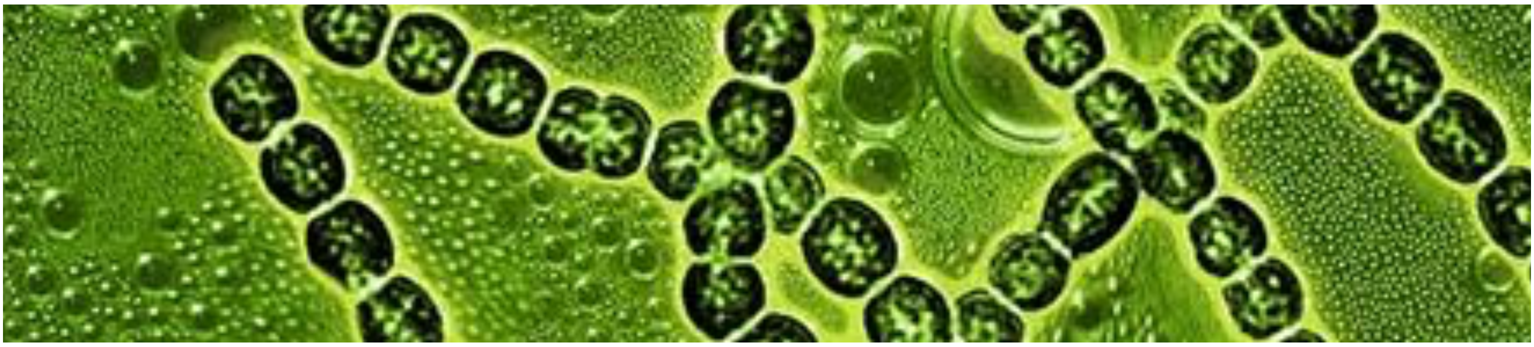
THE FUTURE OF THE CLIMATE:

HOW CAN WE TACKLE CLIMATE CHANGE?

“Harness the Sun to Fuel the World”

Biofuel Production in Cyanobacteria



Ursula Schoellkopf (801181)
Biotechnology Bachelor
Life Sciences and Technology

Beuth Hochschule für Technik Berlin, Sprachenpreis 2015

ABSTRACT

Climate change is caused by increasing greenhouse gas emissions and has several effects on our earth. Global warming leads to retreating glaciers, a rising sea level or other climate catastrophes like floods and droughts. Several attempts have been made in order to reduce greenhouse gas emissions, amongst them the production of biofuels. Limited fossil fuels have to be replaced due to their contribution to atmospheric carbon dioxide levels. Conventionally produced biofuel, however, is somehow controversial since it depends on food-based feedstock and the availability of arable land as well as freshwater. The use of Cyanobacteria is a novel alternative of biofuel production and meets the demands on economic sustainability. Genetically engineered cells are able to directly convert carbon dioxide into ethanol by oxygenic photosynthesis. This innovation combines several advantages over traditional biofuel production and has been put on the market by a company called Algenol which opened its first commercial facility in 2015.

Contents

1 Introduction **1**

2 Biofuel Production **4**

2.1 Traditional Biofuel Production 4

2.2 Biofuel Synthesis in Cyanobacteria 5

3 Outlook **10**

4 Literature **11**

1 Introduction

„The shift to a cleaner energy economy won't happen overnight, and it will require tough choices along the way. But the debate is settled. Climate change is a fact." [Barack Obama][1]

This quote of the U.S. president Barack Obama points out that climate change is an omnipresent issue affecting every single person on this planet. But what is "climate"? And more importantly, why is it changing?

In order to understand climate change it is important to explain the term *climate* first. As opposed to the term *weather*, *climate* describes parameters like temperature, precipitation or humidity over a longer period of time. Along with average temperatures, also extremes like the highest or lowest annual temperatures are a crucial factor when determining the climate for a specific area. There are various climate zones in the world defined by different factors like atmospheric pressure, the biosphere, sun radiation or even volcano eruption that are all combined under the term *climate system*[2].

