Agriculture-independent, sustainable, fail-safe and efficient food production by autotrophic Single-Cell-Protein.

Industrial agriculture (IA) is detrimental to the environment, contaminates water and fosters climate change. Moreover, IA 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 waste products of IA. This concept paper proposes autotrophic SCP bioprocess designs which enable sustainable, fail-safe and efficient “primary production” of edible biomass from CO_2 and N_2/NH_3, while also providing flexible options of biomass upgrading. It can be driven by H_2, CO, HCOOH from several sustainable sources. Most promising designs consist of 2-stages. In the 1st stage, homoacetogenic bacteria (HAB) fix CO_2 and secrete fixed C as acetate with unrivaled yields. In the 2nd stage, acetate is transformed to edible biomass by selected microbes. Bacteria have versatile features such as O_2-tolerant hydrogenases and N_2-fixation. Eukaryotic microalgae are approved as food and exhibit oxygenic photosynthesis (O-PS), partly replacing solar-panels, seawater desalination and H_2O-electrolyzers. Photoheterotrophic growth on HAB-derived acetate provides the opportunity to benefit from the highly efficient light-reaction, while avoiding the inefficient dark-reaction. Slow gas mass-transfer, poor light distribution and expensive cell harvest are major challenges arising from the cultivation in liquid media. To cope with these, microbes grow as hydrated biofilms, exposed directly to substrate gases and light. Two such bioreactors are presented and adaptations for 2-stage designs are proposed. One bioreactor was designed for algae growth in wastewater and provides a mechanism of dry cell harvesting. By harvesting microalgae from flowing waters, reduced carbon and nutrients can be obtained, while at the same time counteracting eutrophication. Non-food-grade biomass can be fed to anaerobic digesters (AD) with suppressed methanogenesis. Gaseous H_2, CO_2, NH_3, H_2S, CO_2 and volatile organic acids can be extracted, with the gas

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phase acting as a “cleaning” barrier. This makes it possible to safely upgrade non-food-grade biomass to food. When food-grade waste is subjected to hydrolysis, diffusible PVA-tubes piercing the AD may allow simultaneous extraction of basic monomers and precious soluble nutrients. As an additional provision of CO_2, alkalic pH and hydroxides formed at the cathode during electrolysis may be used to precipitate CO_2 from the air as carbonates. 2-stage designs with solar-powered H_2 generation from seawater, were estimated to exceed productivity of wheat 20-200 fold. It is speculated that, considering the non-utilization of direct and indirect subsidies, autotrophic SCP is far superior to IA not only in ecological but also in economical aspects.

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Abstract

Industrial agriculture (IA) is detrimental to the environment, contaminates water and fosters climate change. Moreover, IA 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 waste products of IA. This concept paper proposes autotrophic SCP bioprocess designs which enable sustainable, fail-safe and efficient "primary production" of edible biomass from CO_2 and N_2/NH_3, while also providing flexible options of biomass upgrading. It can be driven by H_2, CO, HCOOH from several sustainable sources. Most promising designs consist of 2-stages. In the 1st stage, homoacetogenic bacteria (HAB) fix CO_2 and secrete fixed C as acetate with unrivaled yields. In the 2nd stage, acetate is transformed to edible biomass by selected microbes. Bacteria have versatile features such as O_2-tolerant hydrogenases and N_2-fixation. Eukaryotic microalgae are approved as food and exhibit oxygenic photosynthesis (O-PS), partly replacing solar-panels, seawater desalination and H_2O-electrolyzers. Photoheterotrophic growth on HAB-derived acetate provides the opportunity to benefit from the highly efficient light-reaction, while avoiding the inefficient dark-reaction. Slow gas mass-transfer, poor light distribution and expensive cell harvest are major challenges arising from the cultivation in liquid media. To cope with these, microbes grow as hydrated biofilms, exposed directly to substrate gases and light. Two such bioreactors are presented and adaptations for 2-stage designs are proposed. One bioreactor was designed for algae growth in wastewater and provides a mechanism of dry cell harvesting. By harvesting microalgae from flowing waters, reduced carbon and nutrients can be obtained, while at the same time counteracting eutrophication. Non-food-grade biomass can be fed to anaerobic digesters (AD) with suppressed methanogenesis. Gaseous H_2, CO_2, NH_3, H_2S, CO_2 and volatile organic acids can be extracted, with the gas phase acting as a "cleaning" barrier. This makes it possible to safely upgrade non-food-grade biomass to food. When food-grade waste is subjected to hydrolysis, diffusible PVA-tubes piercing the AD may allow simultaneous extraction of basic monomers and precious soluble nutrients. As an additional provision of CO_2, alkalic pH and hydroxides formed at the cathode during electrolysis may be used to precipitate CO_2 from the air as carbonates. 2-stage designs with solar-powered H_2 generation from seawater, were estimated to exceed productivity of wheat 20-200 fold. It is speculated that, considering the non-utilization of direct and indirect subsidies, autotrophic SCP is far superior to IA not only in ecological but also in economical aspects.

Introduction

In 2014, 800 million people suffered from undernourishment (FAO, 2015), and almost 2 billion people suffered from malnutrition (Grebmer et al., 2014). 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 (Food and Agriculture Organization of the United Nations, 2013). With today's requirements for food without feed and fuel, even organic farming, which has a low ecological footprint at slightly lower yields, could potentially provide enough food for the majority, or even the entirety, of the world population (Badgley et al., 2007; Connor, 2008). However, the world's population is expected to grow to about 9 billion by 2050 and will require 60-100% more food (Godfray et al., 2010). 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 (Alexandratos and Bruinsma, 2012). 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 (Challinor et al., 2014; Thornton, 2012; Wheeler and Braun, 2013). 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, 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 (Connor, 2015). IA is likely to partly counteract these challenges by intensifying irrigation, application of fertilizers and pesticides, and by transforming more land into monocultures. Each of these measures has already

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had profound negative effects on freshwater resources, the ecosystem, climate change, public health and society. While the overall IA output has been doubled in the last decades, the scale of these side effects has been often found to grow at a significantly greater rate (Galloway et al., 2003; Van Grinsven et al., 2013; Tilman, 1999). Side effects are expected to increase faster with further IA gains, in the case of N-fertilizers even exponentially (Shcherbak et al., 2014). 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% (Lassaletta et al., 2014), 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 deteriorate public health (Galloway et al., 2003). 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 (Van Grinsven et al., 2013). 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 O2-dependent marine life (Carstensen et al., 2014).

