HYPOTHESIS PAPER

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

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Abstract

Food production with plants consumes large amounts of water, occupies large areas of land and cannot guarantee food security beyond the 21st century. Industrial agriculture in particular, is destructive to the environment, fosters climate change in profound ways, deteriorates public health and causes high "hidden costs". Single-cell protein (SCP) represents an alternative with minimal carbon-, water- and land footprints. However, when grown on biowastes of industrial agriculture, heterotrophic SCP does not truly improve sustainability or food security.

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 or HCOOH from several sustainable sources. Besides H_2O-electrolysis and syngas, surprisingly fossil fuels may provide an effectively carbon-negative and cheap supply of H_2 through the decomposition of CH_4 or oil. Most promising bioprocess designs consist of 2-stages. In the 1st stage, homoacetogenic bacteria fix CO_2 up to 10 more efficiently than plants, and secrete it as acetate. In the 2nd stage, selected microbes grow on the acetate and thereby form edible biomass. Bacteria have unique features including N_2-fixation, H_2S tolerance and O_2-tolerant hydrogenases for fast light-independent growth. Eukaryotic microalgae are already approved as food and exhibit oxygenic photosynthesis which partly replaces solar-panels, seawater desalination and H_2O-electrolyzers. Photoheterotrophic growth on acetate decouples these benefits from inefficient endogenous 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 that are exposed directly to substrate gases, and that can be dry-harvested. 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, for moderate and arid climates respectively. Preliminary cost estimates and data about direct and indirect subsidies of industrial agriculture lead to the hypothesis that 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 Food security and industrial agriculture

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, by abolishing biofuels and by reducing animal farming[3]. With today's actual requirements for food, industrial agriculture (IA) would not be necessary and 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 in total quantity and relative shares of agricultural production [9]. Unlike the previous decades, production of industrial agriculture is not expected to increase substantially due to climate change impacts in tropical, as well as in temperate cli-

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mates [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 and flooding, pollinator decline, freshwater scarcity, desertification, and soil salinization due to inadequate irrigation. 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 the discrepancy between projected demand and projected supply by further intensifying irrigation, application of fertilizers and pesticides, and by transforming 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 fertilizer range between 35-230 billion €, which is more than the economic benefit of fertilizers in agriculture [16]. The overall efficiency of the nitrogen flow is even further impaired by suboptimal nitrogen managment: denitrification not only prevents reclaiming the combined N from waste water, that was obtained with high energy input and causing the mentioned problems, but even requires another substantial energy investment[19]. 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 [20].

The most incisive among the numerous impacts of IA is the destruction of forests because it seriously affects the natural capacity of the biosphere to adapt to climate change impacts now and in the future. This comes in addition to the fact that IA alone causes up to 29 % of all greenhouse gases [21]. Therefore, besides the direct damage done to the globe's biosphere today, these two major climate-change enhancing factors are going to hit the globe's biosphere a second time in the future.

2 Limitations of plants

Evolved to their own survival advantage and not as food producers for humanity, plants in general have inherent properties which make them rather unsuitable for food production and that represent the root cause of several unsustainable practices of IA:

1. very high blue-green water footprint (irrigation, surface, ground and rain water). Global averages of common crops reach about 1.8 m^3 of water per ton crop [22]. More than 90% of the global blue-green water is used in IA [23].
2. low efficiency of the Calvin-Benson-Bassham-Cycle (CBB) of CO_2-fixation (CF). CBB is a major sink of energy in plant[24] and requires several times more ATP than anaerobic prokaryotic pathways [25]. Due to the oxygenase side reaction of the CBB key enzyme RubisCO, evolving O_2 or low CO_2 can further degrade the efficiency of CBB (photorespiration).
3. inedibility of a large fraction of the plant such as ligneous stem, leaves and roots.
4. indigestibility of a fraction of edible biomass, such as cellulose.
5. photosynthesis efficiency is commonly decreased due to uneven light distribution, i.e. either light limitation or excess light [24].
6. presumably, the penalties incurred by CBB inefficiency, photorespiration, uneven light distribution and inedible / indigestible fractions of the biomass, do not simply sum up but increase in a time- and mass-dependent fashion, since each living cell has to perform aerobic respiration outside daylight hours, consuming storage compounds produced during daylight hours.
7. high vulnerability to abiotic stress such as drought, flooding and salinity.
8. high vulnerability to biotic stress, such as insects, weeds and fungal pests. IA monocultures favor pests and require extensive pesticide treatment. Pesticides are often also toxic to farmers and affect the surrounding environment in a radius enlarged through run-off and aquifer contamination.
9. low productivity per surface and time due to all previous points. 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.

10 high dependency on N fertilizer (with the exception of legumes), for the reasons elaborated above, are among the main causes of the high ecological footprints of IA.
11 strict dependency on light as the only possible energy source restricts the overall productivity to daylight hours.
12 strict limitation to CO_2 as the only carbon source, excluding usage of organic substrates or wastes.

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.
2 produce food reliably, regardless of harsh climate conditions and preferably, also at night.
3 provide the option of complete independence from agriculture, but nonetheless also provide the option to safely utilize biowastes for food production.
4 require a bare minimum of water and fertile land.
5 do no harm to the environment.
6 not foster climate change.
7 minimize dependency on unsustainable or unreliable external factors, such as fertilizers. Provide the option of biological N_2 fixation.
8 operate with diverse sustainable energy sources, including H_2, synthesis gas and sunlight.

