HYPOTHESIS PAPER

# Agriculture-independent, sustainable, fail-safe and efficient food production by autotrophic single-cell protein

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**Abstract**

**Background:** Industrial agriculture is destructive to the environment, pollutes large amounts of water, fosters climate change and cannot guarantee food security for the 21st century. Single-cell protein (SCP) represents a safe alternative with minimal carbon and water footprints, but does not truly improve sustainability or food security when grown on waste products of industrial agriculture.

**Proposal:** This hypothesis paper proposes autotrophic SCP bioprocess designs which enable sustainable, fail-safe and efficient production of edible biomass from $CO_2$ and $N_2$ or $NH_3$. They can be driven by $H_2$, CO, HCOOH from several sustainable sources and provide versatile options of biowaste upgrading. Most promising designs consist of 2-stages. In the 1$^{st}$ stage, homoacetogenic bacteria fix $CO_2$ and secrete it as acetate with unrivaled yields. In the 2$^{nd}$ stage, selected microbes transform the acetate to edible biomass. Bacteria have various unique features including $O_2$-tolerant hydrogenases, $N_2$-fixation and $H_2S$ tolerance. Eukaryotic microalgae are approved as food and exhibit oxygenic photosynthesis which partly replaces solar-panels, seawater desalination and $H_2O$-electrolyzers. Photoheterotrophic growth on acetate allows to decouple the efficient light-reaction from inefficient $CO_2$ fixation. Slow gas mass-transfer, poor light distribution and expensive cell harvest are major challenges arising from the cultivation in liquid media. To cope with this, microbes grow as hydrated biofilms, exposed directly to substrate gases and light. Two suitable bioreactors are presented and adaptations for 2-stage designs are proposed. Since provision with substrates is expensive, two strategies are proposed for the safe extraction of substrates from food-grade as well as non-food-grade biowastes via partial anaerobic digestion. Additionally, alkalic pH and hydroxides formed at the cathode during electrolysis may be used to precipitate $CO_2$ from the air as carbonates. In two use cases, 2-stage designs with solar-powered $H_2$-generation from seawater were estimated to exceed productivity of wheat 20-200 fold. Since for that particular bioprocess design electricity is the dominant cost driver, overall product cost can be estimated to 0,5-1 € per kg dry SCP. When direct and indirect subsidies are taken into account, autotrophic SCP likely outperforms industrial agriculture not only in ecological but also in economical aspects.

**Keywords:** single cell protein; autotrophic; anaerobic digester; food science; environment; industrial agriculture

## 1 Background

In 2014, 800 million people suffered from undernourishment [1], and almost 2 billion people suffered from malnutrition [2]. Nowadays, hunger could be largely eliminated by alleviating poverty, by reducing food wastage, and by substantially reducing the significant portion of grains dedicated to animal feed and biofuels [3]. With today's requirements for food, even organic farming, which has a low ecological footprint at slightly lower yields [4, 5], could potentially provide enough food for the majority, or even the entirety, of the world population [6, 7]. However, the world's population is expected to grow to about 9 billion by 2050 and will require 60-100% more food [8]. The demand of meat, dairy products and biofuels are also projected to increase, not only in total quantity, but also in relative shares of agricultural production [9]. Unlike the previous decades, production of industrial agriculture (IA) is not expected to increase substantially due to climate change impacts in tropical, as well as in temperate climates [10, 11, 12]. Food security is threatened by poor harvests and even crop failures that are becoming more likely due to climate change impacts, such as droughts

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and flooding, freshwater scarcity, pollinator decline, soil salinization due to inadequate irrigation and other forms of environmental degradation. The most important resource, freshwater, is already urgently lacking in many countries and is becoming increasingly unreliable and scarce [13]. IA is likely to partly counteract these challenges by further intensifying irrigation, application of fertilizers and pesticides, and by transforming even more land into monocultures. Each of these measures has already had profound negative effects on freshwater resources, the soil ecosystem, biodiversity in general, climate change and public health. While the overall IA output has been doubled in the last decades, the scale of these side effects has been found to grow at a significantly greater rate [14, 15, 16]. Side effects are expected to increase substantially faster with only minor gains in IA output, in the case of N-fertilizers even exponentially [17]. N-fertilizers (for simplicity referred to as fertilizer) are a comprehensive example of IA-associated effects: Commercial production by Haber-Bosch consumes large amounts of fossil-fuels, causing about 1-2% of all greenhouse gases. Nitrogen use efficiency of common crops is below 50% [18], and the remaining half of fertilizer has various destinies: it is partly washed out, causing eutrophication, it is partly transformed to the highly potent greenhouse gases $N_2$ O, and it is partly degraded to nitrate or $NO_2$ which both deteriorate public health [14]. Estimates of the annual cost of pollution by agricultural fertilizer range between 35-230 billion €, which is more than the economic benefit of fertilizers in agriculture [16]. Counterintuitively, a surplus of nutrients entails a loss in biodiversity and ecosystem services. As an extreme example of the impacts of eutrophication, dozens of thousands of square kilometers of the baltic sea are hypoxic and unable to sustain $O_2$-dependent marine life [19].
The numerous environmental and social impacts of IA cannot be treated here comprehensively. However, among the most incisive, the destruction of forests and the drainage of moors, and the subsequent transformation into monocultures, seriously affect the natural capacity of the biosphere to adapt to climate change impacts now and in the future. This is remarkable also in the light of IA alone causing up to 29 % of all greenhouse gases [20]. Besides the direct damage done to the globe's biosphere, these two climate change enhancing effects alone are likely to cause damage to the globe's biosphere a second time over a long period.

Besides the unsustainable practices of IA, plants in general have several inherent disadvantages that represent the root cause of the mentioned negative effects of IA:

1 very high blue-green water footprint (irrigation, surface, ground and rain water). Global averages of common crops reach about 1.8 m3 of water per ton crop [21]. More than 90% of the global blue-green water is used in IA [22].
2 high vulnerability to abiotic stress such as drought, flooding and salinity.
3 High vulnerability to biotic stress, such as insects, weeds and fungal pests. IA monocultures favor pests and require extensive pesticide treatment. Pesticide are toxic to farmers and affect the surrounding environment in a radius enlarged through run-off and aquifer contamination.
4 inedibility of a large fraction of the plant such as ligneous stem, leaves and roots.
5 indigestibility of a fraction of edible biomass, such as cellulose.
6 Low efficiency of the Calvin-Benson-Bassham-Cycle (CBB) of $CO_2$-fixation (CF). CBB is a major sink of energy in plants and also acts as a valve for energy during the rather common event of excessive light exposure. CBB requires several times more ATP than anaerobic prokaryotic pathways [23]. Due to the oxygenase side reaction of the CBB key enzyme RubisCO, evolved $O_2$ can further degrade the efficiency of CBB (photorespiration).
7 Low productivity per surface and time. Global average of cereal productivity amounted to a mere 0,39 $kg/m^2$ in 2013 (Faostat, 2015). To meet demand, arable land is created by forest clearing and moor draining, during which biodiversity is destroyed and substantial amounts of GHG are released.
8 high dependency on nitrogen fertilizer with the mentioned high impacts of production and degradation (with the exception of legumes).
9 strict dependency on light as the only possible energy source. Biomass formation is limited to daylight hours and parts of the day's production are consumed outside daylight hours.
10 limitation to $CO_2$ as the only carbon source, excluding usage of organic wastes.

## 2 Single-cell protein as alternative food source

Because of the inherent drawbacks of plant-based food production, this work concentrates on edible microorganisms or "single-cell protein" (SCP) for the production of raw foodstuff. SCP has several advantages:

1 it grows several orders of magnitude faster than crops.
2 wastage of water and nutrients as happening in agriculture [24] are virtually eliminated, since

evaporation, transpiration, drainage and runoff are completely avoided.

3 production itself does not have any ecological impact, as SCP production is self-contained in a bioreactor such that no leakage to the environment occurs. Pesticides are not required.

4 selected strains have high nutritional quality [25, 26]. For example, the share of protein of *Rhodobacter* biomass is often well above 60% of the cell dry mass and the amino acid composition of the protein is highly favorable, i.e. comparable to that of a hen's egg, including methionine contents [27, 28, 29]. *Rhodobacter* produce vitamins including B1, B2, B12, E, biotin, niacin, folic acids [27]. *Rhodovulum* also contains the two precious $\omega$-3 fatty acids docosahexaenoic and eicosapentaenoic acid [30, 31] which are commonly obtained from fish oil which, however, is often contaminated by heavy metals.