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, causes significant loss of biodiversity and seriously affects the natural capacity of the biosphere to adapt to climate change impacts now and in the future. Last but not least, IA alone causes up to 29 % of all greenhouse gases (Vermeulen et al., 2012).

Besides the unsustainable practices of IA, plants in general have inherent drawbacks that make them rather unsuitable as the *only* means of food production:

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 (Mekonnen and Hoekstra, 2014).
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. Agroindustrial monocultures favor pests and require extensive pesticide treatment. Pesticide toxic to farmers and the surrounding environment in a wide radius 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 excess energy. CBB requires several times more ATP than anaerobic prokaryotic pathways (Bar-Even et al., 2010). 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).
8. To meet an increasing demand, increased requirements for arable land are met by forest clearing and moor draining, during which biodiversity is destroyed and substantial amounts of GHG are released.
9. high dependency on nitrogen fertilizer with the mentioned high impacts of production and degradation.
10. 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.
11. limitation to CO_2 as the only carbon source, excluding usage of organic wastes.

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 food material. SCP has several advantages:

1. it grows several orders of magnitude faster than crops.
2. waste of water and nutrients is virtually eliminated, as transpiration, drainage and runoff can be avoided completely and evaporation only occurs in open-pond systems where it can be easily mitigated (Rockström et al., 2003).
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 (Amata, 2013; Nasseri et al., 2011). 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 (Noparatnaraporn and Nagai, 1986; Sasikala and Ramana, 1995; Vrati, 1984). Furthermore, *Rhodobacter* produce vitamins including B1,B2,B12, E, biotin, niacin, folic acids (Noparatnaraporn and Nagai, 1986). *Rhodovulum* also contains the two precious ω−3 fatty acids, namely docosahexaenoic and eicosapentaenoic acid (Loo et al., 2013a, 2013b).

Fungal SCP was used as emergency food during both world wars in Germany and was temporarily commercialized on larger scales during the 1960-1980s (Nasseri et al., 2011). 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.

Typically, 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 (Nasseri et al., 2011), and the low consumer acceptance of SCP as “food”. SCP is proposed as a filling ingredient for traditional foods, such as is the case with bakers' yeast. 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 by heat treatment, speeding up endogenic degradation by nucleases (Nasseri et al., 2011). Bad consumer acceptance of SCP can be circumvented by mixing SCP with traditional foodstuff, by extracting 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 rather simple and fast commercialization. In the long term, SCP appears promising as an alternative to 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 displace production of fruits and vegetables.

Several autotrophic single-cell-protein production schemes are proposed that form biomass from CO_2, H_2 and N_2 or NH_3. They are composed of either a single bacterial bioprocess (such as microalgae), or two interdependent stages. Challenges related to low solubility of the gaseous substrates and biomass harvest are addressed in two specific bioreactor proposals.

Aims

Considering the important shortcomings of agriculture, a sustainable, efficient, fail-safe and agriculture-independent food production systems is proposed that should:

1. provide for healthy food.
2. produce food reliably regardless of future harsh climate conditions.
3. operate with various energy sources including sunlight and H_2.
4. offer the option to use sun light, yet also work at night.
5. provide the possibility to upgrade residual biomass to food.
6. have a minimal dependency on external unreliable or unsustainable factors, such as fertilizers.
7. require a bare minimum of water and land.
8. not depend on toxic substances.
9. not harm the environment
10. not foster climate change.

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 proposes to fulfill the aims defined above, can rely on neither products nor wastes of industrial agriculture. Such a bioprocess must be itself *autotrophic*, i.e. it has to be capable to form edible biomass by fixing CO_2 and forming edible biomass.

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 (Boyle and Morgan, 2011) 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 (Bar-Even et al., 2010).
2. growth rates of autotrophic bacteria far exceed plant cells.

3. *diazotrophic* bacteria are capable of biological N_2 fixation (BNF) and are thus independent of the 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 with sustainable N-fertilizers.
5. Several bacteria are able to use H_2 in the presence of O_2 ("Knallgas-bacteria"), for ATP-regeneration, and some bacteria can directly reduce NAD using H_2. Notably, autotrophic Knallgas bacteria can grow in the dark and achieve higher growth yields by avoiding respiration of 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).
7. Some bacteria perform anoxygenic photosynthesis which utilizes light for ATP-regeneration without evolving O_2. This allows to power O_2-sensitive BNF and WL pathways, and doubles biomass yields during growth on acetate, since respiration of organic substrates for ATP-regeneration can be avoided to some extent.
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 (Bar-Even et al., 2010; Erb et al., 2007).
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 (Griesbeck et al., 2000). 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 rich in protein with high nutritional value, low in carbohydrates and contains far less indigestible cell components than plant or fungal cells (no lignin or cellulose).
11. some bacteria can produce cobalamine (vitamine B12), whereas most eukaryotic organisms cannot produce significant amounts themselves.

Cyanobacteria are special among bacteria because they are the only bacteria that have oxygenic photosynthesis and effective physiological adaptations allowing for BNF driven by either o-PS or the Knallgas reaction (Bothe et al., 1977, 1978; Vyas and Kumar, 1995). However, cyanobacteria do neither fix CO2 efficiently, nor assimilate acetate well *(Pearce and Carr, 1967)*. Notably, a multitude of cyanobacteria are known to form cyanotoxins such as microcystins, anatoxins, saxitoxins, hepatotoxic cyclic peptides (Lyra et al., 2001; Nybom, 2013). and ß-N-methylamino-L-alanine (BMAA) (Cox et al., 2005; Holtcamp, 2012). While a few strains were found to not form particular cyanotoxins, none was found that does not form any toxin at all. Generally, uncertainty remains whether toxins tested negative may form under untested conditions.