4 Single-cell protein as alternative food

Due to the inherent drawbacks of plants, the defined aims cannot be achieved with plant-based food production. Therefore, this work concentrates on edible microorganisms, called "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 SCP biomass is entirely edible and no biowastes such as leaves, roots or stems are produced. Furthermore, SCP microbes are often more digestible than plant cells. For these reasons, any SCP has substantial efficiency gains over plants.
3 wastage of water and nutrients as happening in agriculture [26] are virtually eliminated, since evaporation, transpiration, drainage and runoff are completely avoided.
4 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. Nutrients do not leak, Pesticides are not required.
5 selected strains have high nutritional quality [27, 28]. 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 [29, 30, 31]. *Rhodobacter* produce vitamins including B1, B2, B12, E, biotin, niacin, folic acids [29]. *Rhodovulum* also contains the two precious ω-3 fatty acids docosahexaenoic and eicosapentaenoic acid [32, 33] 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 [28]. 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 an example of a successful fungal meat replacement. In the long term, SCP appears promising as an alternative to soy and grains, which represent a large fraction 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.

There is one commonly cited concern with SCP: the high content of nucleic acids can cause gout when ingested in large quantities [28]. It 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 easily. This can be achieved by alkalic treatment, salt treatment and/or a heat treatment that speeds up endogenic degradation by nucleases [28]. 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 a particularly high ecological footprint. A major problem not commonly cited is that SCP grown on substrates derived from industrial agriculture simply inherits the ecological footprint, water footprint and food insecurity of IA products. Therefore, a SCP production that is to fulfill the aims defined above, at most can utilize biowastes of organic agriculture, but not on wastes of industrial agriculture. Instead, the bioprocess must be itself autotrophic, i.e. it has to include at least one step in which a microbe fixes CO_2 and either forms edible biomass itself, or allows other microbes to grow on secreted products.

5 Microbes for autotrophic SCP

Established SCP microbes are mostly eukaryotic microbes such as yeasts and microalgae. Only few bacteria have been used for SCP, including the cyanobacteria *Spirulina* and the methanotrophic bacterium *Methylococcus capsulatus*. The -underappreciated- capabilities of bacteria most relevant to autotrophic SCP include:

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 [34] 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 [25].

2 growth rates of autotrophic bacteria can exceed those of plant cells.

3 diazotrophic bacteria are capable of biological N_2 fixation (BNF) and are independent of external N-fertilizers. Growth in medium deplete of a source of combined nitrogen also represents an effective barrier against a large class of potential contaminants.

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 a source of combined N.

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 directly reduce NAD+ or ferredoxin using H_2. Notably, the Knallgas reaction allows autotrophic bacteria to grow in the dark at practicable speed. The Knallgas reaction may 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 crotonyl-CoA-carboxylase, the fastest known carboxylating enzyme [25, 35].

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 [36]. 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, usually contains less indigestible cell components than plant or fungal cells (no lignin, cellulose or chitin) and is rich in protein with an amino acid profile of high nutritional value. Some bacteria produce nutrients such as cobalamine (vitamine B12), of which eukaryotic organisms cannot produce significant amounts.

Established eukaryotic SCP microbes on the other hand, have the following advantages over bacteria:

1 they are often already commercially available and therefore escape the legal and financial problem of obtaining a new approval for human consumption.

2 they already have a fairly good consumer acceptance, whereas bacteria tend to provoke resilience.

3 microalgae are the only microbes safe for direct food usage that exhibit oxygenic photosynthesis. While cyanobacteria also exhibit this growth mode, for the time being they have to be considered unsafe as direct foodstuff.

4 microalgae are suitable producers of plant-like starchs or lipids, and therefore may provide drop-in replacements of common ingredients such as flour and vegetable oil.

5 yeast provide very high acid tolerance, counteracting the problem of contamination. Yeast also exhibit fast growth..

Despite the enormous metabolic diversity, no single bacterial or eukaryotic species could be found that strictly achieves all aims. For a detailed comparison, see Table 1. This leads to the conclusion that either minor compromises have to be made or a given bioprocess has to be composed of more than one stage (see next section).

A thorough comparison of all candidate microbes, both bacteria and eukaryotes, can be found in Table 1.

6 Single-Step bioprocess 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 such a compromise is related to biological nitrogen fixation (BNF). BNF corresponds to the aim of eliminating the dependency on N-fertilizer and the associated problems described above. 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*). Furthermore, the high ATP requirements of BNF conflict with its O_2-sensitivity which hampers ATP-generation via aerobic respiration or oxygenic photosynthesis. Therefore, BNF restricts production to daylight when powered by anoxygenic photosynthesis or allows respiration only at low microaerobic conditions, leading to slow growth. Another important reason to abstain from BNF is that the overuse of fertilizers already has led to an excess of combined nitrogen. Therefore it is sensible to recycle combined nitrogen instead of adding new combined N to the global N-pool. Furthermore, sustainable means of novel NH_3 production exist and simply consists in the Haber-Bosch processes driven by renewable energy, sun heat and sustainably generated H_2[37, 38]. For these reasons, microbes without BNF such as 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 [39, 40]. However, cyanobacteria fix CO_2 with CBB and do not assimilate acetate well [41, 42]. Above all, a multitude of cyanobacteria are known to form cyanotoxins such as microcystins, anatoxins, saxitoxins, hepatotoxic cyclic peptides [43, 44] and β-N-methylamino-L-alanine [45, 46]. 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. Even if such a strain was found, uncertainty would remain whether toxins tested negative may form under untested conditions, especially if the synthesis of that toxin is not elucidated, or if it cannot be excluded with reasonable certainty that isozymes remain undiscovered. Overall it is concluded that 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).