Fungal SCP was used as emergency food during both world wars in Germany and was temporarily commercialized on larger scales during the 1960-1980s [26]. Today, bakers' yeast (*Saccharomyces cerevisiae*), microalgae such as *Chlorella vulgaris* and Cyanobacteria like *Spirulina platensis* are used as nutritional supplement. The Quorn$^{tm}$ group of products is a successful example of fungal meat replacement.

However, SCP are grown on agricultural products or wastes, and therefore inherit the ecological footprint as well as the insecurity of IA. Furthermore, there are two commonly cited problems with bacterial or fungal SCP: the high content of nucleic acids which can cause gout when ingested in large quantities [26], and the low consumer acceptance of SCP as "food". SIt is sufficient for the sake of this study that the SCP is suitable as emergency food, since nucleic acids can be reduced to safe levels by heat treatment, speeding up endogenic degradation by nucleases [26]. Bad consumer acceptance of SCP can be circumvented by mixing SCP with traditional foodstuff or by prior extraction of valuable nutrients. Usage of SCP as feed for fish or animals would replace soy which is commonly produced with particularly high ecological footprint. It would also allow simpler and faster commercialization. In the long term, SCP appears promising as an alternative to soy and grains, which represent the majority of agricultural production. Besides caloric input, grains have only moderate nutritional value and also have a secondary role in giving taste to meals. SCP is not intended to replace fruits and vegetables.

In this work, several autotrophic SCP bioprocess designs are proposed that form biomass from $CO_2$, $H_2$ and $N_2$ or $NH_3$. They are composed of either a single step, or two subsequent stages. Challenges related to low solubility of the gaseous substrates and biomass harvest are addressed in two specific bioreactor proposals. The problem of substrate provision is tackled by partial anaerobic digestion of biowastes.

## 3 Aims

Considering the important shortcomings of plant-based food production in general and IA in particular, a sustainable, fail-safe and efficient agriculture-independent food production systems should:

1 produce healthy food reliably, regardless of crop failures due to harsh climate conditions. Provide the option of complete independence from agriculture.

2 require a bare minimum of water and fertile land.

3 not harm the environment.

4 not foster climate change.

5 minimize dependency on unreliable or unsustainable external factors, such as fertilizers. Provide the option of biological $N_2$ fixation.

6 operate with various energy sources including $H_2$, synthesis gas and sunlight.

7 provide the option to use sunlight, yet also work at night.

8 provide the option to safely utilize biowastes for food production.

## 4 Microbes

Microbes grown with substrates derived from industrial agriculture inherit the same ecological footprint, water footprint and food insecurity of IA products. Therefore a bioprocess that is to fulfill the aims defined above, can rely on neither products nor wastes of IA but rather must be itself autotrophic, i.e. the bioprocess has to be capable of fixing $CO_2$ and forming edible biomass from it.

Unlike established SCP species, which are mostly eukaryotic microbes such as yeasts and microalgae, bacteria have several advantages:

1 anaerobic $CO_2$-fixation pathways are considerably more efficient than CBB. The Wood-Ljungdahl pathway (WL) and the reductive tricarboxylic acid cycle (rTCA) require between 2 and 6 times [32] less ATP than CBB with respect to the synthesis of common metabolites from $CO_2$. When considering realistic conditions, i.e. the presence of oxygen leading to losses due to the oxygenase side-reaction of CBB, WL consumes up to 10 times less ATP with respect to the formation of GA3P [23].

2 growth rates of autotrophic bacteria far exceed plant cells.

3 diazotrophic bacteria are capable of biological $N_2$ fixation (BNF) and are independent of external N-fertilizers.

4 autotrophic bacteria, unlike plants, are generally not strict autotrophs and grow on alternative carbon sources at far greater growth rates. Similarly, diazotrophic bacteria can grow faster when given N-fertilizer.

5 several bacteria are able to use $H_2$ in the presence of $O_2$ ("Knallgas-bacteria"). It is used for regeneration of ATP, and provides reductant. Some bacteria can even directly reduce NAD+ or ferredoxin using $H_2$. Notably, the Knallgas reaction allows autotrophic bacteria to grow in the dark at relevant speed. The Knallgas reaction can also contribute to achieve higher growth yields by avoiding respiration of an organic substrates for energy supply.

6 bacteria, in particular those with the WL pathway, can efficiently grow on carbon monoxide (CO), an important alternative source of carbon and energy that can be obtained by gasification of recalcitrant organic wastes such as lignin, to synthesis gas (CO, $H_2$ and $CO_2$). WL bacteria can also efficiently grow on formic acid (HCOOH) without prior oxidation of HCOOH. Both, CO and HCOOH can be obtained from electrolysis of $CO_2/H_2O$ and provide alternatives to $H_2$ as electrolytic electron donor.

7 some bacteria perform anoxygenic photosynthesis which utilizes light for ATP-regeneration without evolving $O_2$. This allows co-cultivation of acetate-consuming microbes with HAB and yield of photoheterotrophic growth on acetate is twice that of aerobic heterotrophic growth, since respiration of organic substrates for ATP-regeneration can be avoided to some extent. Furthermore, it allows to power $O_2$-sensitive pathways such as BNF.

8 some bacteria utilize the Ethylmalonyl-CoA pathway (EMC) for acetate-assimilation. EMC promotes carbon use efficiency by co-fixation of two $CO_2$ molecules for every three acetate molecules assimilated using the fastest known carboxylating enzyme[23, 33].

9 some bacteria can tolerate or even utilize hydrogen disulfide ($H_2S$) as source of hydrogen and sulfur using the Sulfide-Quinone Reductase (SQR) enzyme [34]. This is relevant when using the gases evolving during anaerobic digestion as a substrate gas mixture containing $H_2S$, which is toxic to most microbes.

10 bacterial biomass is low in carbohydrates, contains far less indigestible cell components than plant or fungal cells (no lignin or cellulose) and is rich in protein with an amino acid profile of high nutritional value.

11 some bacteria produce nutrients such as cobalamine (vitamine B12), of which eukaryotic organisms cannot produce significant amounts.

Although bacteria show a great diversity of biochemical capabilities, no single species of bacteria could be found that achieves all aims. This leads to the conclusion that either compromises have to be made or a given bioprocess has to be composed of more than stage (see next section).

Eukaryotic microbes have three central advantages over bacteria: established SCP strains like yeast of microalgae are often already approved for human consumption and already have a fairly good consumer acceptance. Since cyanobacteria for the time being have to be considered unsafe for food purposes (see next section), microalgae are the only microbes that exhibit oxygenic photosynthesis. Microalgae maybe suitable producers of plant-like starch or lipids, whereas yeast provide fast growth and versatile metabolism.

A thorough comparison of all candidate microbes, both bacteria and eukaryotes, can be found in Table 3 (appendix).

## 5 Single-Step-designs

Bioprocess designs that are composed of a single step, relying on a single species, accept compromises with the defined aims in favor of greater simplicity (see Figure 1).

One example of compromise is related to biological nitrogen fixation (BNF). BNF corresponds to the aim of eliminating the dependency on N-fertilizer and all associated problems. However, BNF is rarely found in bacteria with efficient $CO_2$ fixation, acceptable growth rate or practical growth conditions (besides bacteria of the genus *Chlorobium*). BNF also causes several complications that overly restrict the bioprocess designs. The high requirements of ATP conflicts with its $O_2$-sensitivity which hampers ATP-generation via aerobic respiration. Therefore, BNF restricts production to daylight when powered by anoxygenic photosynthesis or allows respiration only at very low microaerobic conditions, leading to impracticably slow growth. Another important reason to abstain from BNF in the near future is that the overuse of fertilizers already has led to an excess of combined nitrogen. Therefore it is sensible to recycle nitrogen compounds instead of adding new combined N to the global N-pool. Furthermore, sustainable means of $NH_3$ supply exist: $NH_3$ can be recycled from biowastes (see below) to some extent, and if need arises, $NH_3$ can be produced in Haber-Bosch like processes driven by renewable energy, sun heat and sustainable $H_2$[35, 36].

For these reasons, selected microbes without BNF such as the microalgae or *Aquificales* are nonetheless considered.

