

# **Prize Winner**

# Science Writing Year 11-12

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## THE APPLICATIONS AND LIMITATIONS OF ALGAL BIOREACTORS AND ALGAL BIOFUELS

#### **INTRODUCTION**

Climate change is one of the biggest challenges of our time and resulting heatwaves combined with long periods of drought were linked to Australian bushfires of unprecedented magnitude in the past few years, burning over 12 million acres of land. The UN estimates that burning fossil fuels accounts for 76% of our greenhouse gas emissions (mainly carbon dioxide  $- CO_2$ ), which are the main cause for climate change. Despite the knowledge about the detrimental effects of fossil fuels, it has been predicted by the International Energy Agency (IEA) that they will still account for 77% of all fuels used by 2040. It is therefore critical to develop novel effective ways to reduce greenhouse gas emissions and decrease the atmospheric  $CO_2$  burden.

The application of algae could be the solution against climate change as they absorb large amounts of  $CO_2$  (about 1.8kg of  $CO_2$  per kg of algae biomass gained) and can be used for the production of sustainable ecofriendly fuel.<sup>4</sup> In particular hydrogen produced from algae as a clean source of energy could mitigate the environmental problems caused by the use of fossil fuels. While until recently the growth of algae had its technical limitations, improvements in algae bioreactor technology and culture conditions now allow an upscaling of algae production. The application of algae could therefore have the potential to fundamentally change the way energy is produced, while reducing atmospheric  $CO_2$  concentrations.<sup>5</sup>

#### **BACKGROUND**

Algae are a diverse group of aquatic organisms ranging from large seaweed (macroalgae) to microscopic organisms (microalgae) such as *Spirogyra* which can be seen in figures 1 + 2.

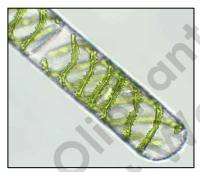


Figure 1. Algae Spirogyra under a light microscope (x40)

(Aytar, E., 2020. Spirogyra Sp. Algae Under Microscopic View x40 - Chlorophyta. [image] Available at: <a href="https://www.dreamstime.com/spirogyra-sp-algae-under-microscopic-view-chlorophyta-image179352431">https://www.dreamstime.com/spirogyra-sp-algae-under-microscopic-view-chlorophyta-image179352431</a> [Accessed 1 July 2021])

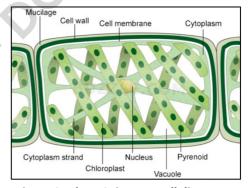


Figure 2. Algae Spirogyra cell diagram (QS study, 2020. Diagram Of Spirogyra. [image] Available at:

<a href="https://www.qsstudy.com/biology/describe-with-labelled-diagram-the-structure-of-spirogyra">https://www.qsstudy.com/biology/describe-with-labelled-diagram-the-structure-of-spirogyra>
[Accessed 1 July 2021])

Most algae are capable of photosynthesis and utilize light energy to convert CO₂ and water into biomass and oxygen.<sup>6</sup> The chemical reaction for photosynthesis can be seen below.

$$6CO_2 + 6H_2O \xrightarrow{Chlorophyll \ and \ light} C_6H_{12}O_6 + 6O_2$$

<sup>&</sup>lt;sup>1</sup> United Nations Sustainable Development. 2020. Climate Change. [online] Available at: <a href="https://www.un.org/sustainabledevelopment/climate-change/">https://www.un.org/sustainabledevelopment/climate-change/</a> [Accessed 28 June 2021].

<sup>&</sup>lt;sup>2</sup> Gobler, C., 2020. Climate Change and Harmful Algal Blooms: Insights and perspective. *Harmful Algae*, 91, p.101731.

<sup>&</sup>lt;sup>3</sup> Steg, L., 2018. Limiting climate change requires research on climate action. *Nature Climate Change*, 8(9), pp.759-761.

<sup>&</sup>lt;sup>4</sup>Budgen, J. and Le-Clech, P., 2020. Assessment of brewery wastewater treatment by an attached growth bioreactor. *H2Open Journal*, 3(1), pp.32-45. <sup>5</sup>Ibid

<sup>&</sup>lt;sup>6</sup> Steg, L., 2018. Limiting climate change requires research on climate action. *Nature Climate Change*, 8(9), pp.759-761.

