in batch or continuous bioreactors. There are several different types of bioreactors, such as stirred-tank, bubble column, trickle-bed, membrane-based and U-loop reactors.). In addition to the various technological approaches for splitting water using electricity it is also possible to produce H_2 and O_2 externally or internally. External water electrolysis uses a separate unit process outside the bioreactor and internal electrolysis is performed directly in the cultivation medium in the bioreactor.

Supplying the required gases from outside the reactor is a more conventional method of growing H_2-oxidizing bacteria, but the challenge of this approach is the low mass transfer of H_2 and O_2 to the aqueous solution [3]. In addition, H_2 and O_2 can produce flammable mixtures in the reactor headspace, which may be ignited, for instance, by measurement instruments in the bioreactor and cause an explosion. Therefore, controllable gas solubility is essential. Problems with the mass transfer of gases and safety in the cultivation of H_2-oxidizing bacteria can be avoided, at least to some extent, by performing the electrolysis directly in the bioreactor. The application in the studied case employs internal water electrolysis, and thus, only CO_2 is supplied from outside the bioreactor.

Direct air capture of CO_2: CO_2 is required as the carbon source for the growth of bacteria. CO_2 supplied to the reactor can be taken from many sources. Flue gases from combustion process and by-products of fermentation or air are a few examples of sources of CO_2. The studied case application uses DAC, which is an emerging technology for the collection of CO_2 directly from the atmosphere. Ambient air passes through either a solid or a liquid medium in which CO_2 molecules are retained. When energy is introduced into the medium, concentrated CO_2 is released, enabling it to be collected, stored, or used. Absorption refers to the case in which a liquid medium is used in the capture process, and adsorption is defined as the process in which a solid medium is used in the capture process. Most DAC systems are based on an adsorption/desorption. The scheme of an adsorption-based direct air capture device is illustrated in **Figure 2.**

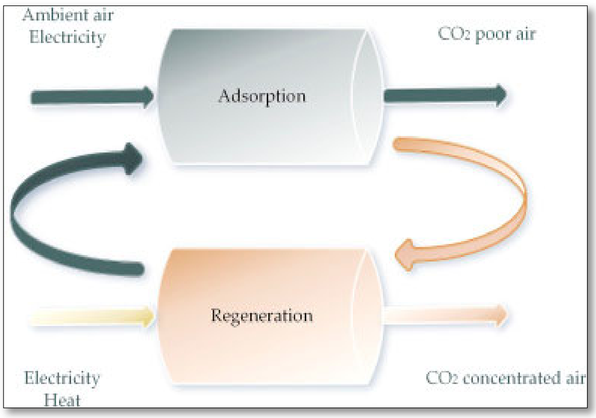

Figure 2. Adsorption-based direct air capture device.

In principle, a pure CO_2 source could be omitted, and atmospheric air could be used as the carbon source for bacteria. According to scientists [2], the electricity-to-biomass efficiency decreased from 54% to 20% when atmospheric air was supplied to the headspace of a reactor instead of 100% CO_2, even though the partial pressure of CO_2 was reduced by 2500 times.

Electrolysis of water: Currently, alkaline water electrolysis is the most mature and widely exploited water electrolysis technology for water splitting. However, proton exchange membrane (PEM) technology is currently challenging alkaline technology by producing higher current densities, thereby offering a more compact design and higher efficiency.

Direct electrolysis in a bioreactor was invented in the 1960s but owing to its high voltage requirement, its conversion efficiency was poor. An electricity-to-biomass efficiency of 54% has been achieved by applying direct electrolysis in a bioreactor, which would approximately correspond to a 10% solar-to-biomass efficiency, assuming 18% efficiency for photovoltaics [2]. Similar kind of electricity-to-biomass efficiency has been achieved by using external water electrolysis [3]. The challenge in direct electrolysis is to find the balance between a suitable environment for bacteria in the cultivation medium, a reasonable efficiency of electrolysis, and a sufficient H_2 formation rate [2]. Therefore, the design of electrode structures, anode and cathode materials and the optimization of growth conditions play key roles in improving the conversion efficiency. In addition, when using internal water electrolysis to grow bacterial biomass for food or feed purposes, it must be ensured that no toxic components are dissolved from the electrodes or from salts used in the cultivation medium. A typical electrode material in bio electrochemical systems has been rare and expensive platinum, but new materials have also been found, such as cobalt phosphate CoP_i for the anode and NiMoZn, stainless steel [2].