It is noteworthy that cyanobacteria do have oxygenic photosynthesis and also provide effective physiological adaptations allowing BNF to be driven by either o-PS or the Knallgas reaction [37, 38]. However, cyanobacteria fix $CO_2$ with CBB and do not assimilate acetate well [39, 40]. Above all, a multitude of cyanobacteria are known to form cyanotoxins such as microcystins, anatoxins, saxitoxins, hepatotoxic cyclic peptides [41, 42] and $\beta$-N-methylamino-L-alanine [43, 44]. While a few strains were found to not form particular cyanotoxins, no study was found that rigorously tested all known toxins at the same time and consequently, no strain was found that does not form any toxin at all. Even if such a strain was found, uncertainty would remain whether toxins tested negative may form under untested conditions. Overall it is concluded, cyanobacteria are unsuitable for direct consumption at the moment for safety reasons. However, substrates for safe SCP production may be extracted from biomass containing even toxic cyanobacteria (see below).

Microalgae are contradicting defined aims in several other ways: First, they have inefficient CBB $CO_2$ fixation. Second, they do not have any means of autotrophic growth independently of light and, lacking $O_2$-tolerant hydrogenases, are only capable of heterotrophic growth at night. Third, wildtype microalgae have indigestible cell-walls and cell-wall free mutants [45] may not get full consumer acceptance. Microalgae are nonetheless also considered for single-step bioprocess designs since it remains to be elucidated in a later work whether the mentioned disadvantages may be outweighed by the following advantages of oxygenic photosynthesis (o-PS): under suitable conditions, o-PS transforms sunlight and water into protons, electrons and $O_2$ with efficiencies above 25% [46, 47]. Photovoltaic-driven water electrolysis reaches at best 16% (¡20% and ¡80% respectively) and it is likely that some energy most be lost for the charge separation that has to occur at the uptake hydrogenase to split $H_2$ into protons and electrons. If $H_2$ is not produced on site, one further has to include up to 85% energetic loss for $H_2$-pressurization [48] and $H_2$-transport. While o-PS works with impure or even sea water, water electrolysis requires deionized water. Therefore, o-PS reduces both capital and operating costs and also improves the ecological footprint since it replaces solar-panels, water desalination unit and water electrolyzer. CBB, although inefficient when only considering CF as such, has the added benefit of extracting $CO_2$ from the air and therefore saves the energy and cost associated with acquiring anoxic $CO_2$. It is noted however, that the favored bioprocess designs consist in microalgae in two-stage designs presented below.

The green sulfur bacterium *Chlorobium tepidum* is one of the few organisms found that has both efficient CF and a very active BNF at the same time [49] . *C.t.* also provides a fast growth rate [50] supported by anoxygenic photosynthesis and exhibits moderate thermophily. However, *C.t.* has a very low oxygen tolerance, and strict dependency on light. Artificial illumination by flashing LEDs within a narrow spectrum matching the absorption spectrum [51] may allow to minimize the losses that would occur at night by fermentation of reserve compounds. Although *C.t.* appears unsuitable for continuous production, a light-dependent design may be acceptable in particular for arid-climates.

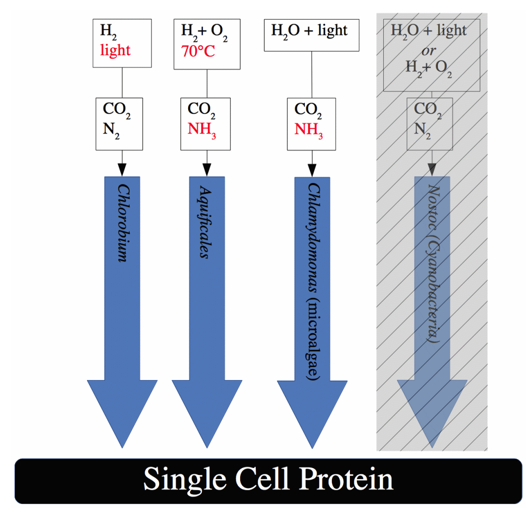


**Figure 1** Single-Step bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims. Cyanobacteria are shown greyed out, since they are not considered safe at the time of writing.

## 6 Two-stage designs

Two-stage designs are composed of a first stage, dedicated to fixation of $CO_2$ and formation of acetate, and a second stage in which acetate is transformed to edible biomass (see Figure 2).

The first stage is performed by homoacetogenic bacteria (HAB) that utilize the most efficient CF pathway found in nature, the Wood-Ljungdahl-Pathway (WL). Besides being efficient [23, 32], organisms with WL have the remarkable quality of secreting the vast majority of the fixed carbon as acetate into the medium. Besides $H_2$ and $CO_2$, and a variety of sugars, several HAB can also utilize CO, formic acid (HCOOH) and methanol as sole source of carbon and electrons. Growth on mixtures of CO and HCOOH might accelerate acetate formation by HAB [52], since CO and HCOOH are the two first CF-products of the WL pathway which are likely to be rate-limiting, but would reduce the advantage of efficient CF.

Although acetate is a universal central metabolite in all living beings, the biomass yield on acetate varies considerably. For example, *Saccharomyces cerevisiae* and *Candida utilis* are two established SCP yeast that both posses an active efficient acetate assimilation mechanism, namely the glyoxylate cycle. However *Saccharomyces cerevisiae* reaches a biomass yield of 29 g cell dry weight /g acetate whereas *Candida utilis* reaches 39 g cdw/g [53].

The chemolithoautotrophic bacterium *Xanthobacter autotrophicus* exhibits the efficient EMC acetate assimilation pathway described above. Furthermore, the growth yield on acetate was shown to improve when an additional dedicated electron donor such as HCOOH (likely also $H_2$) is added besides acetate [54]. The Knallgas reaction allows to oxidize less acetate via TCA for the regeneration of ATP and reductant and also enables BNF by the reduction of the local $O_2$ partial pressure [55].

Photoheterotrophic growth of purple nonsulfur bacteria (PNSB) such as *Rhodobacter* and *Rhodovulum* can supply ATP by photophosphorylation instead of respiration of organic substrate without evolution of $O_2$. This feature avoids inhibition of HAB with $O_2$ and also allows rapid BNF. However at night, either production of biomass would need to be suspended or some form of respiration would need to take place. Since nitrate or iron respiration seem unsuitable or unlikely to allow a sufficient growth rate, some form of $O_2$-dependent respiration may need to be performed. The Knallgas reaction provides ATP and reducing equivalents and may allow heterotrophic growth at high yields at night by limiting respiration of organic substrate.

The PNSB *Rhodovulum* exhibits several unique features that make it the most promising bacterial candidate: it is tolerant to salt-water and therefore not dependent on scarce freshwater, it produces rare $\omega$ -3 fatty acids, it exhibits autoflocculation which may facilitate harvest, it is likely to have similar features as *Rhodobacter* and it is the only among the selected microbes to completely oxidize $H_2S$ to soluble sulfate. This latter point is most relevant when using $H_2S$ containing subtrate gases mixtures, since the other $H_2S$ oxidizing bacteria including *Rhodobacter* deposit insoluble elemental sulfur on the outside of the cell, which may be unsuitable for long-term cultivations.

Oxygenic phototrophs growing photoheterotrophically on acetate such as the established SCP microalgae offer the remarkable opportunity of combining the mentioned advantages of the o-PS light-reaction with efficient CF [56]. However, presence of glyoxylate cycle enzymes does not indicate to what extent acetate is actually assimilated *in-vivo* via the glyoxylate cycle and to which extent acetate is assimilated via the citrate synthase of the oxidative TCA. In the first case, acetate is effectively channeled to biomass formation whereas only 1 NADH+$H^+$ is liberated per two acetate molecules. This leaves a major need for PS-II supplied reductant. In the latter case however, oTCA liberates three NADH+$H^+$ molecules and one $QH_2$ per acetate molecule. With the NAD-pool reduced by acetate oxidation, state transition favors ATP-generation via cyclic photophosphorylation, channelling light energy away from the desired water splitting reaction. Both phenotypes are indeed found: PS-I driven but PS-II independent acetate photoassimilation [57], as well as high PS-II activity [56]. Strain selection maybe used to select suitable strains with high PS-II activity and high growth rates, or metabolic engineering may be applied: Pyruvate dehydrogenase complexe (PDH) and isocitrate dehydrogenase (IDH) are two key enzymes responsible for involved oxidation/decarboxylation reactions. Since acetate is supplied in the medium and PS-II can provide for reduced ferredoxin, downregulation of PHD may reduce avoidable decarboxylations and may also decrease the metabolic burden incurred by the synthesis and maintenance of such a big enzyme complexe. Since $\alpha$-ketoglutarate is an important TCA-intermediate for protein synthesis that can not be replenished without IDH, downregulation of IDH maybe an option for targeting production of biomass rich in lipids or starch.