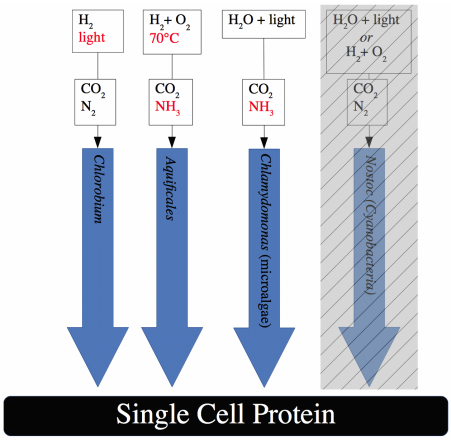

Figure 1 Single-Step bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims. Cynanobacteria are shown greyed out, since they are not considered safe at the time of writing.

Microalgae are contradicting defined aims in several 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 lossy, heterotrophic growth at night. Third, wildtype microalgae have indigestible cell-walls and cell-wall free mutants [47] may not get full consumer acceptance. Microalgae are nonetheless also considered for single-step bioprocess designs the mentioned disadvantages may be outweighed by the following advantages of oxygenic photosynthesis (o-PS): under optimal conditions, the light-reaction of the o-PS transforms light energy and water into protons, electrons and O_2 at an efficiency above 25% [24, 48]. This efficiency drops sharply below 10% when including the inefficient dark-reaction, i.e. the CBB cycle of CO_2 fixation. This is only slightly less than H_2 generated by photovoltaic-driven water electrolysis, which currently reaches at best 16% (less than 20% and 80% for solar panels and water electrolyzers, respectively). However, for several practical reasons, the efficiency of the solar-powered H_2 generation decreases significantly: first, it drops to around 10% due to mismatch losses between solar panel and electrolyzer [49, 50]. While o-PS works with impure or even sea water, water electrolysis requires deionized water, i.e. a water desalination step upfront. H_2-liquefaction can incur between 20 and 86% exergy loss [51], which is not required with o-PS. Transport of liquefied H_2 as well as distribution in the bioreactor can also be omitted. Importantly, solar-powered H_2 generation does not do two essential performances of o-PS: 1) extracting CO_2 at low concentration from the air,

Table 1 Selected microbes and their functions in the proposed bioprocess designs.

Organism	Advantages	Disadvantages	Function
Single-Step Microbes			
Chlorobium	▪ efficient CO_2 & fast N_2 fixation ▪ fast growth ▪ potential health benefits ▪ H_2 S utilization (SQR)	▪ strict light-dependence ▪ O_2 -intolerance ▪ edibility uncertain ▪ partial H_2 S oxidation to solid S0	single step: $H_2 + CO_2$ + light H_2 S + CO_2 + light → SCP
Aquificales	▪ O_2 -tolerant rTCA ▪ Knallgas, NAD+ reducing ▪ H_2 S utilization (SQR)	▪ no active BNF ▪ thermophile ▪ edibility uncertain ▪ partial H_2 S oxidation to solid S0	single step: $H_2 + O_2 + CO_2 + NH_3$ → SCP
Two-Stage Microbes			
homoacetogenic bacteria	▪ most efficient CO_2 fixation ▪ secretes C as acetate ▪ H_2 S tolerant ▪ often allow CO fermentation	▪ O_2 -intolerance	first stage: $H_2 + CO_2$ -or- CO → acetate
Rhodobacter	▪ Knallgas and O_2 -tolerant BNF ▪ high yield (photoheterotrophic) ▪ edible	▪ slow microaerobic dark growth ▪ partial H_2 S oxidation to solid S0	2nd stage: Acetate+H_2 +N_2 +Light/O_2 → SCP
Rhodovulum	▪ tolerance to salt water ▪ produces DHA and EPA ▪ autoflocculation ▪ complete H_2 S-oxidation to $SO4^-$	▪ O_2 -tolerance of hydrogenase ? ▪ O_2 -tolerance of nitrogenase ?	2nd stage: Acetate+H_2 +N_2 +Light/O_2 → SCP
Xanthobacter	▪ Knallgas and O_2 -tolerant BNF ▪ e^--donor improves yield	▪ edibility ?	2nd stage: Acetate + H_2 + N_2 + O_2 → SCP
Nostoc	▪ Knallgas + oxyg.-photosynthesis ▪ o-PS driven nitrogenase ▪ good H_2 -usage by nitrogenase	▪ inefficient CF ▪ poor acetate assimilation ▪ uncertainty about toxicity	single-step: H_2 +N_2 + CO_2 + light/O_2 → SCP
Chlamydomonas	▪ oxygenic photosynthesis ▪ efficient acetate assimilation ▪ approved SCP ▪ fair consumer acceptance ▪ direct biophotolysis	▪ inefficient CF ▪ no BNF ▪ no Knallgas ▪ wildtype: cell wall indigestible	single-step H_2 + CO_2 +light/ O_2 → SCP 2nd stage: Acetate + Light / O_2 → SCP
Candida utilis	▪ established SCP ▪ fair yields without light	▪ no BNF, ▪ no Knallgas ▪ wildtype: cell wall indigestible	2nd stage acetate+O_2 +NH_3 → SCP

and 2) fixation of CO_2. Ultimately, energy, equipment and therefore complexity, ecological footprint and cost of solarpanels, water desalination units, water electrolyzers as well as several other accessory devices and infrastructure can be saved. Nontheless, the favored bioprocess designs with microalgae are presented in the following section.

The green sulfur bacterium *Chlorobium tepidum* is one

of the few organisms found that has efficient CO_2 fixation and an active BNF at the same time [52] . *C.t.* also provides a fast growth rate [53] supported by anoxygenic photosynthesis and exhibits moderate thermophily. However, the absence of aerobic or anaerobic respiration and strict dependency on light would allow biomass production only with artificial illumination. Due to at least three lossy energy transformations (primary energy source to electricity, electricity to light and light to chemical energy), this is a highly inefficient option, even with flashing LEDs within a narrow spectrum matching the absorption maxima [54]. Alternatively, *Chlorobium tepidum* could be permitted its natural behaviour, the fermentation of the reserve compounds accumulated in the cell during the daylight hours[55]. Fermentation products could be stored and then be fed back during the day.