Although bacteria show a great diversity of biochemical capabilities, no single species of bacteria could be found that achieves all aims. Utilization of some capabilities impose considerable restrictions on the bioprocess (es). For example, BNF is a very desirable feature because it eliminates the dependency on N-fertilizer. However, BNF is rarely found in useful MOs with efficient CF and also complicates cultivation: BNF high requirements of ATP conflicts with its O_2-sensitivity which hampers ATP-generation via aerobic respiration and oxygenic photosynthesis. Therefore, BNF imposes restriction to daylight when powered by anoxygenic photosynthesis or allows respiration only at microaerobic conditions, hence slower growth. Furthermore, several sustainable ways of producing ammonia from N_2 do exist (Li and Licht, 2014), including Haber-Bosch operated with renewable energy and H_2 from sea water electrolysis (Morgan et al., 2014). Considering that overuse of fertilizers led to large scale eutrophication, it is sensible to recycle nitrogen compounds instead of adding new combined N to the global N-pool. This could be achieved by using the off-gas of anaerobic digesters, which contains O_2-free mixtures of NH_3, CO_2 and H_2 (see below).

Eukaryotic MOs have three central advantages: established SCP strains like yeast of microalgae are often already approved for human consumption and have already a fair consumer acceptance. Since cyanobacteria for the time being have to be considered unsafe for food purposes, microalgae are the only MOs that provide oxygenic photosynthesis. Microalgae maybe suitable producers of plant-like starch, which may replace cereal-starch, whereas yeast provide fast growth and good nutritional value.

A comparison of all candidate microbes can be found in Table S2.

Single-Step-designs

Bioprocess designs that rely on a single species accept compromises at set aims in favor of greater simplicity (Fig.1). MOs without BNF such as *Chlorella* or *Aquificales* are deemed acceptable because sustainable means of NH_3-production or NH_3-recycling exist (Li and Licht, 2014; Morgan et al., 2014). Microalgae do not have any means of autotrophic growth independently of light, as they lack O2-tolerant hydrogenases. Furthermore, wildtype microalgae have indigestible cell-walls. While cell-wall free mutants exist (Hyams and Davies, 1972), these may not have full consumer acceptance. Inefficient CO_2-fixation (CF) via the Calvin-Cycle (CBB-CF) is deemed acceptable in combination with oxygenic photosynthesis (o-PS). This appears justified because the losses caused by the inefficiency of the CBB-CF are at least partially outweighed by the gains in efficiency as well as the economic advantages conferred by o-PS. In optimal conditions, o-PS transforms sunlight and water into protons, electrons and O2 with efficiency up to 28% (Taiz and Zeiger, 2007), whereas photovoltaic-driven water electrolysis reaches at best 16% (<20% and <80% respectively). If H_2 is not produced on site, one further has to include up to 85% energetic loss for H_2-pressurization (Christopher and Dimitrios, 2012), H_2-transport and H_2-distribution in the bioreactor.

While o-PS works with impure or even sea water, water electrolysis requires deionized water which calls for a water desalination unit. Therefore, O-PS also reduces cost since it replaces solar-panels, water desalination unit and water electrolyzer. CBB, although inefficient when only considering CF per se, has the added benefit of extracting CO_2 from the air and therefore saves the energy and cost associated with acquiring anoxic CO_2.

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 (Wahlund and Madigan, 1993). *C.t.* also provides a fast growth rate (Eisen et al., 2002) supported by anoxygenic photosynthesis and exhibits moderate thermophily. However, *C.f.* has a very low oxygen tolerance, and strict dependency on light. Artificial illumination by flashing LEDs within a narrow spectrum matching the absorption spectrum (Bertling et al., 2006) may allow to minimize the losses that would occur at night by fermentation of reserve compounds. Although *Chlorobium* appears unsuitable for continuous production, a light-dependent design may be acceptable in particular for arid-climates.

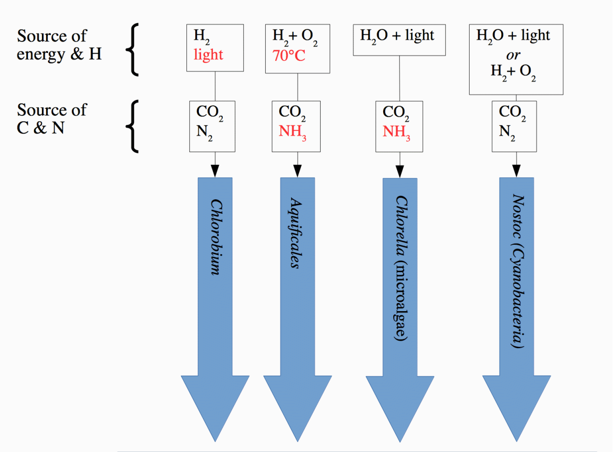

Fig 1: Single-Step bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims.

a) Two-stage designs

Two-stage designs are composed of a first stage, in which CO_2 is metabolized to acetate and a second stage in which acetate is transformed to edible biomass (see Fig. 2). The first stage is performed by a single group of bacteria: homoacetogenic Bacteria (HAB) that utilize the most efficient CF found in nature, the Wood-Ljungdahl-Pathway (WL). Besides being efficient (Bar-Even et al., 2010; Boyle and Morgan, 2011), 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. H_2, CO_2, CO and HCOOH can be produced in a variety of sustainable ways (see Fig. 3). The second stage can be performed by most bacteria, yeast and microalgae because acetate is a universal central metabolite in all living beings. However, the biomass yield on acetate generally varies considerably compared to more reduced substrates such as glucose, because a higher fraction of the substrates has to be consumed for ATP generation. *Saccharomyces cerevisiae* and *Candida utilis* are two established SCP yeast that both posses an active efficient acetate assimilation mechanism, namely the glyoxylate cycle. However, due to impaired energy conservation at site I, *Saccharomyces c.* has to spend more substrate for energy needs and reaches a biomass yield of 29 g dry cell weight /g acetate whereas *Candida utilis* reaches 39 g DCW/g (Verduyn et al., 1991). The chemolithoautotrophic bacterium *Xanthobacter autotrophicus* (*X.a.*) exhibits the efficient EMC acetate assimilation pathway and the growth yield on acetate was shown to improve when an inorganic electron donor such as HCOOH (likely also H_2) is added (Croes et al., 1991). Aerobic respiration of H_2 (the so-called