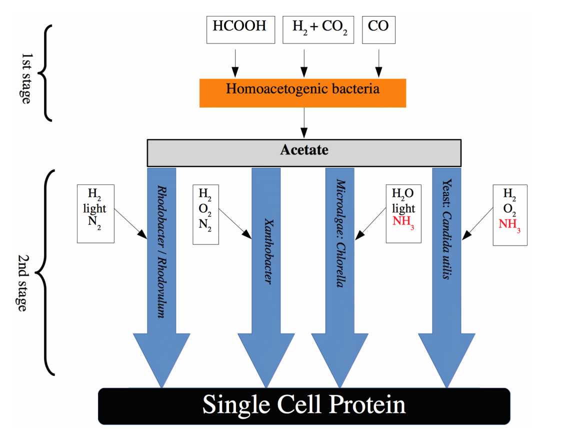


**Figure 2** Two-stage bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims.

## 7 Sources of Energy

All bioprocesses have at least one autotrophic stage and therefore need sources of inorganic carbon and substantial amounts of reductant. Reductant may be

provided as $H_2$, carbon monoxide (CO), formic acid (HCOOH) and mixtures thereof, all of which can be obtained by electrolysis [58, 59, 60, 61]. Electrolysis and several other sustainable are summarized in Figure 3.
A promising technology is the physical-chemical gasification of intractable organic wastes such as lignocellulose to synthesis gas, a mixture of CO, $H_2$ and $CO_2$ that can be fed directly to HAB. Compared to (dry) gasification, hydrothermal gasification (HTG), hydrothermal liquefaction (HTL) and hydrothermal carbonization (HTC) accept wet organic wastes [62], differ in temperature, pressure and retention time, and yield a mixture of gases, fuels and inert carbon respectively.

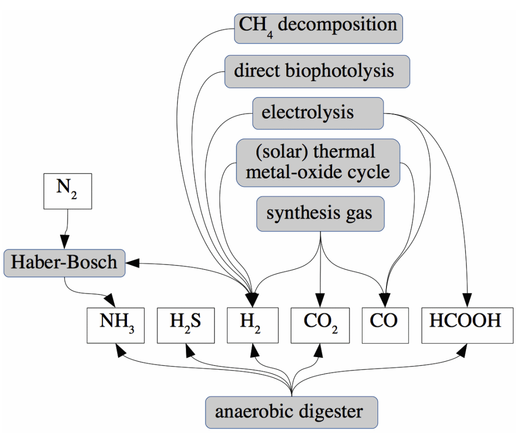


**Figure 3** Overview of sustainable sources of energy, i.e. $H_2$, $CO_2$,CO, formic acid, as well as $NH_3$ and $H_2S$

Counterintuitively, also usage of fossil fuel can provide an eco-friendly source of $H_2$: unlike the currently used method of steam reforming, methane decomposition, also called the “thermal black” process, decomposes methane into $H_2$ and importantly, carbon black instead of $CO_2$ [63, 64, 65, 66]. Carbon black is chemically inert, i.e. it durably prevents the carbon to be released as greenhouse gas and may be used as soil additive or marketed in various products such as inks. Existing methane-based SCP bioprocesses also deserve reconsideration since they conveniently solve the supply problem of both $H_2$ and $CO_2$. They were largely abandonded due to difficulties of gas mass transfer and may also benefit from the bioreactors presented below. However, this would add the methane-C to the global carbon cycle, whereas driving autotrophic SCP with $H_2$ from methane decomposition would essentially be “double carbon-negative”: the methane-C is prevented from being released and one is fixed by HAB with the two $H_2$ that each $CH_4$ decomposes to.

$H_2$ may also be produced from sun without photovoltaic and subsequent water electrolysis: the solar-thermal metal-oxide cycle [67, 68, 69] reach up to 20% efficiency for water splitting and may also be combined with electrolysis [70, 71].
Direct biophotolysis is attractive since it allows the releases of $H_2$ from o-PS mediated water splitting, induced for example by sulphur starvation, knockout of CBB or a lack of $CO_2$ [72, 73, 74, 75]. Inactivation of PS-II and low cell viability are of lesser importance, since microalgae may be simply harvested as SCP. However, even with PDH and IDH downregulated, it maybe hard to control that there is not net flux of reductant from acetate or organic storage metabolites to $H_2$.

## 8 Sources of Substrates and Nutrients

The availability of substrates and nutrients is likely critical for the feasibility of SCP production under economical constraints, or in distant regions. Surprisingly, one of the most urgent challenges and cost drivers lies in the provision of $CO_2$ in larger amounts, in particular anoxic $CO_2$ [76]. While the atmosphere represents an endless supply of $N_2$ and $CO_2$, the concentration of $CO_2$ in the air is too low for an economical extraction using available physico-chemical methods [76]. A commonly cited option is the usage of $CO_2$ from coal power plants but this would indirectly support this harmful technology and create an undesired dependency. Furthermore, cleaning and distribution of $CO_2$ from coal, cement and steel industry would be costly, ecologically questionable and would provide nonetheless insufficient amounts for possible large-scale implementations [76].
Despite the disadvantages described for plants, microalgae and cyanobacteria have the attractive capacity to extract $CO_2$ from the air. Large-scale implementation of their photoautotrophic cultivation is hindered by technological and economical challenges and cyanobacteria are inherently unsafe for food purposes. However, a large untapped resource consists in large surface areas of public waters such as rivers, lakes and the open sea with unobstructed sun exposure, where big amounts of plankton biomass grows for free from atmospheric $CO_2$. Harvesting this biomass was likely hampered by the expensive dewatering, may be well feasible with the rotating bioreactor. Although the biomass is not suitable for direct use as food, extracting it has several benefits: It would avoid the emission of the greenhouse gases that the decomposition of that biomass would cause. Furthermore, it represents an effective means of de-eutrophication, i.e. the removal of excessive nutrients from the waters which,

as described above, causes biodiversity loss and a decrease of ecosystem services. For the extraction of useful substrates from the biomass, different strategies for upgrading of food-grade and non-food-grade biomass to food are presented in the following section (for an overview, see Figure 4).

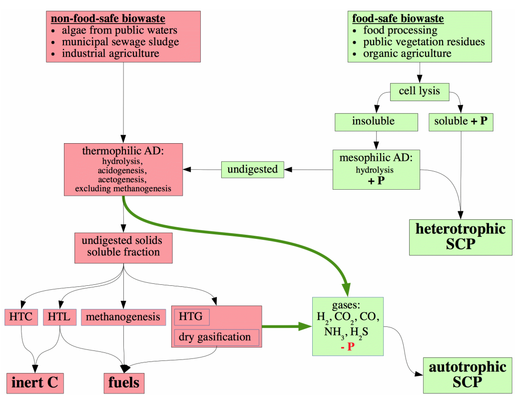


**Figure 4** Substrate and nutrient provision through upgrading of food-grade and non-food-grade waste biomass, respectively. Opportunities of using nutrients from non-food-grade sources for food are highlighted as bold green arrows. (Non-) Recuperation of precious soluble nutrients such as phosphorous are indicated with P in (red) bold.

Non-food-grade biowaste such as algae from eutrophic rivers, municipal sewage sludge or also wastes of industrial agriculture are available in large quantities and it is hypothesized here that nutrients can be extracted safely for food purposes as the gases that evolve during a sequence of biomass degradation steps. First, thermophilic anaerobic digestion (AD) with suppressed methanogenesis allows mineralization to $H_2$, $CO_2$, $NH_3$ and $H_2S$. Suppression of methanogenesis avoids consumption of these gases by methanogens and may be achieved through the removal of those gases, the regulation of the pH below 6, injection of inhibitory levels of $H_2$, or even phages specific to methanogens. Residual $CH_4$ should not disturb SCP production and may even be used in a separate $O_2$-dependent SCP production step involving the established SCP methanotroph *Methylococcus capsulatus*. Spores of pathogens may potentially be carried in the gas phase, but this risk can be addressed by two measures: first, anaerobic digestion, in particular thermophilic AD, is known to deactivate pathogens and spores contained in biowastes [77]. Second, remaining levels of spores can be filtered with commercial filters [78]. Whereas CO, $H_2S$ and $NH_3$ are highly toxic gases for humans, they can metabolized safely by specific microbes (see Table 3) to SCP. Solid and soluble residues may be simply composted as the cheapest option, or further processed in some form of dry or hydrothermal treatment. Hydrothermal gasification is a novel method which allows to completely decompose wet residual organic wastes to $H_2$, $CO_2$ and some $CH_4$, while avoiding expensive drying necessary in conventional gasification[79]. This allows to convert a greater fraction of the biowaste to gases which can be used for growing autotrophic SCP. Again, there is the option to use the $CH_4$ in a separate $O_2$-dependent SCP production step with *Methylococcus capsulatus*. Hydrothermal carbonization yields heat which can be used to heat the AD, and inert carbon, which can be used as a soil additive and at the same time represents a stable carbon sink.