7 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) which utilize the most efficient CF pathway found in nature, the Wood-Ljungdahl-Pathway (WL)[25, 34]. Since CO_2 is at the same time carbon source and the terminal electron acceptor in carbonate-respiration, organisms with WL have the remarkable quality of secreting the vast majority of the fixed carbon as acetate, other organic acids or alcobols 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. Related organisms are also able to metabolize cellulose. If there is a temporary electricity surplus, it can be invested in electrochemical reduction of CO_2 to CO and HCOOH. Growth on mixtures of CO, HCOOH and H2 might accelerate acetate formation by HAB [56], since CO and HCOOH are the first products of the two CO_2 fixation reactors in WL (FDH and CODH/ACS), which are likely to be rate-limiting. However, this temporary increased productivity would come at the cost of forgoing the efficient catalysis by these enzymes.

Although acetate is a universal central metabolite in all living beings, the biomass yield on acetate as substrate 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 [57].

The chemolithoautotrophic bacterium *Xanthobacter autotrophicus* exhibits the EMC acetate assimilation pathway described above and reaches good yields of 35g cdw/g acetate. Furthermore, the growth yield on acetate was shown to improve substantially with the additional of HCOOH as additional electron donor besides acetate [58]. It was caused by a decrease in the activity of the isocitrate dehydrogenase, an enzyme catalyzing the undesired decarboxylation of isocitrate. This ICDH downregulation is likely enabled by shifting regeneration of $NADH+H^+$ from acetate oxidation to formate oxidation via a NAD-reducing formate dehydrogenase operating in *Xanthobacter* [59]. Similarly, it is likely that uptake of H_2 with hydrogenases, regeneration of ATP by aerobic respiration, and regeneration of reductant may allow to oxidize less acetate via the oxidative TCA, and also enables BNF by the reduction of the local O_2 partial pressure [60]. However, since *Xanthobacter* does not encode a NAD-reducing hydrogenase, the increase of the biomass yield with acetate + H_2 is likely to be less pronounced than with the substrate mixture acetate + HCOOH.

Purple nonsulfur bacteria (PNSB) such as *Rhodobacter* and *Rhodovulum* perform anoxygenic photosynthesis, which similarly to *Chlorobium*, generates proton motif force from light without evolving O_2 and ATP is regenerated from the pmf. Unlike *Chlorobium*, however, NAD can be reduced only inefficiently through reverse electron flow. Since O_2 is not evolved or required, inhibition of HAB by O_2 is avoided 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 are impracticable, some form of O_2-dependent respiration may need to be performed. The aerobic respiration of H_2 (Knallgas reaction) provides ATP and reducing equivalents at the quinone level, and may allow growth on acetate or on reserve compounds accumulated in the cells during the day. However, special measures need to address the incompatibility with HAB due to (micro-) aerobic conditions.

In addition to *Rhodobacter*, the PNSB *Rhodovulum* exhibits several unique features that make it a particularly promising candidate: it is tolerant to salt-water and therefore not dependent on scarce freshwater, it produces rare ω -3 fatty acids, it exhibits autoflocculation which may facilitate harvest, 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

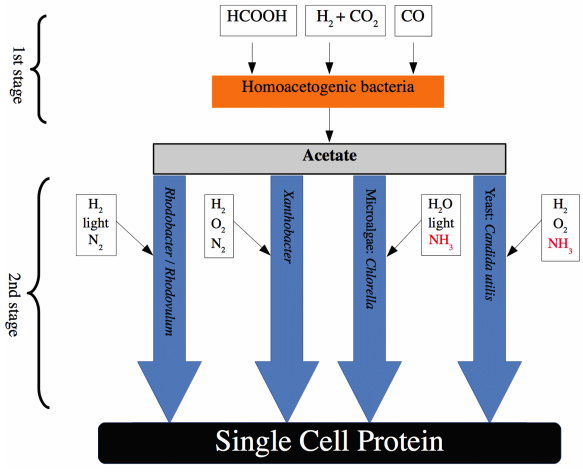

Figure 2 Two-stage bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims.

long-term cultivations.

Like PNSB, oxygenic phototrophs such as microalgae are able to grow photoheterotrophically on acetate. This growth mode offers the remarkable opportunity of combining the mentioned advantages of the o-PS light-reaction with efficient CO_2 fixation through HAB derived acetate [61]. This means that the efficiency of photosynthesis reaches up to 27% [24, 48] and is not reduced by inefficient CBB CO_2 fixation. 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 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, and therefore an occasion to benefit of, PS-II mediated generation of reductant from water splitting. In the latter case however, oTCA oxidizes acetate to CO_2 and liberates three $NADH+H^+$ molecules and one QH_2 per acetate molecule. With the NAD-pool reduced by acetate oxidation, an adaptive mechanism called "state transition" shifts light utilization towards ATP-generation via cyclic photophosphorylation (PS-I), channelling light energy away from the desired water splitting reaction (PS-II). Both phenotypes have been reported: PS-I driven but PS-II independent acetate photoassimilation [62], as well as high PS-II activity [61]. Adaptative selection maybe used to select for strains with high PS-II activity and high growth rates. Alterantively, 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 the TCA-intermediate α-ketoglutarate is an important precursor 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.

8 Sustainable 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), H_2S and mixtures thereof. H_2, CO and HCOOH can be obtained by electrolysis [63, 64, 65, 66]. Besides electrolysis, several other sustainable sources of energy 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, hence help to avoid expensive drying [67]. HTG, HTL and HTC differ in temperature, pressure and retention time, and yield a mixture of gases, fuels and inert carbon respectively. Inert carbon represents an occasion of carbon sink and may therefore contribute to provide a carbon-negative SCP production.