Knallgas reaction) provides ATP. This allows to oxidize less acetate via TCA for ATP generation and also supports BNF by the reduction of the O_2 partial pressure (Robson and Postgate, 1980). Photoheterotrophic growth by anoxygenic phototrophs such as *Rhodobacter* and *Rhodovulum* can supply ATP by photophosphorylation instead of respiration of organic substrate. Besides ATP generation, O_2-tolerant hydrogenases also provide reducing equivalents, yet at varying degrees of energy conservation. For example, hydrogenases of *Aquifex* are capable to reduce NAD whereas *Rhodobacter* only reduce quinones.

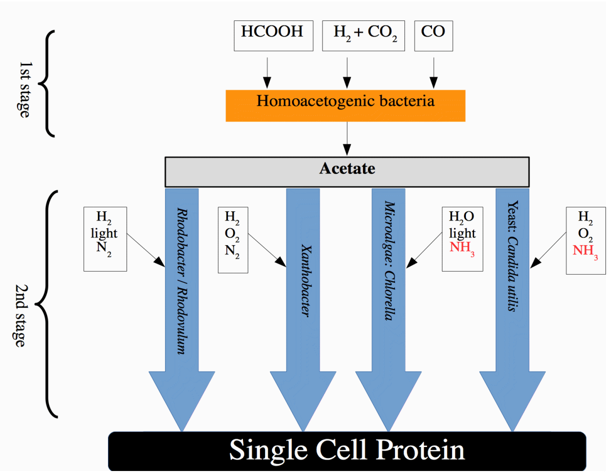

Fig 2: Two-stage bioprocess designs and microbes with cultivation requirements. Red indicates discrepancy from aims.

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 (Terauchi et al., 2010). However, *in-vitro* activity 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 (Johnson and Alric, 2013), as well as high PS-II activity (Terauchi et al., 2010). Strain selection maybe used to select suitable strains with high PS-II activity and high growth rates. Pyruvate dehydrogenase (PDH) and isocitrate dehydrogenase (IDH) are two key enzymes responsible for oxidation/decarboxylation reactions. PDH may be downregulated because acetate is supplied in the medium and PS-II can provide for reduced ferredoxin. IDH may only be downregulated for production of biomass rich in lipids or starch, since alpha-ketoglutarate, an important TCA-intermediate for protein synthesis, which may not be replenished by glyoxylate cycle. Once a suitable strain has been found, the concomitant O_2-evolution may disturb the O_2-sensitive HAB of the first-stage. This may be addressed in the following ways: 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 low growth rate and also partial utilization of organic substrate as electron donor. Alternatively, the 2nd stage maybe comprised of mixtures of microalgae with *Xanthobacter*, which consumes O_2 by respiration of H_2 instead of organic substrates. Recently, several HAB were found to be moderately O_2-tolerant such as *Sporomusa aerivorans (Boga et al., 2003)* or *Clostridium magnum (Karnholz et al., 2002)*, which can quickly reduce O2-partial pressure and continue acetate secretion in the presence of low levels of O2. However, it is unclear whether the largely unknown H_2-dependent mechanism of O2 consumption is energy-conserving. Yet another option would be a gas-tight separation of 1st from 2nd stage, which however, likely involves a more complicated and expensive setup.

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

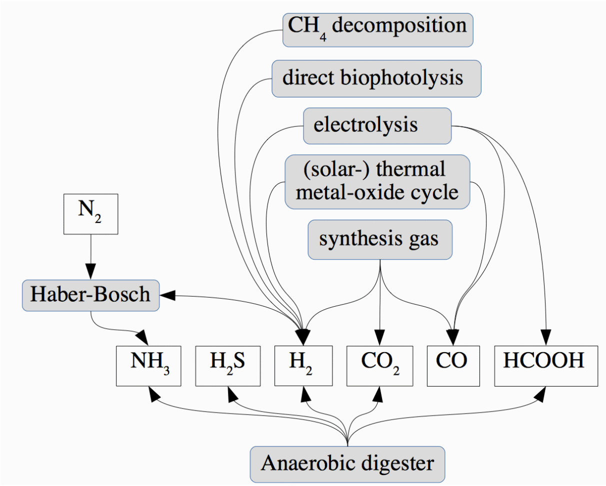

Fig 3: Sustainable sources of the gaseous substrates H_2, CO_2, CO, formic acid, as well as NH_3 and H_2S.