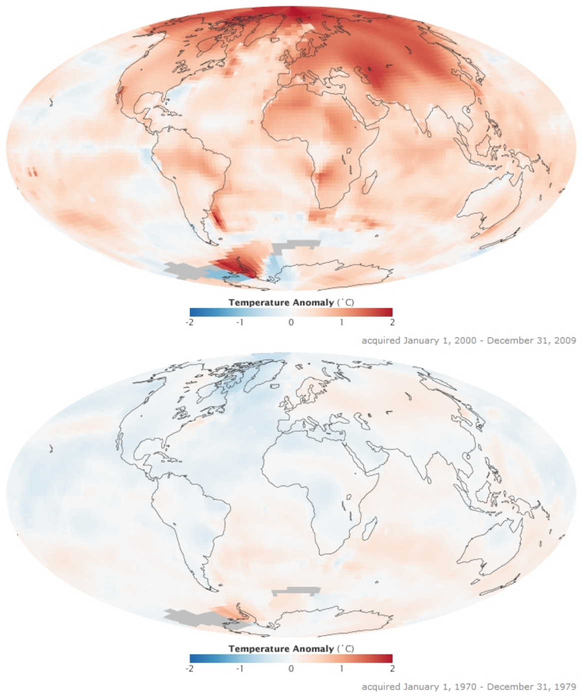


Figure 1 Temperatures across the world between 2000-2009 (top) and 1970-1979 (bottom), as compared to average temperatures from 1951 to 1980 [NASA]

Around 1988 people started acknowledging that the climate is changing and researchers tried to find explanations[3]. Since the beginnings of industrialization in the 19th century the average world temperature has increased by approximately 0.8 K (Fig. 1). Although that might seem like a relatively low amount, the consequences of the rising temperatures speak for themselves.

Higher temperatures cause retreating glaciers and the decrease of the area covered by sea ice in the arctic. Between 2002 and 2009 over 1,600 billion tons of ice retreated in the area of Greenland and Antarctica. In addition, ocean temperatures are rising which causes the water to ex-

[1] whitehouse.gov (2014) State of the Union Address

[2] Storch et al. (1999). Das Klimasystem und seine Modellierung: Eine Einführung

[3] Dessler (2012). Introduction to Modern Climate Change

pand. Both factors contribute to the rising sea level affecting mostly coastal regions. During the last 40 years the sea level has increased by about 1.8 mm per year[1].

Thus, climate is heading towards a more threatening level. As a result floods or droughts occur more frequently. Heat waves present a serious health risk, especially for kids and the elderly, whereas intensive rainfalls have a negative impact on water supply and water quality. Moreover, the ecosystem is affected and on top of that the lifecycle of plants and animals in terms of their reproduction and migration patterns[2].

The cause of climate change has been narrowed down to greenhouse gas emission. Greenhouse gases, amongst them carbon dioxide and methane, are gases that trap heat in the atmosphere. A result is the so-called greenhouse effect causing the temperatures to rise. Carbon dioxide emission has increased because of fossil fuels that are burnt for heat and energy, clearing forests and industrial production[3] (Fig. 2). Studies show that elevated carbon dioxide levels can lead to down-regulation of photosynthesis in plants[4].

The United Nations Climate Change Conference in 2010 stated the 2 K-target, which implies that the temperature should not rise more than 2 K against pre-industrial temperature levels[5]. This aim can only be achieved by reducing greenhouse gas pollution. Fig. 3 shows that a vast percentage of greenhouse gas emission is caused by industry and transportation.