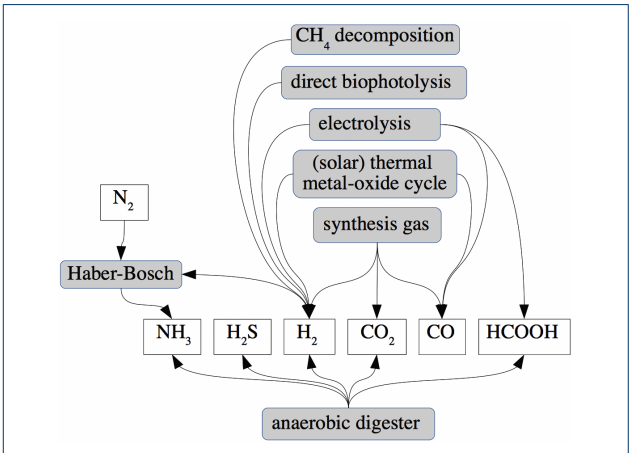

Figure 3 Overview of sustainable sources of energy, i.e. H_2, CO_2,CO, formic acid, as well as NH_3 and H_2S

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 [68, 69, 70, 71]. 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 [72, 73, 74] reach up to 20% efficiency for water splitting and may also be combined with electrolysis [75, 76].

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 [77, 78, 79, 80]. Eventual 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 down-regulated, it maybe hard to control that there is not net flux of reductant from acetate or organic storage metabolites to H_2.

9 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 [81]. 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 [81]. 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 [81].

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, an untapped resource consists in 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, but may be well feasible with the rotating bioreactor presented below. 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 Figure 4.

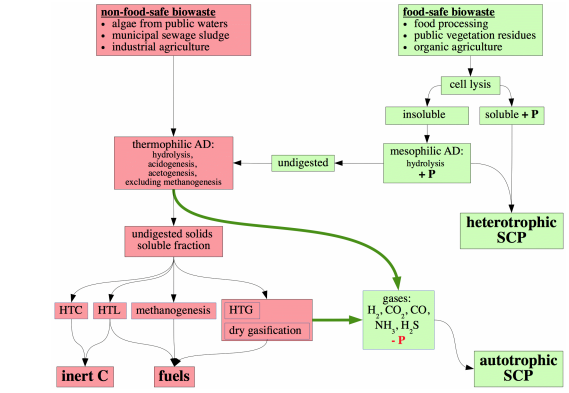

Figure 4 Substrate and nutrient provision through upgrading of food-grade and non-food-grade waste biomass, respectively. Opportunities of using nutrients from non-food-grade sources for food are highlighted as bold green arrows. (Non-) Recuperation of precious soluble nutrients such as phosphorous are 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 timely recovery 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 to some extent [82]. Second, remaining levels of spores can be filtered with commercial filters [83]. 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, yielding soil substratum. Alternatively, residues may be further processed in some form of dry or hydrothermal treatment. As described above, hydrothermal gasification is a novel method which allows to decompose wet residual organic wastes to H_2, CO_2 and some CH_4, while avoiding expensive drying necessary in conventional gasification[84]. 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 contributing to a carbon-negative balance.

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 achieved 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, polynucleotides 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 improved energy conservation can be expected. 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. For that purpose, 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 [85]. 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. In the case that electrolyzers are operated for generation of H_2, CO or HCOOH, side products of electrolyzis may be used in that aim: 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 effluent in a separate compartment than the cathode chamber, since O_2 may otherwise participate in futile cycle 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 $CaCO_3$ (chalk) 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.

10 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. Photobioreactors typically suffer at the same time from an excess of light in the upper layers, namely light inhibition, and 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 [86, 87] and of soluble products from liquid media [88]. 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: by growing and harvesting biofilms instead of suspending cells in large amounts of liquid media, cells 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, i.e. acetate formed in the 1st stage and thereby prevents end-product inhibition. In the case of two-stage designs, the extraction of the soluble product is 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 [89]. However, buffering implies an increase in ionic strength which has a negative effect on the reaction kinetic of CO_2 fixation by HAB [25]. 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 [90].

Besides facilitated supply of gaseous substrates or light, another central advantage of biofilms is that they can be harvested dryly, for example by scraping, and that expensive cell dewatering is avoided. This should occur frequently, since matured biofilms form complex structures which do not necessarily have good properties of diffusion.

Two gas-fermenting, hydrated biofilm-bioreactors (called "contactors"), implementing these features are presented in the following two subsections (also see Figures 5 & 6).

Rotating biological contactors

Rotating biological contactor (RBC) are a kind of established bioreactors often used in waste water treatment that are half immersed in liquid hydration media and half exposed to the substrate gases [91][92]. These rotating cylinders achieve good hydration as well as good gas mass transfer and at the same