This strategy for non-food-grade biowaste (red in Figure 4) would also allow to benefit of cyanobacteria even when containing cyanotoxins, not as direct SCP, but at least as source of substrate.

An important drawback of substrate extraction from non-food-grade biowaste is that nutrients which remain dissolved and do not occur in the evolved gases, such as phosphor, vitamins and trace elements, are missing for autotrophic SCP production. This can be addressed by nutrient extraction from food-grade biomass: First, the biomass can be subjected to cell lysis and the soluble fraction may be used for SCP growth either directly or after some form of pretreatment such as short heat treatment or simple incubation for autolysis to occur, in which endogenic hydrolytic enzymes such as peptidases, nucleases and amylases degrade proteins, RNA /DNA and carbohydrates to their respective monomers. This would preserve a lower entropy in the substrates compared to complete mineralization and subsequent autotrophic growth on $H_2$+$CO_2$+$NH_3$. As a consequence, faster growth, higher growth yield and overall energy conservation are improved considerably. The insoluble fraction of lysed cells may be fed to mesophilic anaerobic digesters (AD) for a limited time, such that predominantly hydrolysis and to some limited extent acidogenesis break up polymers to monomers. Again, minimizing degradation would minimize time and heating energy, and at the same time allow benefitting of nutrients at lower entropy. It would be even conceivable to co-cultivate SCP-producing microbes within the same reactor with immobilized hydrolyzing consortium, but this seems unlikely to work since the latter require direct contact to substrates particles and often operate best at higher temperatures. However, medium or a SCP-microbes containing suspension may flow through the AD-reactor in tubes made of polyvinylalcohol (PVA), which block particles and microbes but allow diffusion of metabolites from hydrolysis into the

tube [80].
Solube or unsoluble residues from the hydrolysis stage of mesophilic-AD may then be subjected to the same sequence of treatments as for non-food-grade biowastes described above.
Since organic waste biomass may not be accessible in sufficient quantities in an economically viable manner, and since greenhouse gases continue to rise in the atmosphere, extraction of $CO_2$ from the air is desirable. For that aim, side products of electrolyzers, operated for the generation of reductant, may be used: alkalic pH and hydroxides form at the cathode and degrade performance by increasing the overpotential. These could be consumed to extract $CO_2$ from the air by precipitation as carbonates. Air must be bubbled into alkalic, hydroxide enriched catholyte in a separate compartment than the cathode chamber, since $O_2$ may otherwise participate in reactions occurring at the cathode, such as a wasteful proton reduction to water. To transport the inorganic carbon to the HAB, the carbonate-enriched catholyte solution may be fed directly to the HAB bioreactor. To avoid a continuous loss of water and electrolytes, Ca- and Mg-based electrolytes may be used which yield chalk ($CaCO_3$) and $MgCO_3$, two carbonates that precipitate. Sedimented precipitates may be fed to HAB with minimal loss of water and other electrolytes. Indeed, a similar technology is used by the US company Skyonic.

## 9 Bioreactors

Gas-dependent autotrophic cultivations in liquid media are typically suffering from substrate limitation due to the poor water-solubility of gaseous substrates such as $H_2$, $CO_2$ or syngas. Similarly, photobioreactors typically suffer at the same time from an excess of light, namely light inhibition, in the upper layers and a light limitation in the lower layers from mutual shadowing by the cells. Slow gas diffusion seriously hampers bioprocess kinetics by substrate limitation ($CO_2$) and in the case of oxygenic phototrophs product removal ($O_2$), as well. An important economical obstacle is posed by the expensive extraction of biomass from liquid medium [81, 82] and of soluble products from liquid media [83]. Clearly, cultivation in liquid media in 3D-vessels is troublesome for both gas-dependent and light-dependent cultivations. These challenges are addressed in the following way: the problem of biomass dewatering is addressed by growing and harvesting biofilms instead of suspending cells in large amounts of liquid media. Biofilms are exposed directly to gas and light, thereby reducing limitation of gas diffusion or light incidence. The biofilms are hydrated by intermittent immersion of the biofilm in a liquid hydration media bath, or a thin continuous flow of liquid hydration media on the biofilm (see Figure 5 and 6). This supplies nutrients to the biofilm, removes the soluble products formed in the 1$^{st}$ stage and thereby prevents endproduct inhibition. The extraction of the soluble product is

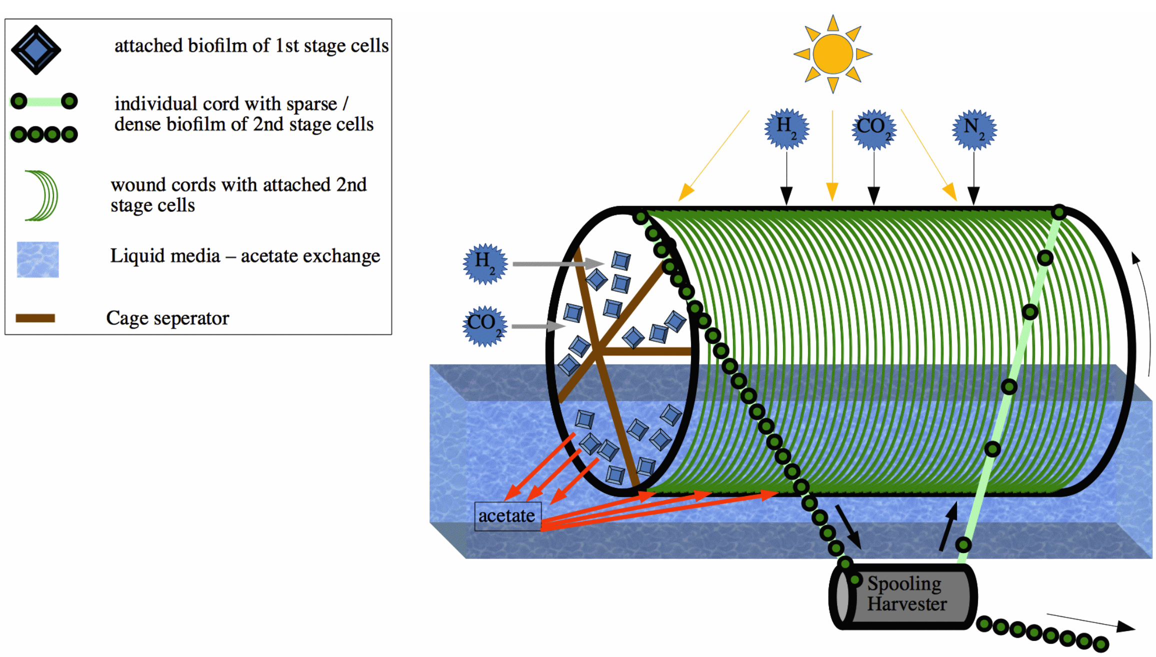


**Figure 5** Proposed adaptation of two rotating biological contactors for 2$^{nd}$ -stage designs, harboring first-stage inside and second-stage outside of a single shared cylinder.