Algae sequester  $CO_2$  naturally during photosynthesis and approximately half of the algae biomass dry weight consists of carbon which is derived from  $CO_2$  (figure 4).<sup>7,8</sup> Thus, photosynthetic algae have the potential to be used to sequestrate  $CO_2$  out of the atmosphere.<sup>9</sup> Microalgae are of particular interest for researchers as they double their biomass every 24 hours and during their exponential growth phase can even double in mass as quickly as every 3.5 hours.<sup>10</sup>

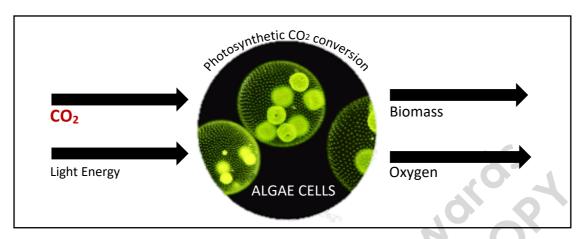


Figure 4. Photosynthetic conversion of CO<sub>2</sub> into microalgae biomass

Adapted from: Kativu, E., 2014. *Carbon Dioxide Absorption Using Fresh Water Algae And Identifying Potential Uses Of Algal Biomass.* [online] Core.ac.uk. Available at: <a href="https://core.ac.uk/reader/39669356">https://core.ac.uk/reader/39669356</a>> [Accessed 20 July 2021].

Large-scale microalgae culture has been successfully achieved with green algae species such as *chlorella* and *spirulina*. <sup>11</sup> Open ponds are the most common means of cultivating algae as they are simple to construct and are low cost. <sup>12</sup> However, they face a variety of limitations, including low biomass conversion and loss of CO<sub>2</sub>. <sup>13</sup> Therefore, for effective CO<sub>2</sub> sequestration and to maximise algae growth, photobioreactors have been developed. Photobioreactors contain large volumes of algae in an enclosed environment and vertical arrangement (figure 4).

Large-scale algae production still remains technically challenging, due to a variety of factors limiting algae culture (see table 1), as well as rapid, difficult to control algal growth.<sup>14</sup> However, recently the company Hypergiant from Texas, USA, developed an artificial intelligence (AI) bioreactor (figure 5) which not only uses AI to maximise algal growth by maintaining optimum temperatures and light intensities, but also monitors algal development without human intervention through computer learning.<sup>15</sup> Bioreactors such as the Hypergiant bioreactor allow the culture of algae in a controlled large-scale system for CO<sub>2</sub> sequestration and synthesis of products such as biofuel.<sup>16</sup>

Table 1. Factors limiting microalgae growth

Biotic factors	Presence of pathogens (viruses, bacteria and fungi) and competition from other algae.
Abiotic factors	Light, temperature, pH, salinity, presence of toxic chemicals, oxygen concentration,
	bioavailability of nutrients.

<sup>&</sup>lt;sup>7</sup>Phillips, B., 2018. *Algae As Energy: A Look To The Future - Climate, Energy, And Society - College Of Liberal Arts - Auburn University*. [online] Cla.auburn.edu. Available at: <a href="https://cla.auburn.edu/ces/energy/algae-as-energy-a-look-to-the-future/">https://cla.auburn.edu/ces/energy/algae-as-energy-a-look-to-the-future/</a> [Accessed 21 July 2021].

<sup>8</sup>Steg, L., 2018. Limiting climate change requires research on climate action. Nature Climate Change, 8(9), pp.759-761.

<sup>9</sup> Ihid

<sup>&</sup>lt;sup>10</sup>Marimuthu, D. and Jayaraman, A., 2018. Isolation and Growth Characterization of the Fresh Water Algae Chlorosarcinopsis Eremi on Different Growth Media. *Journal of Pure and Applied Microbiology*, 12(1), pp.389-392.

<sup>&</sup>lt;sup>11</sup>Kativu, E., 2014. Carbon Dioxide Absorption Using Fresh Water Algae And Identifying Potential Uses Of Algal Biomass.. [online] Core.ac.uk. Available at: <a href="https://core.ac.uk/reader/39669356">https://core.ac.uk/reader/39669356</a> [Accessed 20 July 2021].

<sup>&</sup>lt;sup>12</sup>Concas, A., Pisu, M. and Cao, G., 2018. Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO2 sequestration with microalgae. *Chemical Engineering Journal*, 157(2-3), pp.297-303.

<sup>13</sup> lbid

<sup>&</sup>lt;sup>14</sup>Jalilian, N., Najafpour, G. and Khajouei, M., 2020. Macro and Micro Algae in Pollution Control and Biofuel Production – A Review. *ChemBioEng Reviews*, 7(1), pp.18-33. <sup>15</sup>Hypergiant. 2020. *Green R&D -HYPERGIANT EOS BIOREACTOR*. [online] Available at: <a href="https://www.hypergiant.com/green/">https://www.hypergiant.com/green/</a> [Accessed 1 July 2021].

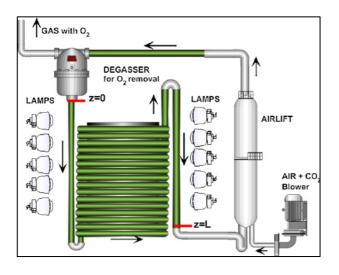


Figure 4. Novel simulation model of photobioreactors for CO<sub>2</sub> sequestration of microalgae

(Concas, A., Pisu, M. and Cao, G., 2018. Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO<sub>2</sub> sequestration with microalgae. *Chemical Engineering Journal*, 157(2-3), pp.297-303.)