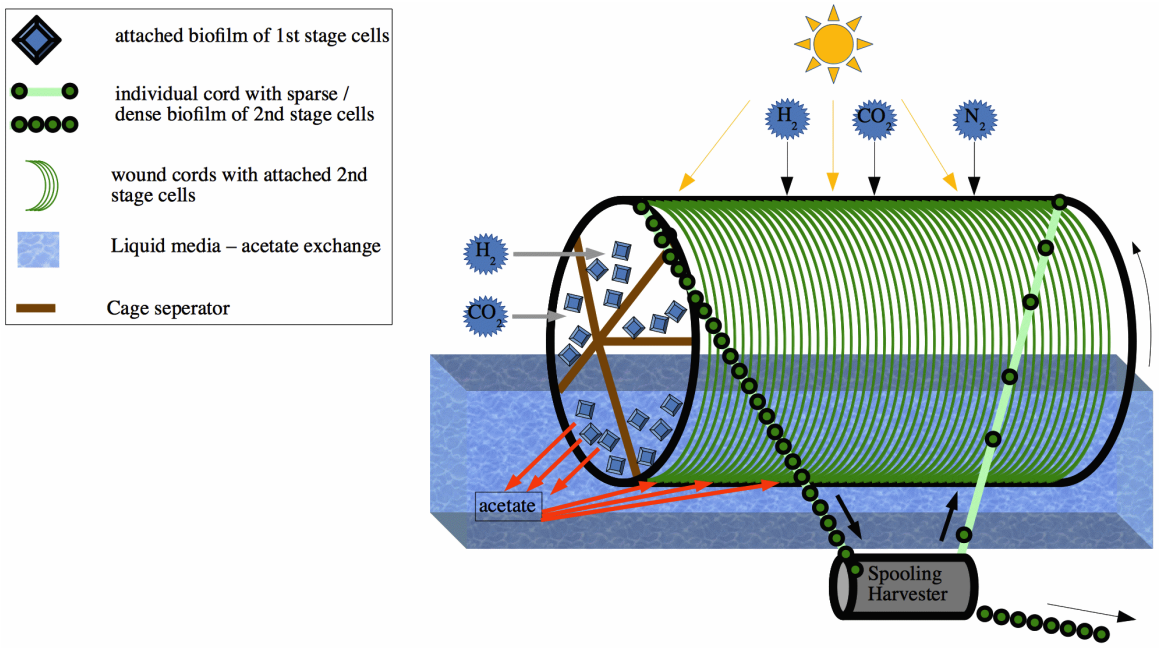

Figure 5 Simplified rendering of the proposed adaptations of two rotating biological contactors for 2nd -stage designs, harboring first-stage inside and second- stage outside of a single shared cylinder.

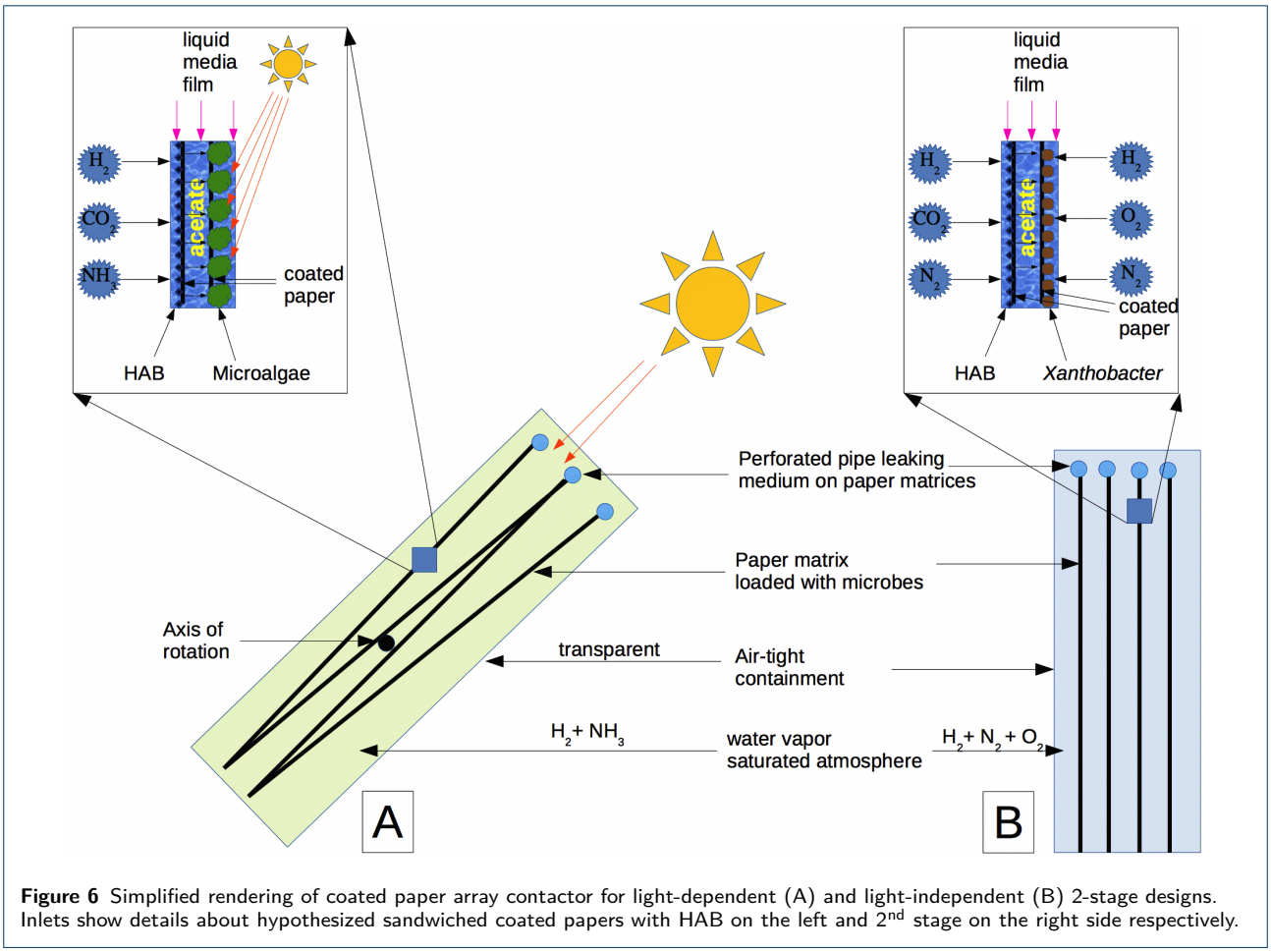

Figure 6 Simplified rendering of coated paper array contactor for light-dependent (A) and light-independent (B) 2-stage designs. Inlets show details about hypothesized sandwiched coated papers with HAB on the left and 2nd stage on the right side respectively.