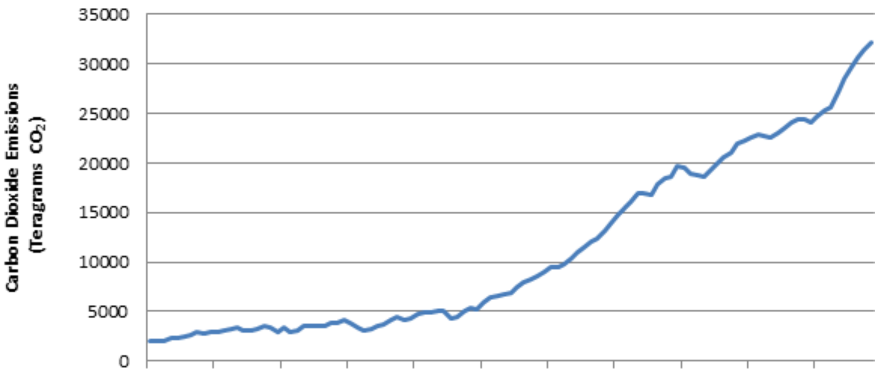


Figure 2 Global carbon dioxide emissions from fossil fuels 1900-2008 [epa]

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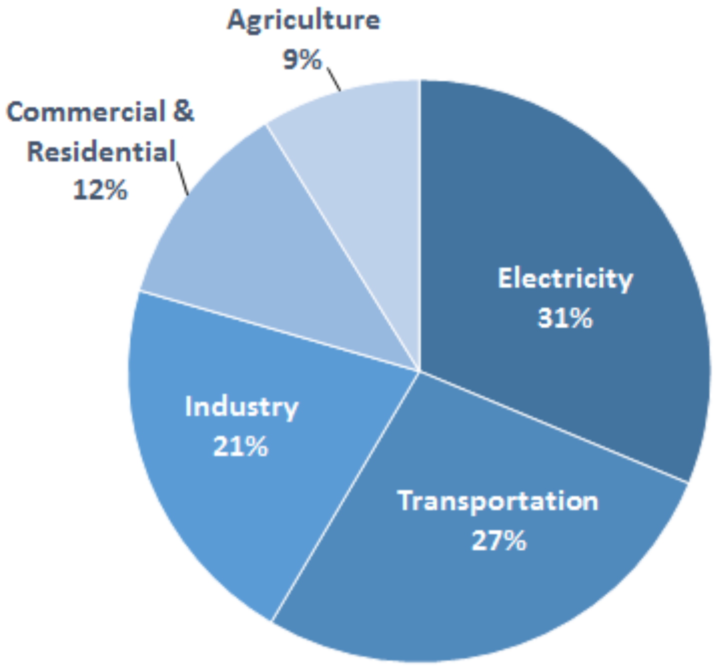
[1] Dessler (2012). Introduction to Modern Climate Change

[2] U.S. Environmental Protection Agency (2015). Future Climate Change

[3] U.S. Environmental Protection Agency (2015). Climate Change Science Overview

[4] Newman et al. (2011). Climate Change Biology

[5] Geden SWP-Studie (2012). Die Modifikation des 2-Grad-Ziels



In order to address this problem an alternative form of fuel is needed so that unsustainable fossil fuels can be replaced. Several attempts to produce biofuel have been made in the interest of “*the shift to a cleaner energy economy”*, as the quote above implies.

Figure 3 total U.S. greenhouse gas emissions by economic sector in 2013 [epa]

2 Biofuel Production

2.1 Traditional Biofuel Production

In order to reduce air pollution, the U.S. government designed the so-called "Clean Air Act" in 1970. One purpose of this comprehensive federal law was to increase the use of biofuels and was followed by an expansion in research to optimize biofuel production concerning greenhouse gas emission and environmental impact[1]. Ethanol represents the most commonly used biofuel and can be found in 10 %-blends with gasoline (E10). The traditional production of bioethanol involves the usage of corn, sugar cane, sugar beets, cereal crops etc. as feedstocks, which are converted into ethanol through fermentation, distillation or enzymatic processes[2]. The basic idea here is to replace fossil fuels with renewable biological materials. However, there are many doubts as to whether traditional biofuel production is really helping to stop climate change. Various studies discuss the lifecycle energy balance, which basically compares the needed amount of energy for the production of ethanol with the amount of energy the fuel actually provides[3]. In order to achieve a positive energy balance, the quantity of fuel needed for transportation or production processes of bioethanol has to be decreased. If there is a need for initial input of fossil energy, the energy balance is negative; the biofuel production is thus unsustainable.