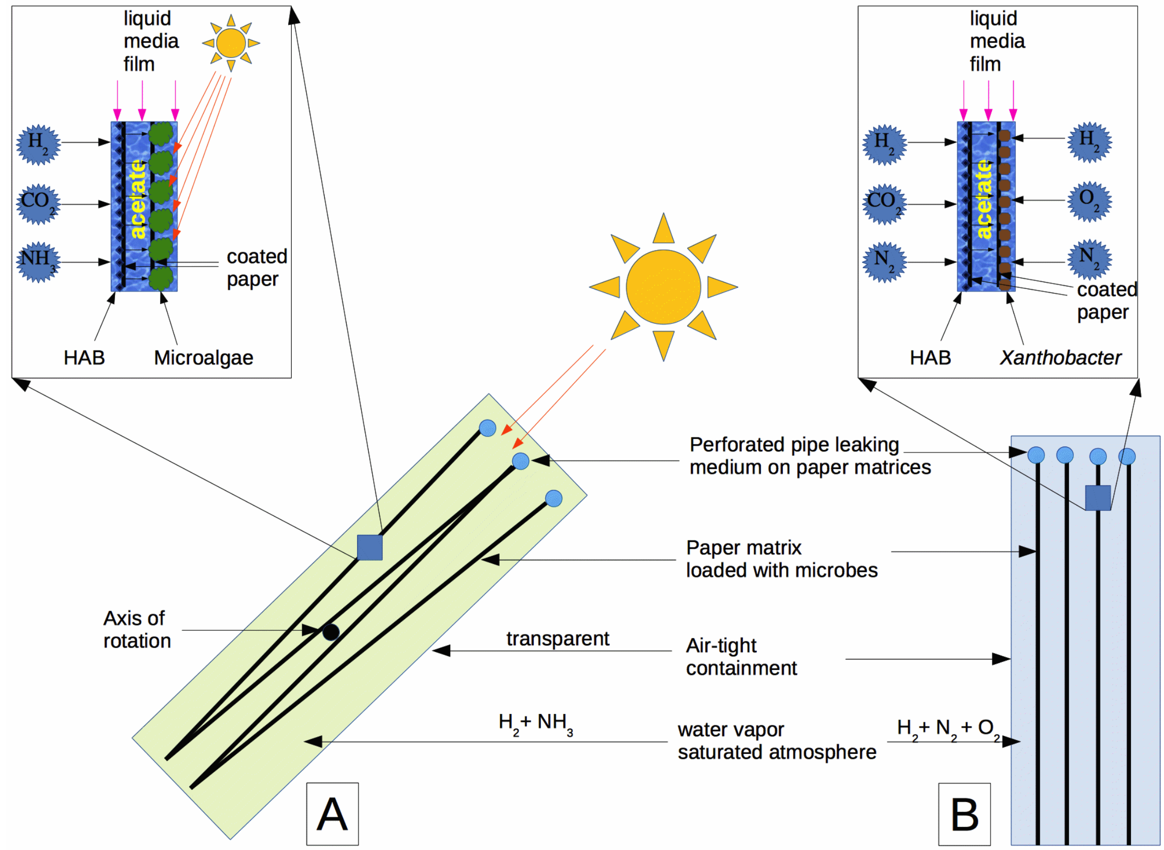


**Figure 6** Coated Paper Array Contactor for light-dependent (A) and light-independent (B) 2-stage designs. Inlets show details about hypothesized sandwiched coated papers with HAB on the left and 2nd stage on the right side respectively.

entirely avoided and left to microbes of the 2nd stage, which selectively extract the acetate from the hydration media. This indirect "co-cultivation" with acetate as intermediate also keeps its concentration low compared to a batch reactor, which has benefits to both producer and consumer of acetate: to prevent endproduct inhibition of HAB by the undissociated form at pH «7, acetic acid, strong buffer systems are usually used to maintain neutral pH [?]. However, buffering implies an increase in ionic strength which has a negative effect on reaction kinetic [23]. By keeping the acetate concentration low, buffer capacity and therefore ionic strength and the limiting effect on reaction kinetic can also be kept low. On the consumer side, a low concentration of acetate keeps osmotic pressure low and contributes, together with adequate buffering, to a low concentration of acetic acid which can be toxic to the cell at higher concentrations [84].
Two gas-fermenting, hydrated biofilm-bioreactors (called "contactors"), implementing these features are presented in the following two subsections.

### Rotating biological contactors

Rotating biological contactor (RBC) are established bioreactors that are half immersed in liquid hydration media and half exposed to the substrate gases [85][86]. They achieve good hydration as well as good gas mass transfer and at the same time present a low-tech, low-cost option with stable long-term operation[87, 88]. In light-dependent single-step as well as all second-stage bioprocesses, microalgae were shown to grow as biofilm attached on cords that are wound around the cylinder [87]. For harvest, the cord is continuously unwound from and rewound to the cylinder by a "spool harvester" and the biomass grown on the cord is dry-harvested by a scraping mechanism [87]. The light-independent first-stage (HAB), which merely acts as biocatalyst and whose biomass is not a desired product have been successfully immobilized on special biofilm carriers which are inserted into cages in the inside of the rotating cylinder[88]. Alternatively, HAB have also been successfully immobilized within hydrogels [89], which may be applicable also for gas fermentation. As a moderate gas diffusion barrier, it may help HAB to cope with low levels of $_2$.

Coated Paper Array Contactor

Coated Paper Array Contactor consist of arrays of thin matrices made of coated paper, which provide support for biofilm growth [90][91]. They are in a sealed gas-tight container with controlled atmosphere consisting of substrate gases such as $H_2$ + $CO_2$ + $NH_3$ or $N_2$, and a saturated water gas phase. Besides water vapor, hydration of the cells can be achieved by applying liquid media on the top of the matrices, flowing down inside the matrix and as thin liquid film on the outside of the matrix. Although cell harvest of 2$^{nd}$ stage cells is not established, the development of a scraping mechanism appears realistic. Coated Paper Arrays Contactors may also already be useful when acetate production by HAB shall be uncoupled from the 2$^{nd}$ stage which may become necessary if measures to protect HAB from $O_2$ turn out insufficient.

## 10 Biomass yields compared to wheat

Grains constitute the most important group of IA products, grain products are ubiquitous in cuisine but being rich in carbohydrate, poor in protein or vitamins, grains have a rather unbalanced nutritional value. Therefore, autotrophic SCP aims at replacing grains, but not fruits, vegetables or spices. In order to compare autotrophic SCP with grains, wheat is chosen because it is among the most important cereals cultivated in Europe. High yields of wheat of 7 tons/hectar*year are reached in a moderate climate such as Germany (FAOSTAT), corresponding to 0,08 g/m$^2$*hour. In arid climates, such as Iran, solar irradiance is double (2000 vs 1000 kWh/m$^2$ ) whereas average wheat yield is about one forth (0,02 g/m$^2$ *hour). Solar panels allow a sensible comparison, with the local solar irradiance as basis of comparison. Considering the known power consumption of water desalination units [92] and water electrolyzers [58], it is possible to calculate the amount of $H_2$ gas which can be produced from the electricity derived from solar panels in that particular region. From the amount of $H_2$ one can calculate the amount of acetate that can be produced by HAB [93] and the subsequent biomass formation from acetate by *Chlamydomonas reinhardtii*[32, 56](see Table 1 for overview, Table 2 in appendix for details). This microalgae as 2$^{nd}$ stage was chosen for the following reasons: 1) it has the "generally recognized as safe" status by the US institution FDA and similar microalgae such as *Chlorella vulgaris* are approved and sold as food. 2) the dry harvest mechanism and growth on cord for the RBC described above, was established for microalgae [87]. 3) Unlike anoxygenic photosynthesis performed by *Rhodobacter*, o-PS has several advantages described above, in particular, the o-PS apparatus generates and tolerates $O_2$ which allows to switch seamlessly between anaerobic/microaerobic photoheterotrophic (daylight) and aerobic heterotrophic (night) growth conditions without damages and lengthy adaptations lags. 4) *Chlamydomonas* has good growth yields for both growth modes and has high specific growth rates [32, 56].

Besides solar panels also microalgae need light and therefore some light has to be subtracted from solar electricity generation. Since microalgae are fed acetate and do not have to fix $CO_2$, they have substantially reduced requirement for ATP and reductant whereas large amounts of energy are consumed for water electrolysis. Therefore 25% of the light are assigned to photoheterotrophic growth, and 75% to solar panels. Microalgae can use dim, scattered light well which occurs in solar parks between photovoltaic modules. Furthermore, excess acetate produced in the day for which microalgae did not have enough time or light for photoheterotrophic assimilation, can be used for $O_2$-dependent heterotrophic growth at night. This was also respected in one central factor, the growth yield, as the average of photoheterotrophic and heterotrophic growth modes respectively, assuming an average ratio of daylight to darkness of 1 (12 hours each). In case microalgae get more sunlight than needed for photoheterotrophic growth, additional carbonate ions may be fed to the liquid media to allow Calvin Cycle to use excessive energy for $CO_2$ fixation.

The case studies combined geographical as well as technological influences in a single factor. The two countries Iran and Germany vary greatly in wheat yields and solar irradiance. Two sets of technology, common commercial models and cutting-edge devices, considered solar panel, water electrolyzers and water desalination units. In order to underline the variability, cutting-edge technology was assigned to Iran and common technology to Germany. With this comparison setup, high variations were observed depending on region and technology used. The relative influence of regional and technological factors on the overall result were 8 and 1,5 respectively. The yield increase of solar-powered SCP compared to wheat varied by an order of magnitude between the two cases and amounts to 19,5 fold (Germany, common technology) and 234,5 fold (Iran, best technology). Calculations assume source of sea water, solar panels, water desalination unit and water electrolyzers and bioreactors to be all on the same site and do not include pressurization of $H_2$.