provided as H_2, carbon monoxide (CO), formic acid (HCOOH) and mixtures thereof, all of which can be made via electrolysis (Alvin Chan et al.; Jhong et al., 2013; Kumar et al., 2013; Wu et al., 2013). Mixtures of CO and HCOOH might accelerate acetate formation by HAB (Kerby and Zeikus, 1987), since CO and HCOOH are the two first CF-products of the WL pathway which are likely to be rate-limiting. A promising technology that attracted considerable interest is the physical-chemical gasification of intractable organic wastes such as lignin/lignocellulose to synthesis gas, consists of variable mixtures of CO, H_2 and CO_2. CO can be transformed to H_2 and CO_2 in a chemical water-gas-shift reaction or be fed directly to HAB and CO-tolerant 2nd-stage microbes. Compared to gasification, hydrothermal liquefaction accepts wet organic wastes (López Barreiro et al., 2013) and yields a mixture of fuels and gases. A counterintuitive option would be the use of fossil fuel CH_4 as source of H_2: “Methane decomposition” or the “thermal black” process decomposes methane into H_2 and carbon black (Abanades et al., 2014; Abbas and Wan Daud, 2010; Choudhary and Goodman, 2006; Halmann and Steinberg, 1998). Carbon black is chemically inert and may be used as soil additive or marketed in various products such as inks. Notably, CH_4 decomposition allows the use of most of the energy contained in CH_4 as H_2 and durably preventing the carbon to be released as greenhouse gas. Existing methane-based SCP bioprocesses also deserve reconsideration since they conveniently solve the supply problem of both H_2 and CO_2, and may also benefit from the bioreactors presented below. However, this would add the C to the global carbon cycle, whereas driving autotrophic SCP with H_2 from methane decomposition would provide the opportunity to be “double carbon-negative”: for each CH_4 two H_2 are formed with which HAB can assimilate one external CO_2, whereas the methane-C is prevented from being released. H_2 may also be produced from sun without photovoltaic and subsequent water electrolysis: the solar-thermal metal-oxide cycle (Agrafiotis et al., 2015; Muhich et al., 2013; Perkins and Weimer, 2009) may reach up to 20% efficiency for water splitting and may also be combined with electrolysis (Li et al., 2015; Licht et al., 2010). Direct biophotolysis (Benemann, 2000; Dasgupta et al., 2010; Hemschemeier et al., 2008; Rühle et al., 2008) is attractive since it allows the releases of H_2 from o-PS mediated water splitting, induced for example by sulphur starvation. 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.

Supply of Substrates and Nutrients

The availability of substrates and nutrients is likely critical for SCP production at large scales and under economical constraints, or simply in distant regions with a need for autonomy. Surprisingly, one of the most urgent challenges lies in the provision of CO_2, in particular anoxic CO_2 (Chisti, 2013). 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 current physico-chemical methods (Chisti, 2013). A commonly cited option is the usage of CO_2 from coal power plants but this would indirectly support this obsolete 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 insufficient amounts for possible large-scale implementations (Chisti, 2013). Plants, microalgae and cyanobacteria have the capacity to extract CO_2 from the air, but, as described above, their photo*auto*trophic cultivation exhibits several challenges for economical large-scale implementation and cyanobacteria are inherently unsafe for food purposes. However, unused biomass can be found anywhere. An overlooked spot are in public waters such as rivers, lakes and the open sea, where algae grow on huge surface areas and volume. Harvesting this biomass may not be suitable for direct use as food due to the undefined microbial composition and cyanotoxins, but has several advantages: It's not only a source of carbon,

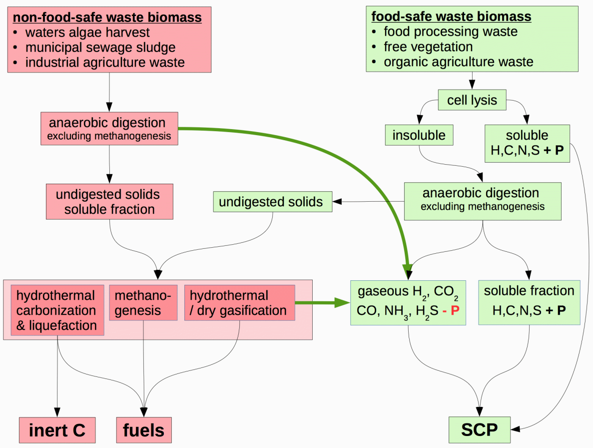

Fig 4: Substrate and nutrient provision through upgrading of food-grade and non-food-grade waste biomass, respectively. Opportunities of using nutrients from non-food-grade sources for food are highlighted as bold green arrows. (Non-) Recuperation of precious soluble nutrients such as phosphorous are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

but of all nutrients including N, S, K and phosphor in the useful proportions. It would also 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 ecological imbalance and biodiversity loss. Different scenarios for upgrading of food-grade and non-food-grade biomass to food are depicted in Fig. 4. Non-food-grade biomass has the advantage that much larger quantities are available. It is hypothesized here that nutrients can be extracted safely from non-food-grade biomass for food purposes via gases evolved during its degradation in anaerobic digestion (excluding methanogenesis), hydrothermal or dry gasification. Its seems unlikely that pathogens, virulence factors or toxic organic or inorganic substances pass through the gas phase. Note that CO, H_2 S and NH3 are relevant examples of gases that are toxic to humans, but in a certain concentration range can be metabolized safely by SCP-microbe (s) to completely harmless SCP. However, these extraction paths exclude those nutrients that remain dissolved and do not occur in the evolved gases, such as phosphor, vitamins and trace elements. Suppression of methanogenesis may be achieved through regulation of the pH below 6, uncontrolled removal of nutrients, injection of inhibitory levels of H_2, or methanogens phages. The pH approach, and likely all approaches, allow only incomplete inhibition (Taconi et al., 2008), but being nontoxic, residual CH_4 should not disturb SCP production. However, the release of exhaust containing CH_4 to the atmosphere should be prevented, considering its potent greenhouse effect.

Food-grade 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 and nucleases degrade proteins, RNA /DNA and carbohydrates to their respective monomers. This would preserve a lower entropy compared to complete mineralization and subsequent autotrophic growth on H_2+CO_2+NH3, allowing faster growth, higher growth yield and overall energy conservation. The insoluble fraction of lysed cells may be fed to 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 energy, and at the same time allow benefitting of nutrients at lower entropy. It would be desirable to cocultivate 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 SCP-producing microbes may flow through the AD-reactor in tubes made of poly (vinyl alcohol) which block particles and microbes but allow diffusion of metabolites from hydrolysis into the tube (Peppas and Wright, 1996). Another advantage would be that the major nutrient phosphorous, vital electrolytes such as potassium, as well as trace metals can be readily absorbed from the soluble fractions of lysed cells, of the effluent of AD-M, and possibly of hydrothermal treatment of undigested solids (the latter is not shown in Fig. 4).