Another controversial matter is the vast amount of land needed for the growth of the feedstock. Suitable land for farming is limited hence biofuel production is in constant competition with food production[3]. This may lead to an increase of food prices affecting mostly poor countries where shortages of food are already an issue[4].

Furthermore, growing solely one type of corn in monocultures optimized for biofuel production can have a negative impact on biodiversity. More pesticides have to be used in order to fight off pests, which can develop multi resistance against the crop protection product[5].

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[1] U.S. Environmental Protection Agency (2015). Summary of the Clean Air Act

[2] IEA Energy Technology Essentials (2007) Biofuel Production

[3] Wang et al. (2007). Life-cycle energy and greenhouse gas emission impacts of different corn ethanol plant types

[4] Donner (2011) TECHNOLOGY REVIEW. Biosprit aus Kohlendioxid

[5] biofuel.org.uk

Thus, when thinking about new ways of biofuel production, not only lifecycle balances have to be considered but also the rising amount of land, water and chemicals used during these processes, which might be in conflict with sustainability.

2.2 Biofuel Synthesis in Cyanobacteria

Although the basic idea of traditional biofuel production to replace fossil fuels with organic renewable material sounds promising, there are various problems that need coping in order to minimize greenhouse gas emission through biofuel usage. Traditional biofuels like corn ethanol have helped big countries, among them the U.S., to become somewhat independent from fossil fuels. However, corn ethanol production takes up more than a third of the crop which adds up to about 30 million acres of land in the U.S. used for corn ethanol production[1]. Thus, the challenge is not only to find the best kind of biomass, which can be converted into biofuel but also to make the process as economically sustainable as possible. Biotechnology introduces a new, more efficient procedure to produce biofuel that combines several advantages over traditional biofuel production. In this process Cyanobacteria are used to efficiently produce ethanol in an environment-friendly way.

Cyanobacteria, also referred to as blue-algae, are autothrophic, nonpathogenic prokaryotes that are able to perform photosynthesis similar to eukaryotes like higher plants. In fact, the theory is that the photosynthetic organelles in eukaryotes have developed from cyanobacteria, which have been absorbed by a phagotrophic host through endosymbiosis during evolution[2]. By oxygenic photosynthesis Cyanobacteria convert carbon dioxide and solar energy into high-value organic products. The most significant advantage is that Cyanobacteria have very simple growth requirements and can be cultivated in various habitats like marine, freshwater or terrestrial environments[3]. Therefore, a commercial use of those bacteria for biofuel production is possible and can become profitable as well. Since Cyanobacteria need carbon dioxide in order to produce glycogen as the main form of stored carbon, the motivation is to make use of the harmful carbon dioxide produced by power plants or through exhaust emissions and turn it into biofuel. This would somehow close the circle of fuel usage and make limited fossil fuels obsolete.

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[1] algenol.com: Myths what is true and what is not

[2] Löffelhardt et al. (2004). Molecular Biology of Cyanelles

[3] Lau et al. (2015). Cyanobacteria: Photoautotrophic Factories for the Sustainable Synthesis of Industrial Products

The biggest challenge was to genetically modify Cyanobacteria because they cannot produce ethanol naturally at all - or not as much as necessary. In 1999 Deng et al. published their work about genetic engineering in Cyanobacteria in which they introduce a way to transform *Synechococcus* with foreign DNA in order to create a new pathway for ethanol production. This strain of Cyanobacteria is genetically well characterized and can be transformed by using shuttle vectors. The two key enzymes for ethanol synthesis are pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). Through nonoxidative decarboxylation PDC converts pyruvate into acetaldehyde, which is then turned into ethanol catalyzed by ADH. There are only a few strains of bacteria where the expression rate of those two enzymes is relatively high. One example is *Zymomonas mobilis* that is known as the bacteria for tequila synthesis out of agave sap[1]. Deng et al. were able to transform the genes for ADH and PDC into the chromosome of *Synechococcus* sp. PCC 7942 and thereby achieved accumulation of ethanol in the culture medium of the transformed cells. They successfully transferred a new pathway for ethanol synthesis into the metabolism of *Synechococcus* (Fig. 4). Ethanol accumulation increased along with the growth of the cells as shown in Fig. 5. Their ethanol yields showed a concentration of approximately 5 mM after four weeks of growth, which is very low in compar-