The production of 1 kg dry SCP is calculated to require about 10 kWh. With a levelized cost of (solar) electricity of 0,055 €/kWh for Iran [94] and 0,08 [95] €/kWh for Germany, this would result in a raw electricity cost of 0,54 and 0,93€ per kilogram of dry SCP, respectively. Although a multitude of initial capital costs and operational costs are not taken into account, a study examining economics of a comparable bioprocess came to the conclusion that electricity costs make up more than 90% of overall costs due to the high energy consumption of water electrolysis [96]. Therefore, estimates of electricity costs do provide a reasonable basis for a first estimate of overall costs.

It is unlikely that autotrophic SCP will be able to compete directly with IA products on today's distorted marketplace in the near future. Instead, it would be fair to compare the entirety of costs holistically i.e. the costs of production invested by the producer as well as all the costs hitting society from the entire life cycle of the product, including costs from deterioration of public health and environmental damages. No such holistic economic investigation was found for IA products but there are numerous indications, i.e. from studies limited to negative effects of N-fertilizers, that the costs to society are likely to be quite high. Assuming autotrophic SCP does not cause any such life cycle costs to society, there are three possible effects which may actually reduce the costs to society rather than only not adding to it: first, simply by replacing IA products which would have caused such costs. Second, if biomass is extracted from eutrophic rivers and lakes for substrate provision and proving effective for de-eutrophication as mentioned above. Third, by providing a protein-rich, carbohydrate-poor food with excellent nutrient content, it contributes to a decrease in the prevalence of common diseases such as diabetes or coronary artery disease, and thereby also contributes to lower health costs.

**Table 1** Preliminary calculations of SCP yield of a 2-stage bioprocess design compared to wheat in Germany and Iran with two sets of technology. Relative influence of regional and technological variation on the overall result are marked red. For detailed table, see Table 2 (Appendix). For explanations, see text.

| Climate | Arid | Moderate | |
|---|---|---|---|
| Country | Iran | Germany | |
| wheat yield | 0,020 | 0,081 | $g/m^2/h$ |
| solar radiation | 2000 | 1000,00 | $kWh/m^2/yr$ |
| regional diff. factor | 8,06 | 1,00 | |
| Technology | Best | Common | |
| solarpanel efficiency | 0,22 | 0,17 | |
| solar electricity | 430,00 | 170,00 | $kWh/m^2/yr$ |
| desal. + electrolysis | 0,088704 | 0,104833 | kWh/mol $H_2$ |
| $H_2$ | 4847,58 | 1621,63 | mol $H_2/m^2/yr$ |
| tec. diff. factor | 1,49 | 1,00 | |
| acetate | 68,70 | 22,98 | $kg/m^2/yr$ |
| SCP | 4,71 | 1,57 | g $cdw/m^2/h$ |
| increase to wheat | 234,54 | 19,48 | fold change |
| overall | | | |
| energy requirement | 9,484 | 11,208 | kWh/kg cdw |
| electricity cost | 0,52 | 0,90 | €/kg cdw |

## 11 Challenges and Perspectives

Several suitable yeasts and microalgae are approved for human consumption and as such there are viable options for immediate implementation. However, none of the proposed bacteria have any legal approval. Some microbes have been used as animal feed and are likely to be suitable as human food, or even have been conceived for human food [29][97], but none have undergone a sufficiently thorough analysis which would allow legal approval. *Chlorobium* and *Aquificales* are not pathogenic, and *Chlorobium* has been used as fish probiotic [98]. Further research, legal work and experiments need to be done until proposed bacteria are approved as food.

The dry-harvesting mechanism for rotating bioreactor ("spooling-harvester") has been tested for microalgae, but has yet to be tested for the proposed bacteria which do have a considerably smaller cell diameter and may be more difficult to scrape from the cord.

It was shown for *Xanthobacter* grown on acetate, that the addition of formic acid (HCOOH) as a dedicated electron donor substantially increases the biomass yield per acetate. It was caused by a decrease in the isocitrate dehydrogenase, an enzyme catalyzing the undesired decarboxylation of isocitrate which. However, no such data could be found for the other microbes, and for the growth conditions acetate + $H_2$ or acetate + $H_2$ + $N_2$. Given an alternative source of reductant, it would likely represent a survival advantage to those cells that channel a higher fraction of C into biomass formation rather than energy supply. Therefore, it is likely that either WT cells do exhibit that behavior, or that prolonged cultivation might select for suitable strains.

Both proposed interventions in anaerobic digesters (AD) remove the products of the desired process. Subsequent steps are not intended to occur and kinetics and stability should rather be improved than disturbed. However, AD are complex and sensitive bioprocesses, and therefore it is uncertain to which extent gases or liquid products can be removed without a collapse of the AD.

Consumer acceptance and psychological requirements of consumers are essential for commercial success, but have been largely ignored in this study. Whereas some of the proposed designs and substrate provisions do rely on microalgae which do already have a fair user acceptance, autotrophic SCP with a tolerance of $H_2S$ or with biological $N_2$ fixation do require bacterial microbes as SCP. Consumer acceptance for bacterial SCP is possibly reluctant and might require consumer information campaigns, hindering commercialization. Another step for achieving consumer acceptance and sufficient market penetration consists in food engineering of suitable food building blocks and developing suitable replacements for existing products. Similarly to soy, wheat or fungi based meat replacement products, it will take time to develop techniques of food processing that remove unpleasant tastes, that helps at providing texture and that give satisfactory taste. Contrary to soy and wheat, SCP provides far greater possibilities of tuning the raw production instead of merely processing the a rather constant vegetable raw product. For example, different yeast and microalgae can form starch or lipids as storage compounds, and their exact composition may be tuned further by either strain selection or genetic engineering to more closely mimick flour or vegetable oil respectively. Furthermore, heterologously expressing synthetic genes of inactive synthetic proteins may allow the targeted overproduction of specific amino acids. At night, and with microalgae also during the day, $O_2$ required for respiration or evolved during o-PS in the second stage may disturb the $O_2$-sensitive HAB of the first stage. This may be addressed in the following ways: for microalgae, the light level can be adjusted to the so-called compensation point such that the microalgae produce as much $O_2$ during photosynthesis as is consumed by simultaneous respiration. However, this would imply a lowered growth rate and also a partial utilization of acetate as electron donor. Alternatively, the 2$^{nd}$ stage maybe comprised of mixtures of microalgae with a second microbe which vigorously consumes $O_2$ and thereby reduces $O_2$ partial pressure levels sufficiently low for HAB. For that purpose, $O_2$-tolerant diazotrophic bacteria can be grown without a source of combined N, since they were frequently shown a particularly high $O_2$ consumption in order to protect the nitrogenase from $O_2$ and to regenerate ATP. *Xanthobacter autotrophicus* or *Azotobacter vinelandii* respire $H_2$ and alternatively, *Methylococcus capsulatus* can be used to metabolize $CH_4$ remaining from AD or hydrothermal gasification (see above). Recently, several HAB were found to be moderately $O_2$-tolerant such as *Sporomusa aerivorans* [99] or *Clostridium magnum* [100], which can quickly reduce $O_2$-partial pressure and continue acetate secretion even in the presence of low levels of $O_2$. However, it is unclear whether the unelucidated $H_2$-dependent mechanism of $O_2$ consumption is energy-conserving. Yet another option would be a gas-tight separation of 1$^{st}$ from 2$^{nd}$ stage, which however, likely involves a more complicated and expensive setup and possibly a disturbed exchange of acetate.