Post-processing of bacterial biomass: Separation of the bacterial biomass from the cultivation medium is challenging, as the bacterial cells are small (1-2 µm), their density is close to that of water (1.003 kg l^{-1}), and the cell dry weight densities at the end of cultivation are relatively low (10–30 g l^{-1}) [5]. The separation is typically carried out by centrifugation but microfiltration via a membrane can also be used. After solid–liquid separation, other post-treatment processes, such as thermal disruption, acid treatment, flash drying, and grinding, can be implemented depending on the end-product properties desired. Various cell components such as lipids or polyhydroxyalkanoates can also be separated from the protein fraction using solvent extraction. To estimate the energy needed for post-processing, centrifugation and evaporation were chosen as example processes.

Material and energy requirements of the case technology: The amount of required input substances can be approximated by the stoichiometry of the bacteria. [2] reported the following stoichiometry for *C. necator* biomass formation when the bacteria were cultivated using direct electrolysis in the bioreactor:

$$CO_2 + 0.24NH_3 + 0.525H_2O \rightarrow CH_{1.77}O_{0.49}N_{0.24} + 1.02O_2$$

According to this stoichiometry, 0.38 kg of H_2O, 1.76 kg of CO_2, and 0.16 kg of NH_3 are required to produce 1 kg of biomass. In addition, 1.31 kg of O_2 is produced per kilogram of biomass. Usable protein content of 50–65% of dry biomass are typical estimates for *C. Nectator*. Assuming that the protein content of the biomass is 60%, the water consumption is approximately 0.6 L $kg_{protein}^{-1}$. The actual water consumption is higher because there is always some residual moisture after solid–liquid separation that is then removed via drying. In addition to H_2, O_2, and CO_2, bacteria also require a mineral salt medium containing essential nutrients, such as nitrogen, phosphorus, and sulphur.

The estimated material and energy flows required to produce 1 kg of bacterial biomass are illustrated in **Figure 3.** The energy input for producing bacterial biomass consists of the energy required for the electrolysis of water, CO_2 capture and post-processing of the biomass. An electricity-to-biomass efficiency of 54% for *C. necator* bacteria has been achieved using direct electrolysis, assuming the free energy gain from CO_2 to biomass to be $\Delta_r G° = 479$ kJ mol^{-1} [2], which corresponds to an electric energy consumption of the electrolysis of 9.86 kWh per 1 kg of produced biomass. The energy consumption of CO_2 capture depends on the technology applied. Climeworks announced that their CO_2 capture device has a thermal energy demand of 1.8–2.5 kWh kg^{-1} CO_2 and an electric energy demand of 0.35–0.45 kWh kg^{-1} CO_2. According to the stoichiometry, the average energy consumption of CO_2 capture would thus be 0.71 kWh electric energy and 3.78 kWh thermal energy per 1 kg of produced biomass. At the end of the cultivation process, the biomass concentration in the medium can be assumed to be 2.5% [5] estimated an average value of 1.35 kWh m^{-3} for centrifugation to obtain a biomass concentration of 20%. Therefore, to harvest 1 kg of biomass via centrifugation, 40 kg of reactor solution must be treated, which leads to 0.05 kWh $kg_{biomass}^{-1}$. If evaporation is used to achieve a biomass concentration of 90%, 3.89 kg of water has to be evaporated. With the heat requirement for water vaporization of 2260 kJ kg^{-1} and the estimated thermal efficiency of 84%, 2.91 kWh of heat is needed for evaporation per 1 kg of biomass. As the electricity consumption for water evaporation is 87 kWh m^{-3}, 0.34 kWh is needed per 1 kg of biomass.