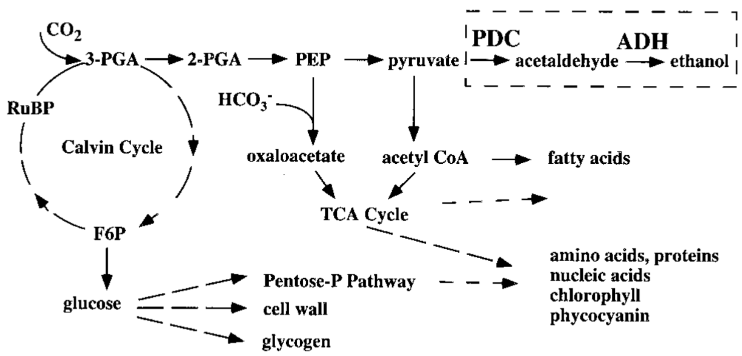

Figure 4 Photosynthesis and photoassimilate metabolism in cyanobacteria. The pathway at the upper right is the added pathway for ethanol synthesis

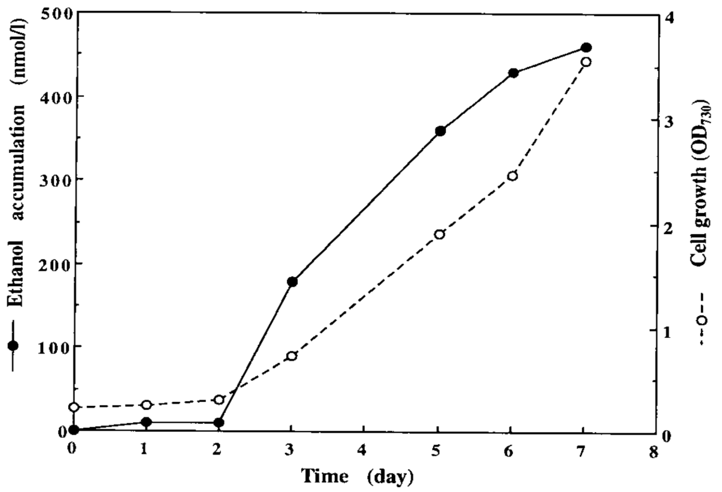

Figure 5 Cell growth and ethanol synthesis in transformed *Synechococcus*

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[1] Sprenger (1996). Carbohydrate metabolism in *Zymomonas mobilis*

ison to ethanol yields of about 1 M achieved through microbial fermentation processes[1]. However, this was the first study where Cyanobacteria could be transformed into a photoautotrophic organism that is able to produce a detectable amount of ethanol through oxygentic photosynthesis. Following studies by different research groups had the aim to improve ethanol production in Cyanobacteria by changing various parameters.

In 2009 Dexter et al. experimented with a different strain of Cyanobacteria called *Synechocystis* sp. PCC 6803. This was the first completely sequenced photoautotrophic prokaryote[2]. In addition, the use of antibiotics during the selection process of the transformed cells was avoided. Instead, Dexter et al. used PCR based essays and ethanol essays in order to select transformants. The advantage here is that there is no need for any antibiotics in the culture media for maintaining the cells. The ethanol yield in this study peaked at about 12 mM, which is more than twice as much as Deng et al. achieved. Nonetheless, ethanol production still has to be increased in the interest of making it profitable. Assumptions are that another pathway in the metabolism of Cyanobacteria is competitive to the pathway of ethanol synthesis via ADH and PDC and that this could be one reason why ethanol yields are not as successful as expected.

Oxygenic photosynthesis basically involves two processes to convert carbon dioxide into other metabolites; one is light dependent, the other one is light independent. During the light dependent reactions the energy of light is used to produce high-energy molecules like ATP and NADH. This form of stored energy is used in the second process during which carbon fixation reactions occur in order to convert carbon dioxide into carbohydrate and other molecules[3]. As shown in Fig. 4 pyruvate is not only used to produce ethanol in the genetically modified cells, but also serves as a substrate for acetyl-CoA. Furthermore, acetaldehyde can be rapidly metabolized into acetate. All those reactions are in competition with the novel inserted pathway for ethanol hence more research is needed concerning the regulation of those pathways.