Since organic waste biomass may not be available in sufficient quantities, locally or globally, 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 usually form at the cathode and degrade performance by increasing the overpotential. These could be used to extract CO_2 from the air by dissolution or 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, utilization of Ca- and Mg-based electrolytes yields chalk (CaCO3) and MgCO3, 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.

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 limited light penetration into the liquid media and mutual shadowing by the cells, namely light inhibition and light limitation. 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.

Another concern is the expensive extraction of biomass from the liquid media which is a significant factor that renders photobioreactors uncompetitive (Chen et al., 2011; Uduman et al., 2010). Clearly, cultivation in liquid media in 3D-vessels is troublesome for both gas-dependent and light-dependent cultivations. With respect to these challenges, two kinds of biofilm-gas fermenting bioreactors ("contactors") are presented, which function by exposing the biofilm directly to the gas atmosphere:

1) Rotating biological contactors

Rotating biological contactor (RBC) are established bioreactors that are half immersed in liquid media and half exposed to the substrate gases (Cortez et al., 2008; Patwardhan, 2003). 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. In light-dependent single-step as well as all second-stage bioprocesses, microbes grow as biofilm attached on cords or ribbons that are wound around the cylinder (Christenson, 2011). For harvest, the cord or ribbon 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, greatly simplifying the harvest process (Christenson, 2011). The light-independent first-stage (HAB), which merely act 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.

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 (Gosse et al., 2010, 2012). They are in a sealed gas-tight container with controlled atmosphere consisting of substrate gases such as H$_2$ + CO$_2$ + NH3 or N$_2$, and a saturated water gas phase. Besides water vapor, hydration of the cells may 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. However, cell harvest is not established for coated paper but may be useful when acetate production by HAB shall be uncoupled from the 2nd stage.

Biomass yields compared to wheat

Wheat is among the most important cereals cultivated in Europe. Yields of wheat in a moderate climate such as Germany are about 7 tons/hectar*year (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/m2*hour). Solar panels allow a sensible comparison with the local solar irradiance as basis of comparison. Considering the known power

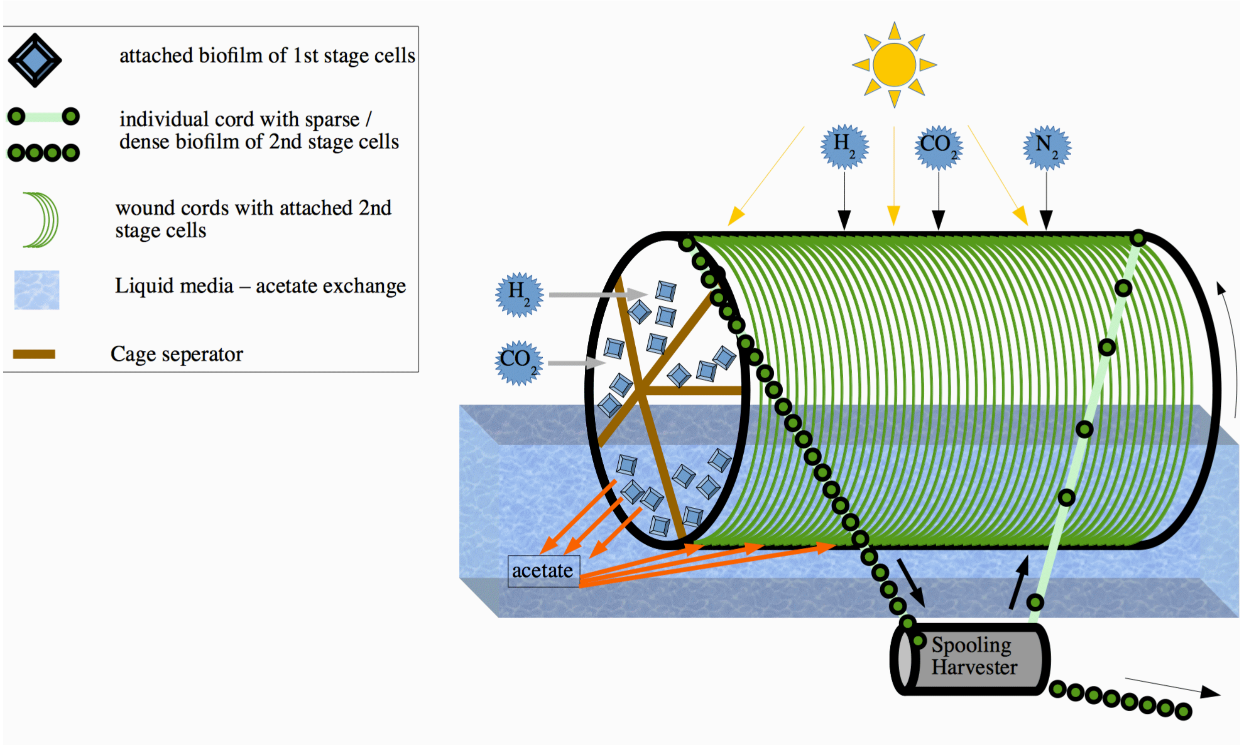

Fig 5: Proposed adaptation of two rotating biological contactor for 2nd -stage designs, harboring first-stage inside and second-stage outside of a single shared cylinder.