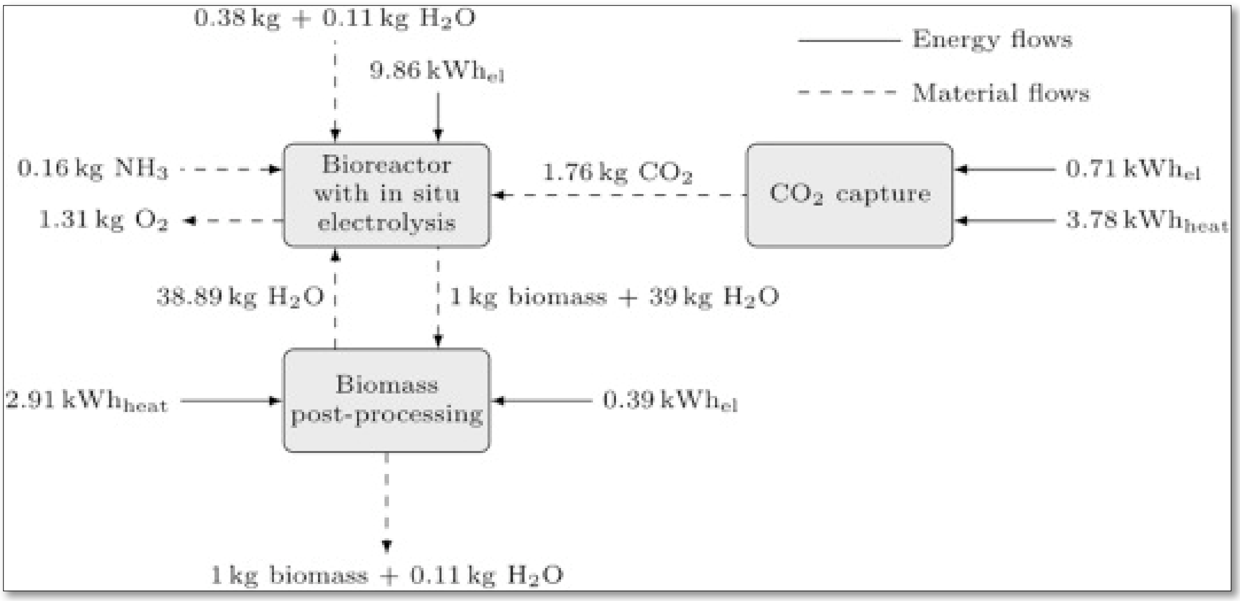

Figure 3. Approximated main material and energy flows required to produce 1 kg of bacterial biomass.

Land use of renewable energy

The direct land area impact of renewables consists of land directly occupied by solar arrays and wind turbine pads, access roads, substations, service buildings and other energy infrastructure physically occupying land area. According to the national renewable energy laboratory, the generation-weighted direct land area impact of solar PV varies within the range of 0.010-0.014 m^2 kWh^{-1} y^{-1} in U.S. In the case of wind energies, the capacity-based direct land area impact is within the range of 0–0.002 m^2 kWh^{-1} y^{-1} in U.S, when the average capacity factor of 0.367 is used.

Land and water use of soybean production

To evaluate the yields of soybean production in U.S, the yields of the last ten years were taken from National Agricultural Statistic Service of United States Department of Agriculture. The top maximum and minimum values were not used for the evaluation as they were counted as exceptions. The range of the yields were 2.4-5.95 m^2 $kg^{-1}y^{-1}$ in the years 2008-2018 in U.S. The soybean protein content varies between 35% and 40% [6]. If the average protein content is used, the yield of soybean protein varies between 6.40-15.86 m^2 $kg^{-1}y^{-1}$. In the case of water use, the range of green-blue water use of soybeans is approximately 2.67-6.67 L $kg_{protein}^{-1}$. when using the average protein content.

Land and water use of another bacterial MP

Scientists [4] evaluated FeedKind® commercial products in terms of water use and land occupation. FeedKind® is produced from bacterium capable of methanotrophy, thus methane is required for the bacterial growth. The land occupation values are in range of 0-0.052 m^2 $kg_{protein}^{-1}$ and the water consumption in the range of 10–29 L $kg_{protein}^{-1}$ depending on how the product is post-processed. The high-water consumption is due to energy used and vegetable oil in the finalized product. The land occupation included only the land requirement of vegetable oil production. For impacts of MP via methane to be comparable with the studied MP, only the impacts of protein production were included in the evaluation. When the impacts of energy and vegetable oil are omitted, the results are approximately 0.5–1.45 L $kg_{protein}^{-1}$ for water consumption and zero for land occupation.