The work of Deng et al. and Dexter et al. show that using Cyanobacteria for biofuel production is in no way a new idea. In fact, researches allegedly have tried to use algae to produce energy during World War II[4]. But all those attempts failed due to the lack of efficiency until today. Algenol is the first industrial biotechnology company to start a commercial facility in 2015 for

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[1] Deng et al. (1999). Ethanol Synthesis by Genetic Engineering in Cyanobacteria

[2] Ikeuchi M. (1996). Complete genome sequence of a cyanobacterium *Synechocystis* sp. PCC 6803

[3] Dexter et al. (2009). Metabolic engineering of cyanobacteria for ethanol production

[4] Donner (2011) TECHNOLOGY REVIEW. Biosprit aus Kohlendioxid

the production of ethanol and other biofuels by making use of Cyanobacteria[1]. The company located in Fort Myers, Florida was founded in 2006 and has spent years on research in the interest of making biofuel production in Cyanobacteria as efficient as possible. They experimented with different strains of Cyanobacteria and various parameters in order to achieve perfect conditions for cultivation. Algenol has various partners, amongst them a subsidiary research company called Cyano Biofuels at the Humboldt University of Berlin. The location of the headquarter, though, is chosen wisely, because there are approximately 266 days with sun per year in Florida[2]. Since solar energy is necessary for biofuel production here, it is important to use as many sun hours as possible in order to achieve high yields. Algenol claims to be able to produce 9000 gallons ethanol per acre per year. In comparison, traditional biofuel production only reaches about 420 gallons with corn and 800 gallons with sugar cane. In addition to the high yields another advantage is that Algenol uses marginal, non-arable land and is thus not competing with agriculture. Cyanobacteria grow in plastic photobioreactors that are perfected to cultivate the cells with as much sun radiation as possible (Fig. 6).

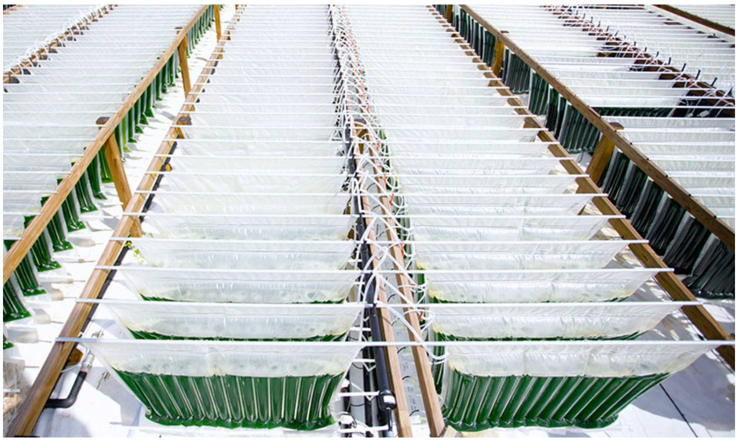

Figure 6 Plastic photoreactors of Algenol in Fort Myers, Florida

A constant carbon dioxide flow into the bioreactors is given so that the cells are able to convert it into ethanol. Ethanol evaporates quickly due to the relatively high temperatures caused by the greenhouse effect. It condenses at the walls of the bioreactor and can then be collected separately (Fig. 7). The bacteria can be cultivated in seawater, hence there is no need