## 12 Conclusion

Industrial agriculture (IA) uses prohibitively large amounts of fresh water, does substantial damage to the environment and fosters climate change by a third of all greenhouse gas emissions. It also deteriorates public health and causes high hidden costs. Furthermore, it is still not sufficiently acknowledged in the public, that there is considerable risk of food shortages in the 21st century. This work challenges plants as only means of food production and presents entirely IA-independent, autotrophic SCP production systems that are sustainable, fail-safe and efficient. It can grow food from $CO_2$, $H_2$ and $NH_3$ or $N_2$, but nonetheless can provide numerous options of upgrading residual biomass. The produced single-cell protein is suitable as emergency ration for the possible eventuality of escalated food insecurity. It contains precious vitamins, fatty acids, antioxidants, and has several health benefits. Therefore, it may also be sold as nutritional supplement, as an ingredient for processed food or as animal feed, replacing soy. Depending on cultivated organisms and cultivation conditions, production can be adapted to favor formation of protein, lipid or starch. As such, it's main destiny is to partly replace grains and oily crops, but not fruit and vegetables. In two case studies, potential productivities were estimated to exceed wheat by one to two orders of magnitude. Considering economics, it is clear that "competitiveness" is neither realistic nor a fair target since current IA product prices are highly distorted by direct subsidies and notably, by indirect subsidies, also called "negative externalities" or "hidden costs". These indirect subsidies consist of public spendings related to diseases, climate change impacts or impaired ecosystem services. No comprehensive study was found that sums up all hidden costs of IA. However, health costs caused by nitrogen fertilizers were estimated for Europe to range between 70 and 320 billion € each year, of which health costs and air pollution constitute about 75% [16]. These big amounts can be explained by the numerous effects of the degradation products of nitrogen fertilizer such as NOx, $NH_3$, $N_2O$ and nitrate. They cause a significant increase of asthma, respiratory disorders, inflammation of airways, reduced lung functions, bronchitis, cancers, infectious diseases and frequency of infestations. It must be emphasized again that these high costs represent the effects of nitrogen fertilizers in Europe in one year only. It can be assumed that in other regions which use more nitrogen fertilizer, such as Asia, comparable or even superior damages are done. Other effects of IA, such as climate change impacts are also causing serious ramifications and high costs. Furthermore IA directly and indirectly consumes large amounts of fossil fuels, which according to recent findings by the International Monetary Fund are estimated to "benefit" of indirect subsidies worth 5 trillion dollars per year worldwide [101]. A comprehensive economic analysis is beyond the scope of this work, but it is important to note that the economical reasons which put an end to SCP in the 1980s, did not take into account trillions $ of indirect subsidies. The proposed autotrophic SCP bioprocess designs are potentially carbon-negative and as such may even hinder climate change. The low content in carbohydrates and high content in protein and vitamins have rather positive impacts on public health. By partially replacing IA, direct damage to the environment can be avoided. Therefore, it is speculated that even though it is likely not competitive with distorted soy prices, the actual economy is superior to IA products when taking into account health and environment, especially when produced and consumed locally.

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**Table 2** Preliminary calculations of SCP yield sof a 2-stage bioprocess design compared to wheat in Germany and Iran respectively, and with two sets of technology (best and common). For details see text.

| Case study | High | Moderate | |
|---|---|---|---|
| Country | Iran | Germany | |
| wheat average yield | 19858,16 | 79979,22 | hg/ha/year |
| wheat average yield (dry weight) | 17574,4716 | 70781,6097 | hg/ha/year |
| wheat average yield (dry weight as $g/m^2$ /h) | 0,0200621822 | 0,0808009243 | $g/m^2$ /h |
| wheat regional ratio | 4,03 | 1 | |
| global radiation Iran / Germany | 2000 | 1000,00 | $kWh/m^2$ /yr |
| global radiation regional ratio | 2 | 1,00 | |
| overall regional difference factor | 8,06 | 1,00 | |
| Technology | Best | Common | |
| efficiency of solarpanel | 0,22 | 0,17 | |
| solar electricity per $m^2$ per year | 430,00 | 170,00 | $kWh/m^2$ /yr |
| electricity requirement of water electrolysis | 44,00 | 52,00 | kWh/kg $H_2$ |
| electricity requirement ofwater desalination | 2,00 | 10,00 | kWh/m3 $H_2O$ |
| overall electricity requirement (per mol $H_2$ ) | 0,088704 | 0,104833 | kWh/mol $H_2$ |
| $H_2$ from sea water | 4847,58 | 1621,63 | mol $H_2$ $/m^2$ /yr |
| overall technology difference factor | 1,49 | 1,00 | |
| 1st stage: homoacetogenic bacteria | | | |
| HAB: yield acetate per $H_2$ | | 0,236 | mol acetate/ mol $H_2$ |
| acetate as mol | 1144,03 | 382,71 | mol acetate/$m^2$ /yr |
| acetate in kilograms | 68,70 | 22,98 | kg acetate/$m^2$ /yr |
| 2nd stage: *Chlamydomonas reinhardtii* | | | |
| yield, photomixotrophic on acetate | 0,80 | 0,65 | g cdw/ g ac |
| yield $O_2$ -heterotrophic on acetate | 0,52 | 0,52 | g cdw / g ac |
| average yield (daylight/dark hours = 1) | 0,635 | 0,585 | g cdw / g ac |
| light used for electricity/acetate | 0,75 | 0,75 | |
| overall yield | 41,22 | 13,79 | kg cdw $/m^2$ /yr |
| as g cell / $m^2$ / hour | 4,71 | 1,57 | g cdw/$m^2$ /h |
| increase compared to wheat | 234,54 | 19,48 | fold change |
| overall energy requirement and cost estimate | | | |
| Energy requirement per kg SCP | 9,484 | 11,208 | kWh/kg cdw |
| Levelized cost of electricity | 0,055 | 0,080 | €/kWh |
| electricity cost per kg SCP | 0,52 | 0,90 | €/kg cdw |

**Table 3** Selected microbes and their functions in the proposed bioprocess designs.

| Organism | Advantages | Disadvantages | Function |
|---|---|---|---|
| Single-Step Microbes | | | |
| *Chlorobium* | • efficient $CO_2$ and fast $N_2$ fixation<br>• fast growth<br>• potential health benefits<br>• $H_2S$ utilization (SQR) | • strict light-dependence<br>• $O_2$ -intolerance<br>• edibility uncertain<br>• partial $H_2S$ oxidation to solid S0 | single step:<br>$H_2 + CO_2$ + light<br>$H_2S + CO_2$ + light<br>→ SCP |
| *Aquificales* | • $O_2$ -tolerant rTCA<br>• Knallgas, NAD+ reducing<br>• $H_2S$ utilization (SQR) | • no active BNF<br>• thermophile<br>• edibility uncertain<br>• partial $H_2S$ oxidation to solid S0 | single step:<br>$H_2 + O_2 + CO_2 + NH_3$<br>→ SCP |
| Two-Stage Microbes | | | |
| *homoacetogenic bacteria* | • most efficient $CO_2$ fixation<br>• secretes C as acetate<br>• slow growth (role of biocatalyst)<br>• $H_2S$ tolerant<br>• often allow CO fermentation | • $O_2$ -intolerance | first stage:<br>$H_2 + CO_2$<br>-or-<br>CO<br>→ acetate |
| *Rhodobacter* | • Knallgas and $O_2$ -tolerant BNF<br>• high yield (photoheterotrophic)<br>• edible | • slow microaerobic dark growth<br>• partial $H_2S$ oxidation to solid S0 | 2nd stage:<br>Acetate+$H_2$ +$N_2$ +Light/$O_2$<br>→ SCP |
| *Rhodovulum* | • tolerance to salt water<br>• produces DHA and EPA<br>• autoflocculation<br>• complete $H_2$ S-oxidation to sulfate | • $O_2$ -tolerance of hydrogenase ?<br>• $O_2$ -tolerance of nitrogenase ? | 2nd stage:<br>Acetate+$H_2$ +$N_2$ +Light/$O_2$<br>→ SCP |
| *Xanthobacter* | • Knallgas and $O_2$ -tolerant BNF<br>• $e^-$-donor improves yield | • edibility ? | 2nd stage:<br>Acetate + $H_2$ + $N_2$ + $O_2$<br>→ SCP |
| *Nostoc* | • Knallgas + oxyg.-photosynthesis<br>• o-PS driven nitrogenase<br>• good $H_2$ -usage by nitrogenase | • inefficient CF<br>• poor acetate assimilation<br>• uncertainty about toxicity | single-step:<br>$H_2$ +$N_2$ + $CO_2$ + light/$O_2$<br>→ SCP |
| Chlamydomonas | • oxygenic photosynthesis<br>• efficient acetate assimilation<br>• approved SCP, consumer acceptance<br>• direct biophotolysis | • inefficient CF<br>• no BNF and no Knallgas<br>• wildtype: cell wall indigestible | single-step<br>$H_2 + CO_2$ +light/ $O_2$<br>→ SCP<br>2nd stage:<br>Acetate + Light / $O_2$<br>→ SCP |
| *Candida utilis* | • established SCP<br>• fair yields without light | • no BNF,<br>• no Knallgas<br>• wildtype: cell wall indigestible | 2nd stage<br>acetate+$O_2$ +$NH_3$<br>→ SCP |