time present a low-tech, low-cost option with stable long-term operation[93, 94]. HAB have been successfully immobilized on biofilm carriers (AnoxKaldnes^{TM} K1). These carriers are particularly suitable for RBC because they provide an openly accessible surface which readily allows gas exchange and biofilm attachment, while also protecting the biofilm from the frequent collisions occurring in the rotating cylinder. Syngas fermentation was successfully demonstrated with *Clostridium carboxidivorans* grown on K1 carriers inserted into cages in the inside of the rotating cylinder[94]. Alternatively, HAB may also be immobilized within hydrogels such as has been demonstrated with LentiCats^{TM} [95]. An advantage of entrapment in hydrogels would be that it may prevent biomass outgrowth and that it may present a moderate diffusion layer that may help HAB to cope with low levels of 0_2 (see section Challenges and Perspectives).

Cells whose biomass is to be harvested, i.e. single-step and 2nd-stage in two-stage bioprocesses, has to be cultivated differently in order to allow cell harvest. Microalgae were shown to grow as biofilm attached on cords that are wound around the cylinder [93]. For harvest, the cord is continuously unwound from and rewound to the cylinder and the biomass grown on the cord is dry-harvested by a "spool harvester" with a scraping mechanism [93].

Taken together, it is hypothesized here that two-stage designs can be cultivated using a single cylinder: in the inside of the cylinders, 1st-stage, HAB, are immoblized on biofilm carriers or entrapped into hydrogels. On the outside of the same cylinder, 2nd-stage cells are cultivated on cords as biofilm support, which can be easily scraped off for harvest. If the 2nd-stage microbes are neither evolving nor requiring 0_2, i.e. if the entire bioprocess is operated anaerobically, then 1st and 2nd stages can share the same atmosphere and medium. For example, this applies for PNSB that are cultivated photoheterotrophically, but not if biomass production has to be continued at night with O^2-dependent respiration. Sharing atmosphere and medium is also unlikely to work when combining HAB with microalgae, because microalgae evolve O^2 during daylight hours and biomass growth at night requires O^2 at night for

respiration (fermentation of reserve compounds would be an anaerobic option without substantial biomass growth).

In order to allow 2-stage designs with both HAB and microbes evolving or requiring O^2, the following adaptions to the RBC setup may contribute to lower the 0_2 partial pressure:

1 The coexistence with the O^2 evolving or requiring the 2^{nd} stage microbes with HAB maybe possible also sharing both atmosphere and medium, if the cord is conducted through openings to a separate chamber with proper illumination or O^2 exposure, and then be conducted back. A pressure gradient may prevent larger amounts of O^2 to flow into the anaerobic chamber.

2 The gas-tight separation of the atmosphere and medium from 1^{st} and 2^{nd} stages. The substrate gas atmosphere destined to consumption by HAB, may be fed into the inside of the cylinder. This may build up a minor pressure gradient and prevent O^2-enriched gas to flow from outside to the inside of the cylinder. The liquid medium into which HAB secrete the acetate and from which the 2^{nd} stage take up the acetate, can at first be contained in the inside of the cylinder and continuously drop out or flow out through holes in the -otherwise gas-tight- cylinder. The following mechanisms remain to be devised: a) gas-tight delivery mechanism to supply substrate gas and fresh medium to the inside of the rotating cylinder. b) device that allows outflow of acetate-enriched medium while preventing uncontrolled outflow of substrate gases or inflow of O^2-enriched gas from the outside.

General solutions to 0_2-related problems that are not specific to the RBC are discussed below in the section "Challenges and Perspectives".

Coated Paper Array Contactor

Coated Paper Array Contactor are composed of paper matrices on which convective assembly of living cells and adhesive latex particles forms colloid-latex biocomposite adhesive coatings [96, 97, 98]. While the paper provides mechanical stability, hydration and diffusion of products, 1^{st} stage bacteria are entrapped at high density in the microporous latex film which prevents biomass outgrowth while still permitting a reasonable exchange of substrate gases and acetate. 2^{nd} stage microbes are not entrapped in latex but half entrapped half immobilized in some kind of coated paper matrix, which does not inhibit biomass outgrowth. This way, 2^{nd} stage microbes may be kept in close contact for good exchange of acetate, while still allowing easy harvest of outgrown biofilm from the coated paper. As illustrated in Figure 6, flat biofilm matrices such as coated paper allow a more space-efficient setup and at the same time, the required light can be distributed on a much larger surface area by orienting the phototrophic film almost parallel to the incoming light. This allows to keep the light intensity per cell low which strongly increases the efficiency of photosynthesis. Coated paper array contactors are proposed to be held 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 paper 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 some form of scraping mechanism appears realistic.