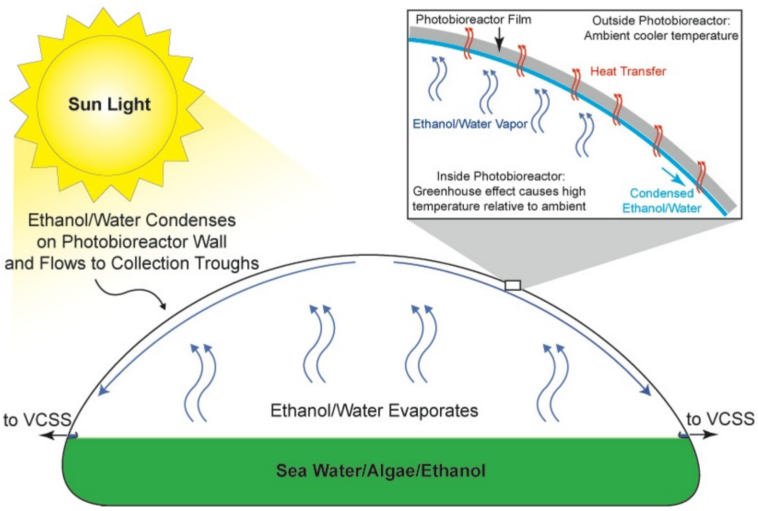

Figure 7 Algenol’s ethanol production circle

[1] algenol.com

[2] currentresults.com

to use freshwater. Freshwater is actually produced during oxygenic photosynthesis as a side product along with oxygen. The biomass waste is converted into other biofuels like biodiesel, gasoline and jet fuel during refinery processes. All this adds up to around 144 gallons of advanced fuels that can be made out of one ton of carbon dioxide. Algenol aims to lower the carbon footprint by 80 % compared to conventional gasoline[1]. This, along with all the other advantages, sounds like Algenol has managed the breakthrough in the efforts of making biofuel production more sustainable. However, there are several open issues that have to be discussed before Algenol will be able to enter the highly competitive fuel industry. For example, there are various regulatory approvals and permissions that have to be obtained before the produced biofuels can be sold. Moreover, customers have to be convinced of this new form of biofuel production. People have uttered concerns about whether their vehicles are compatible with the fuels. Also the term “genetically modified organisms” often has a negative connotation within the population. Algenol is working on tackling those concerns in order to pursue a new way of biofuel production that could help to stop climate change.

[1] Luo et al. (2010). Life Cycle Energy and Greenhouse Gas Emissions for an Ethanol Production Process Based on Blue-Green Algae

3 Outlook

Genetically engineered Cyanobacteria represent a new possibility to produce biofuel more efficiently and sustainable. Chances are that this new procedure will once become a common way to fuel the world. Algenol, as a biotechnological company, has made it practicable to produce ethanol and other biofuels cheaper and without any harm for the environment. The big question is, if the efficiency Algenol promises can be obtained in other countries as well. Germany for example has a lot less sun hours per year compared to Florida. The amount of sun energy is, however, crucial for gaining optimum yields. Furthermore, the rules for genetically modified organisms and their dissemination are stricter in Germany than they are in the U.S.. Thus, it appears that, for the time being, it will be difficult for companies like Algenol to compete with conventional fuel producers.

Above all, oil companies will not be very pleased about new competitors on the fuel market. Thus, monopolistic capitalism stands, as is often the case, in the way of introducing technological advances. It is a fact that the demand for fuel increases along with the growing population and industrialization. Companies like Algenol could therefore help to compensate those needs provided that it will not turn into a monopoly itself. Because when it comes to tackling climate change, the whole world has to work as a team.

People have to become aware of climate change as a serious threat for our future generations. And they have to understand that it is on us to reduce the risk of our climate to get worse. Humans are creatures of habit and that is why it takes time for us to get used to technical innovations. Recent examples are the wide integration of the internet, wind power plants, smart phones, photovoltaic systems or biometric data in passports, all of which are advances that once were criticized or considered unnecessary, whereas today people could not do without them.

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Fig. 2: http://cdiac.ornl.gov/trends/emis/tre_glob.html

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Fig. 4;5: Deng M.-D., Coleman J. R. (1999). Ethanol Synthesis by Genetic Engineering in Cyanobacteria. *Applied and Environmental Microbiology*, Vol. 65, No. 2

Fig. 6: http://www.algenol.com/direct-to-ethanol/direct-to-ethanol

Fig. 7: http://www.buildingindustry.org/algenol

Cover

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