RESULTS

The sum of the electrical energy consumed during the electrolysis and the average value of the range given for CO_2 capture together with the estimated requirement for biomass drying would be 10.96 kWh $kg_{biomass}^{-1}$. The consumed energy per kilogram of produced protein would be 18.26 kWh **(Figure 3).** The bioreactor within-situ electrolyser uses most of the resources in the production process of bacterial protein **(Table 1).** The land use of main components of the case technology originates in the calculation by multiplying the range of direct land occupation of electrical power generation (Refer to Land use of renewable energy) with electricity demand of the main components in the technology **(Equation (1)) .** The water consumption of MP production originates in calculation according to **Equation (2)**.

Table 1. Water and land use of the main components in the case technology.

	DAC	Bioreactor	Post-processes	Total
Land use [$m^2 kg_{prot}^{-1}$]				
MP_{Solar}	0.01-0.018	0.16-0.23	0.0065-0.0091	0.18-0.26
MP_{Wind}	0-0.0026	0-0.033	0-0.0013	0-0.04
Water use [L kg_{prot}^{-1}]				
Input	-	65.82	65.18	-
Output	-	-65.18	-65	-
Net use	-	0.63	0.18	0.82

The land use and water consumption of bacterial MP via studied technology are clearly lower than the land use and water consumption of soybean production and have similar kind of impacts than MP via methane production **(Table 2)**. The direct land occupation of bacterial protein has ten times lower land area requirement per a year than soybean production in the case of the highest land occupation value of solar energy generation. The results show that land occupation of MP production is minimal, especially, when wind energy is relied upon. The MP production consumes approximately one tenth of the water compared to soybean production. The characteristics of MP via methane and the studied MP production are assumed to be the same as both processes can be designed to be closed systems.

The efficiency of electricity-to-biomass of 54% reported in this paper is high compared with other values found in the literature. For instance, if the electricity requirement of the electrolysis would increase by 10% per produced amount of biomass, the increase in the demand for electricity would be approximately 0.99 kWh, which would decrease productivity, land use per produced protein, by approximately 8%. The protein content of the final MP product is also affecting the productivity. When calculating land use by using 50% and 65% protein contents of MP, the productivity decreases by 16% or increases by 10%. Other process sensitivities to the total electricity demand are minor compared with that of electrolysis **(Table 1)**.

Table 2. Water and land use of soybean and MPs with other production characteristics.

Land and water requirement	Soybean	MP_{Solar}	MP_{Wind}	$MP_{Methane}$
Water consumption [L kg_{prot}^{-1}]	2.67-6.67	0.82	0.82	0.5-1.45
Land use [$m^2 kg_{prot}^{-1} y^{-1}$]	6.40-15.86	0.18-0.26	0-0.04	0
Other characteristics				
Sterile production environment	No	Yes[a]	Yes[a]	Yes[a]
Pesticides and herbicides use	Yes	No[b-c]	No[b-c]	No[b-c]
Fertilizer run-offs	Yes	No[b-c]	No[b-c]	No[b-c]
Controllable production process	No	Yes[a-c]	Yes[a-c]	Yes[a-c]
Seasonal dependence	Yes	No[b-c]	No[b-c]	No[b-c]
Need for arable land	Yes	No	No	No

[a]*Lee 2015 [5]*

[b]*Israelidis 1987*

[c]*Pikaar et al. 2017*

DISCUSSION AND CONCLUSION

Land and water use of MP production and other characteristics

The main finding of this study was that MP protein production using CO_2 capture from air and renewables needs many times less land area and water than does soybean production. Although, the studied case technology uses energy intensive DAC devise to provide CO_2 for the process. The electricity-to-biomass efficiency of electrolysis majorly affected the energy demand and, thus, should play a key role in the application design.

Lee [5], simulated avoided land use, nitrogen flows and greenhouse gas emissions in agriculture, if bacterial MPs from different pathways replaces traditional protein sources. The results showed significant reduction potentials. The pathways were MP via water electrolysis, sugarcane-to-MP, cellulose gasification-to-MP and biomethane-to-MP. MP via water electrolysis had the most significant reduction potential from the above-mentioned MPs. The MP via water electrolysis uses similar hydrogen oxidizing bacterium species to produce biomass as the studied case technology, but the production technology was different, e.g., bioreactor, external water electrolysis, and point sources of CO_2 were different. By using DAC instead of point sources to provide CO_2 opens new possibilities for mitigation, adaptation and location purposes for MP production as CO_2 is taken from air and the production facility is not bound to specific locations.