The problem of O_2 described above for the RBC exists equally for the coated paper array contactors, but the approach of the acetate-enriched HAB derived liquid flowing from the 1^{st} to the 2^{nd} stage is not applicable here. Instead, the coated paper is at the same time separator of the two atmospheres, anoxic and oxic. To prevent O_2 diffusion, a layer of O_2-consuming microbes can be positioned between HAB and the oxic layers. In the case of O_2-dependent heterotrophically growing microbes, it may suffice to just grow thicker biofilms of these microbes. In the case of O_2-evolving microalgae, a dedicated layer of O_2-consumers may need to be installed. Biomass growth of these O_2-consumers is not desirable, since it would render the setup and handling more complex and notably, would consume acetate. A sufficiently strong sink of energy, which O_2-consuming respiration can meet without wasting energy, may consist in diazotrophic bacteria with the capability of aerobic H_2-respiration. They were shown a particularly high O_2 consumption in order to protect the nitrogenase from O_2 and to regenerate the big amounts of ATP, including *Xanthobacter autotrophicus* or *Azotobacter vinelandii* [99]. Alternatively, *Methylococcus capsulatus* also consumes large amounts of O_2 when metabolizing CH_4 which may arise from AD-derived substrate gas mixtures or from hydrothermal gasification (see above). Since the BNF reaction is highly regulated and prone to fluctuate based on the actual need of the cell, O_2 removal may be unreliable as soon as the cell does not need any more ammonia. In several diazotrophs including *Rhodobacter capsulatus*, genetic or pharmacological inhibition of the glutamin-synthase were shown to lead to a continuous deregulation of nitrogenase and the subsequent secretion of NH_4^+ to the medium [100, 101, 102, 103]. This may also hold true for aerotolerant diazotrophs such as *Xanthobacter autotrophicus*, *Azotobacter vinelandii* and *Methylococcus*

capsulatus. Besides stabilizing O_2 removal, secretion of NH_4^+ by these microbes may thereby also provide combined nitrogen to microalgae, which are incapable of BNF.

Both reactors may also be uncoupled from each other. For example, acetate may be produced in RBC and consumed with coated paper contactors or vice versa, a possible scenario if measures to protect HAB from O_2 turn out insufficient.

11 Biomass yields compared to wheat

Grains constitute the most important group of IA 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 in 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 [104] and water electrolyzers [63], 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 [105] and the subsequent biomass formation from acetate by *Chlamydomonas reinhardtii*[34, 61](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 [93]. 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 [34, 61].
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 [106] and 0,08 [107] €/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 [50]. Therefore, estimates of electricity costs do provide a reasonable basis for a first estimate of the overall costs of electricity driven bioprocess designs.

Naturally, these preliminary cost estimates do not apply for bioprocess designs that rely on other sources of substrates. For example, SCP production with H_2 from decomposition of natural gas is likely to be much less expensive.

Table 2 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 3 (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	
desal. + electrolysis	0,088704	0,104833	kWh/mol H_2
tec. diff. factor	1,49	1,00	
solar electricity	430,00	170,00	$kWh/m^2/yr$
H_2	4847,58	1621,63	mol $H_2/m^2/yr$
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

12 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 [31][108], 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 [109]. Further research, legal work and experiments need to be done until proposed bacteria are approved as food.

The dry-harvesting mechanism is not established for coated paper biofilm reactors. For rotating bioreactor, the "spooling-harvester" has been tested for microalgae, but has yet to be tested for bacteria which do have a considerably smaller cell diameter and may be more difficult to scrape from the cord.

A fundamental conflict is present in some two-step bioprocess designs: O_2-dependent or O_2-evolving 2^{nd} stages occur together with the 1^{st}-stage, consisting of O_2 sensitive homoacetogenic bacteria. The following hypothetical measures or mixtures of measures to address the problem are likely to require extensive experimental work:

1. O_2-evolution by microalgae can be reduced by limiting the irradiance. At the so-called compensation point, the microalgae produce as much O_2 during oxygenic photosynthesis as they consume by simultaneous respiration. At this low light intensity, efficiency of photosynthesis is highest and these conditions would also be favorable for direct biophotolysis. This would only support a low growth rate and would also imply an increased respiration of acetate and consequently, a decreased growth yield per acetate. However, since acetate was generated with very high efficiency by HAB, and since the released CO_2 can be re-fixed by the HAB biofilm, this maybe acceptable.
2. The 1^{st} and 2^{nd} stages could be separated into two separate atmospheres, connected only through the liquid media through which exchange of acetate occurs (see Figure 5 and inlets in Figure 6). Adapations for the bioreactors are discussed in the respective section.
3. Immobilization of HAB in hydrogels[95] may constitute a limited gas diffusion barrier for O_2. It must be experimentally determined if the benefit of O_2 shielding outweights the cost of also impeding the diffusion of substrate gases such as H_2 and CO_2.
4. Besides these mechanisms to reduce pO_2, several HAB were recently found to be moderately O_2-tolerant, including *Sporomusa aerivorans* [110] or *Clostridium magnum* [111], which do not grow but can quickly reduce pO_2 and continue acetate secretion even in the presence of O_2. However, it is unclear to which extent the H_2-dependent mechanism of O_2 consumption is energy-conserving. Since HAB biomass is hardly usable and HAB outgrowth may eventually also disturb gas exchange, uncontrolled HAB growth has to be considered a disturbance and loss of carbon in the system. Thereby, the unknown amount of energy wasted by the O_2 removal mechanism must be compared to the prevented loss that would have been caused by HAB growth and its management. Experimental work is required to determine these losses for various candiate HAB and for various O_2 concentrations and to which extent these losses undermine the advantage of efficient CO_2 fixation.

13 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 local famines or the possible eventuality of escalated food insecurity in a distant future. 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 [112]. 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.

Assuming autotrophic SCP would indeed not cause any such life cycle costs to society, it may actually reduce the costs to society rather than only not adding to it: first, simply by replacing IA products such costs and direct damage to the environment is avoided. Second, autotrophic SCP is carbon-negative, and the difference of GHG emissions compared to IA food may counteract climate change and contribute to reduce the substantial costs of climate change impacts. 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. Antioxidants and vitamins rather have positive impacts on public health. Fourth, if biomass is extracted from eutrophic rivers for substrate provision, and if de-eutrophication is proving effective, environmental damage and associated costs can be avoided. For these reasons, it is speculated that even though it is likely not competitive with distorted prices of IA products such as soy, 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 3 Preliminary calculations of SCP yields of 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_2 O
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