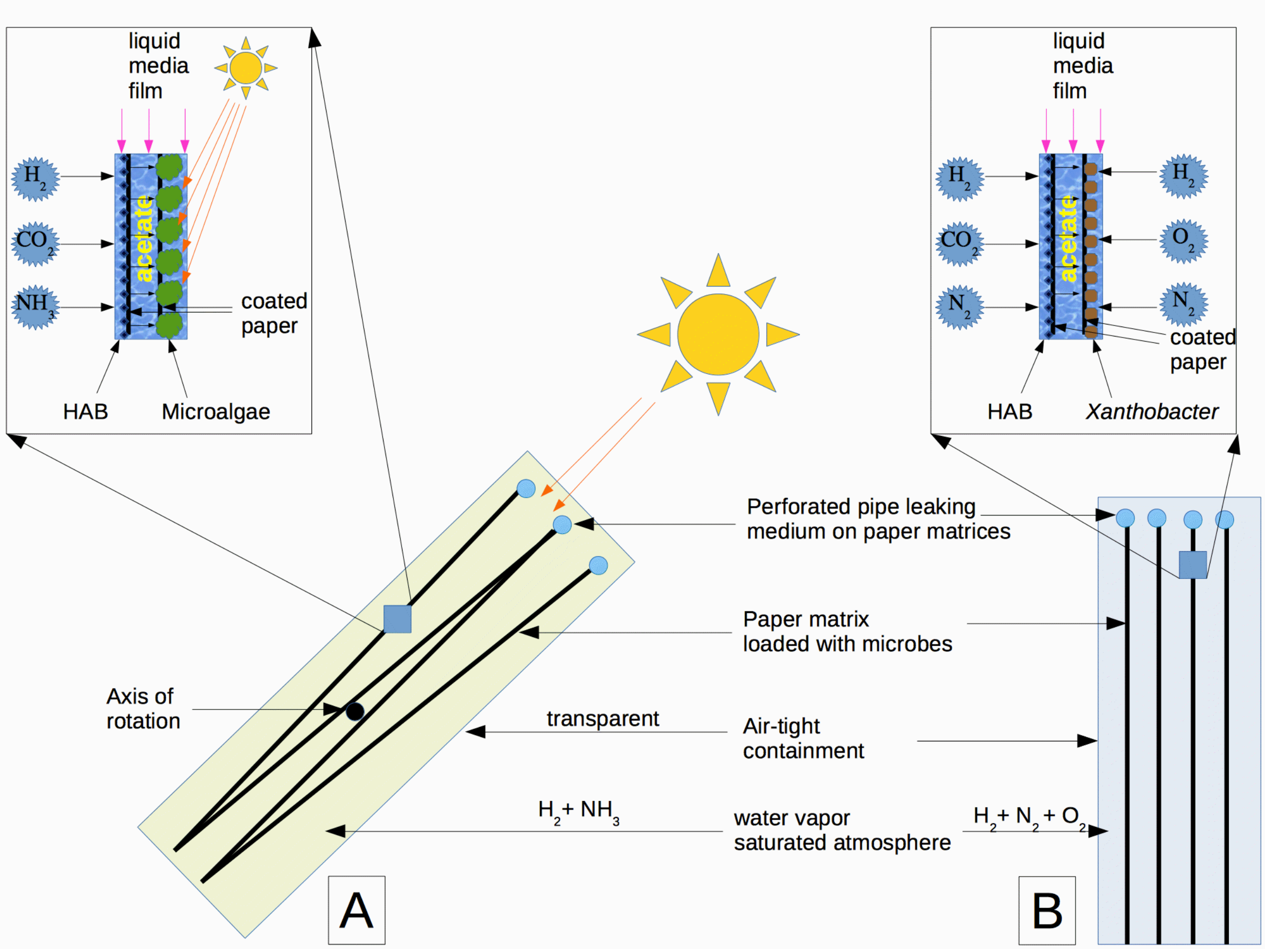

Fig 6: Coated Paper Array Contactor for light-dependent (A) and light-independent (B) 2-stage designs. Inlets show details about hypothesized sandwiched coated papers with HAB on the left and 2nd stage on the right side respectively.

consumption of water desalination units (Elimelech and Phillip, 2011) and water electrolyzers (Alvin Chan et al.), it is possible to calculate an amount of H2 gas which can power the formation of acetate from H2 and CO2 by HAB (Fast and Papoutsakis, 2012) and the subsequent biomass formation from acetate by *Chlamydomonas reinhardtii* (Boyle and Morgan, 2011; Terauchi et al., 2010) (see Table 1 for overview, Table S1 for details). This microalgae as 2nd 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 (Christenson, 2011). 3) Unlike anoxygenic Rhodobacter, o-PS has several advantages, in particular in photoheterotrophic growth on acetate (described above). Furthermore, the photosynthesis apparatus generates and tolerates O2. This allows to switch seamlessly between anaerobic/microaerobic photoheterotrophic (daylight) and aerobic heterotrophic (night) growth conditions without lengthy adaptations lags. 4) Chlamydomonas has good growth yields for both growth modes and has high specific growth rates (Boyle and Morgan, 2011; Terauchi et al., 2010). 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 electrons and reductant. Since very large amounts of energy are consumed for water electrolysis, 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 have enough time or light for photoheterotrophic assimilate, can be used for 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. High variations were observed depending on region and technology used. The yield increase of solar-powered SCP compared to wheat varied by an order of magnitude between two case studies: 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. Relative influence of regional and technological factors on the overall result were 8 and 1,5 respectively. Calculations assume that a salty water source (such as the open sea), solar panels, water desalination unit and water electrolyzers and bioreactors are all on the same site. It can also be calculated that the energy required for the production of 1 kg SCP is about 10 kWh. In the EU28, this would cost about 0,90€/kg or 1,80€/kg for industry prices without tax or consumers prices with tax respectively.

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 details see text and Table S1.

Case: Country+Technology **Country**	**1** **Iran**	**2** **Germany**	
wheat average yield (dry)	0,020	0,081	g/m2/h
wheat regional ratio	4,03	1,0	
global radiation	2000	1000,00	kWh/m²/yr
global radiation regional ratio	2	1,0	
overall regional difference	8,06	1,0	
solar electricity per m² per year	430,00	170,00	kWh/m²/yr
H_2 from sea water (solar)	4847,58	1621,63	mol H_2/m²/yr
technology factor	1,49	1,0	
acetate produced	68,70	22,98	kg acetate/m²/yr
SCP produced	**4,41**	**1,48**	**g/m²/h**
increase compared to wheat	**219,88**	**18,26**	**fold change**

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 this legal status. 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 (Vrati, 1984; Vrati and Verma, 1983), 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 (Dahiya et al., 2012). Considerable legal work and likely 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. This causes that some carbon is lost as CO_2 and that reductant is extracted from organic substrate. 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 makes sense for the cell to 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 a comparatively simple selective adaptation might select for suitable strains.