MP production via the proposed method can be a competitive alternative to conventional protein production also because of other beneficial characteristics and potential environmental advantages related to land and water use. Because the application can be designed as a closed system, climate change has no impact on the growing conditions and it is possible to recycle nutrients until they are completely used, resulting in high nutrient utilization efficiency without run-offs [5]. Another beneficial aspect is that MP production does not require pesticides and herbicides, the use of which might cause biodiversity losses. In addition, it is possible to use wastewaters as a nutrient source, which would reduce the fresh water and nutrient consumption of the application. However, the safety and process sterility of wastewater use has to be ensured. Owing to the abundance and availability of the required raw materials to cultivate MP this method of protein production is highly independent of place and climate and, therefore could be exploited in food production even where there is no arable land. Due to the rapid growth rate of microbes, the method can be fast implemented when food or feed availability becomes challenging.

USABILITY OF MP AS FOOD AND FEED

Until now, the main use of bacterial protein has been a feed for animals, an application that has been widely studied and implemented in an industrial scale, e.g., Methylophilusmethylotrophus using methanol as an energy source. Furthermore, animals are typically inefficient in converting plant-based proteins into animal proteins and thus, a large amount of plant-based proteins are consumed as feed resources. The provision of MP as feed would save plant-based proteins, such as soybean protein, for human consumption or land and water for other uses. However, as showed in their simulations, MPs can replace only a certain amount of nutrition value apart from protein in feed, but with other sustainable practices, MPs could be a part of the solution in transition towards sustainable food systems.

Despite the superior growth rate, low resource needs and high protein content, in general, bacterial biomass still has some disadvantages which limit its wider use. These limiting factors are related to the downstream processes after cultivation, regulation, and safety challenges. For instance, it must be ensured that the selected bacteria are not pathogenic to humans or animals and do not contain toxic compounds. Another challenge is to provide a sterile production environment because contaminating microorganisms typically grow well in the culture medium [5].

MP for example from *Methylophilusmethylotrophus* or *C. necator* have amino acid profiles and a nutritive value comparable with fishmeal and soybean meal but have high nucleic acid concentrations. The high nucleic acid content of the bacterial biomass is not a problem for ruminants, and monogastric animals tolerate it. For humans, the consumption of more than 2 g/d of nucleic acids may lead to kidney stone formation and gout, and the cell walls of bacteria are indigestible for non-ruminant animals. The cell walls can be destroyed, and the nucleic acid concentration can be reduced by mechanical, chemical, or enzymatic means [5]. Bacterial biomass has already been commercialized as animal feed, but the nutritional and toxicological testing required for its approval for food purposes may take several years and cost millions of euros [5]. The use of bacteria as food seems also to be unacceptable to many people, even though, for instance, lactic acid bacteria play a key role in many fermented foods. Some products, such as yoghurts, are even marketed based on the presence of lactic acid bacteria. Examples of successful commercialization of unconventional microbial protein sources, such as the mycoproteinQuorn, exist, indicating that there are commercial possibilities for microbial food products.

RELIABILITY OF THE ANALYSIS AND FUTURE RESEARCH NEEDS

Currently, soybeans are the most important source of plan-based protein for feed purposes, thus, we used only them as a plant-based reference protein. Other commonly used alternatives for fodder are mainly side streams from other products (e.g., sunflower and oilseed rape), the sources are mainly used for crop rotation, specific sources are not used so widely for feed than soybeans are (e.g., legumes) or the sources have low protein contents (e.g., maize and barley). In addition, the content and quality of the studied protein is comparable to soymeal [7].