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, or as an ingredient for processed food. 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 productivities 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 due to health damage, 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% (Van Grinsven et al., 2013). These big amounts can be explained by the numerous effects of the degradation products of nitrogen fertilizer such as NO_x, NH3, N2O and nitrate. They cause a significant increase of asthma, respiratory disorders, inflammation of airways, reduced lung functions, bronchitis, cancers (e.g. colon, neural tube), 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 of 5 trillion dollars per year worldwide (Coady et al., 2015). 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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Appendix

Table S1. Preliminary calculation of yields of a two-stage design compared to wheat.

Constants			
molar mass H2		2,016	g/mol
molar mass H2O		18	g/mol
molar mass acetate		60,05	g/mol
molar mass ethanol		46,07	g/mol
Case study	**1**	**2**	
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
	0,0200621822	0,0808009243	g/m2/h
Technology	**Best technology**	**Common commercial technology**	
Renewable Electricity			
Efficiency of solarpanel	0,22	0,17	
global radiation sahara / southern germany	2044	1100,00	kWh/m^2/yr
solar electricity per m^2 per year	439,46	187,00	kWh/m^2/yr
H2 generation			
Energy requirement of water electrolysis	44,00	52,00	kWh/kg H_2
	0,0887040	0,1048320	kWh/mol H_2
Energy requirement of water desalination	2,00	10,00	kWh/m^3 H_2O
	0,0000001111	0,0000005556	kWh/mol H2O
Energy requirements of water desalination + water electrolysis	0,088705	0,104833	kWh/mol H2
H_2 from sea water (solar)	4954,20	1783,80	mol H_2/m^2/yr
1st stage: *homoacetogenic bacteria*			
HAB: yield acetate per H2		0,236	mol acetate/ mol H2
acetate as mol (solar)	1169,19	420,98	mol acetate/m^2/yr
acetate in kilograms (solar)	70,21	25,28	kg acetate/m^2/yr
kWh/mol acetate	0,376	0,444	kWh/mol acetate
kWh/g acetate	0,00626	0,00740	kWh/g acetate
2nd stage: *Chlamydomonas reinhardtii*			
Biomass yield for photomixotrophic growth on acetate (at ± 1,5 μE/m2/s)	0,75		g cell dry mass / g acetate
Biomass yield for O2-heterotrophic growth on acetate	0,52		g cdw / g acetate
Average yield, assuming daylight hours / dark hours = 1	0,635		g cdw / g acetate
surface/light used for solar electricity/acetate (1- phototrophic growth)	0,75		
yield on solar H2 from desalinated sea water	39,493076695	14,2197832019	Kg /m2/yr
as g cell / m^2 / hour	4,51	1,62	g/m2/h
increase compared to wheat	224,72	20,09	fold change
kWh/kg cdw	9,857	11,649	
kg cdw/kWh	0,10	0,09	

Table S2: Seleected microbes and their functions in the proposed bioprocess designs.

Organism	**advantages**	**disadvantages**	**Function**
Chlorobium	- efficient CO_2 as well as fast N_2 fixation - fast growth - potential health benefits - H_2S utilization (SQR)	- strict light-dependence - O_2-intolerance - edibility uncertain - only partial H_2S oxidation, deposits S^0	*single step:* H_2 + light (strict) → biomass
Aquificales	- aerotolerant rTCA & Knallgas - NAD+ reducing hydrogenase - H_2S utilization (SQR), partial oxidation to S^0	- no active BNF - thermophile - edibility uncertain - only partial H_2S oxidation, deposits S^0	*single step:* $H_2 + O2 + CO_2 + NH_3 \rightarrow$ biomass
Rhodobacter	- Knallgas & moderately O_2-tolerant BNF - high yield with photoheterotrophic growth on acetate - edible with health benefits (pigments, vit B12 [31]) - H_2S utilization (SQR), partial oxidation to S^0	- only moderate microaerobic dark growth - only partial H_2S oxidation, deposits S^0	*2nd stage:* Acetate+H_2+N_2+Light/$O_2 \rightarrow$biomass
Rhodovulum	- tolerance to salt water - produces precious $\omega-3$ fatty acids (DHA & EPA) - autoflocculation - complete oxidation of H_2S to sulfate	- O_2-tolerance of hydrogenase unknown - O_2-tolerance of nitrogenase unknown	*2nd stage:* Acetate+H_2+N_2+Light/$O_2 \rightarrow$biomass
Xanthobacter	- Knallgas & electron donor improve acetate yield - BNF with fairly O_2-tolerant & good H_2-usage - does not need growth factors[32]	- edibility uncertain	*2nd stage:* Acetate +$H_2 + N_2 + O_2 \rightarrow$ biomass
Nostoc	- light-driven H^+/e^- extraction from water (o-PS) - o-PS at day, Knallgas outside daylight hours - good H_2-usage by nitrogenase;	- inefficient CF - bad acetate assimilation - uncertainty about non-toxic strains	*single-step* H_2+N_2+ CO_2 +light/ $O_2 \rightarrow$ biomass
Chlorella *&* *Chlamydomonas*	- light-driven H^+/e^- extraction from water (o-PS) - efficient acetate assimilation via glyoxylate cycle - established SCP, health benefits (pigments), starch / lipids - direct biophotolysis (limited H_2 generation via o-PS)	- inefficient CF, no BNF and no Knallgas - wildtype: cell wall indigestible - low growth rate compared to PNSB	*single-step* $H_2 + CO_2$ +light/ $O_2 \rightarrow$ biomass *2nd stage* Acetate + Light / $O_2 \rightarrow$ biomass
Candida utilis	- established SCP - fairly good yields despite light-independence	- no BNF, no Knallgas, no light utilization - wildtype: indigestible fraction of biomass	*2nd stage* acetate+O_2+$NH_3 \rightarrow$ biomass + CO_2