The study was based on values given in the literature and by technology manufacturers. The energy requirements of the unit processes excluded from the study have only a negligible effect on the total energy consumption. Similarly, the material flows of production process and the impact of the MP production facilities excluded from the analysis on direct land area requirement, are minimal. The potential conservative land and water use estimation of the state-of-the-art technology has many disadvantages compared to soybeans. The nutrient use is more efficient than that of soybeans due to closed production system, the land use of MP production includes the land use of renewables and the production does not require pesticides or herbicides. In addition, the land occupation of energy production depends on the location, used technology and energy sources available, which can have a major effect on the requirements of land area. For example, a significant amount of PV solar power plants can be installed on the roofs of buildings, and wind farms can be located offshore resulting in a minimal direct land area requirement.

The proposed protein production method is highly energy-intensive compared with conventional production practices in agriculture. Approximately 90% of the energy demand of the application comes from the electrolysis process, and thus, the energy requirement is highly dependent on the electrolysis efficiency and the mass transfer of H_2 and O_2. The used electricity-to-biomass efficiency was based on a lab-scale bioreactor; thus, scaling up the process is essential when larger production volumes are preferred. Although it has been shown that scale-up of designs for producing H_2-oxidizing bacteria-based biomass is not a problem [8], piloting a facility with a larger production capacity is essential to validate the results of this study.

With current electricity prices, only the cost of energy required to produce MP using the application is higher than the price of soybeans even if the capital and other operational costs are not taken into account. In the future, the situation may change, as electrobioreactors could be used to provide demand-response services for the grid, electricity prices could go down, or the price of soybeans could go up. The energy-related costs can also be reduced by using different technologies than the ones presented in this method. Thus, techno-economic research is preferred before commercializing the proposed application.

H_2-oxidizing bacteria survive night cycles when no electricity is used to provide substances for biomass growth and continue their growth during day cycles when renewable electricity is available. Thus, the production of bacterial biomass could be balanced according to the varying production and load of the grid, which is known as a demand response, and can lead to reduced electricity costs and/or incentive payments. However, further research is needed to conclude the suitability of the application for use as a demand-response tool. Especially, the time required to achieve the optimum productivity of MP is a topic of interest.

Because the application binds atmospheric CO_2, investigating the greenhouse gas emissions of the entire production process would also be of interest. To study the different environmental impacts over the product's lifetime, a life-cycle approach can be used. A pilot-scale application can help in estimating the actual energy and material flows of the production processes and in estimating reasonable production scales. A cradle-to-grave impact assessment takes into account the supply chains for product distribution, materials for production facilities and other material flows. Another research need would be to estimate the available amount of renewable energy for MP production and to which extent MPs could replace plant-based feeds while considering, for example, the entire nutrient value and post-processing requirements of the alternative feed protein sources.

CONCLUSION

We have shown that this technology exhibits a minor direct land occupation and water consumption compared to traditional protein sources such as soybeans. Currently, MPs are not widely used protein sources; thus, there is much progress that can be made by using the proposed MP production technology to improve food security and sustainability of food or feed chains. Therefore, the technology should be considered as a promising tool for sustainable agricultural development. However, before the commercialization of the studied technology, ensuring the safety as food and piloting with larger production capacities including a techno-economic and a more comprehensive environmental assessment is essential.

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REFERENCES

1. Semba RD (2016) The rise and fall of protein malnutrition in global health. Ann Nutr Metab 69: 79-88.
2. Liu CBC, Colón M, Ziesack PA, Silver DG (2016) Nocera Water splitting-biosynthetic system with CO2 reduction efficiencies exceeding photosynthesis. Science 352: 1210-1213.
3. Yu J (2014) Bio-based products from solar energy and carbon dioxide. Trends Biotechnol 32: 5-10.
4. Cumberlege T, Blenkinsopp J (2016) Clark Assessment of environmental impact of FeedKind protein carbon trust. Available online at: https://www.carbontrust.com/media/672719/calysta-feedkind.pdf
5. Lee BH (2015) Fundamentals of Food Biotechnology (second ed.). John Wiley & Sons, Chichester, UK.
6. Day L (2013) Proteins from land plants - potential resource for human nutrition and food security Trends Food Sci Technol 32: 25-42.
7. Aragno M (1998) Techniques in Microbial Ecology. Oxford University Press, New York, pp: 3917-3933.
8. Reed, J Geller, McDaniel R (2015) Kiverdi CO2 conversion by Knallgas microorganisms California Energy Commission Publication number: CEC-500-2017-005.