Figure 5. Hypergiant Al Algae Bioreactor (Hypergiant 2020. HYPERGIANT EOS BIOREACTOR. Available at: https://www.hypergiant.com/green/)

The main biofuel produced from algae is biodiesel which relies on the lipid content of the organisms.<sup>17</sup> Biodiesel when combusting under optimal conditions compares favourably to petrol diesel, producing less CO<sub>2</sub> per kilogram combusted.<sup>18</sup> However, one issue with biodiesel can be the emission of nitric oxide which is poisonous and can contribute to acid rain.<sup>19</sup>

Recently, the culture of algae for the production of hydrogen as novel biofuel has been of particular interest.  $^{20}$  Biohydrogen is considered clean and sustainable as its combustion only results in  $H_2O$  as an end-product, as can be seen below.  $^{21}$ 

$$2H_2 + O_2 \xrightarrow{Combustion} 2H_2O$$

Biological hydrogen is produced as an intermediate or end product of algal metabolic pathways under defined conditions. There are three potential mechanisms of biohydrogen production in algal cells: 1. Direct bio-photolysis, 2. Indirect bio-photolysis and 3. Dark fermentation (see below).

$$1.2H_2O + light\ energy \rightarrow 2H_2 + O_2$$
 
$$2.6H_2O + C_6H_{12}O_6 + light\ energy \rightarrow 12H_2 + 6CO_2$$
 
$$3.C_6H_{12}O_{12} + 2H_2O \rightarrow 2C_2H_4O_2 + 4H_2 + 2CO_2$$

1. Bio-photolysis 2. Indirect bio-photolysis 3. Dark fermentation

Hydrogen photoproduction via direct photolysis has received increasing attention since it allows the breakdown of  $H_2O$  into molecular  $H_2$  without an output of  $CO_2$ .<sup>22</sup> The pathway is termed 'direct biophotolysis' due to its direct pathway of electrons. During photosynthesis water is split at photosystem II (PSII) into electrons, protons and oxygen. The electrons then move from PSII to photosystem I (PSI) and are used for biomass production by the cell. However, under certain culture conditions, such as sulphur deprivation, the metabolism of green algae switches from biomass production to hydrogen production. The electrons are then utilised to activate the enzyme hydrogenase ( $H_2$ ase) which

 $<sup>^{17}</sup>$  Nandan, S., 2020. Biofuel Production Technology from Bioenergy Crop-Algae Biofuel. SSRN Electronic Journal.

<sup>18</sup> Tsai, D., Chen, P. and Ramaraj, R., 2017. The potential of carbon dioxide capture and sequestration with algae. Ecological Engineering, 98, pp.17-23.

<sup>&</sup>lt;sup>19</sup> Dursun, N. and Gülşen, H., 2019. Methods of Biohydrogen Production and Usage of Bioreactors for Biohydrogen Production. *Journal of the Institute of Science and Technology*, pp.66-75.

<sup>&</sup>lt;sup>20</sup>Khanna, N. and Das, D., 2012. Biohydrogen production by dark fermentation. Wiley Interdisciplinary Reviews: Energy and Environment, 2(4), pp.401-421.

<sup>21</sup> Ihid

<sup>&</sup>lt;sup>22</sup> Fakhimi, N., Gonzalez-Ballester, D., Fernández, E., Galván, A. and Dubini, A., 2020. Algae-Bacteria Consortia as a Strategy to Enhance H2 Production. *Cells*, 9(6), p.1353.

catalyses the synthesis of H<sub>2</sub> from H<sup>+</sup> (see figure 6). Algal hydrogenase, however, is strongly inhibited by O<sub>2</sub> produced by PSII which is a limiting factor for hydrogen synthesis in algae.<sup>23</sup>

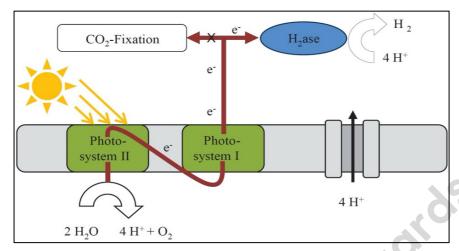


Figure 6. Electron transport reactions of hydrogen production in microalgae. (Seibert, M. and Torzillo, G., 2018. Microalgal Hydrogen Production: Achievements And Perspectives. Royal Society of Chemistry.)

Different strategies have been developed to overcome hydrogenase inhibition by  $O_2$  and maximize hydrogen production, such as co-culturing algae with various bacteria. A significant benefit of co-culturing heterotrophic bacteria with algae is that they can efficiently remove  $O_2$  from the growth media. Furthermore, the provision of fermentative metabolites such as acetic acid from bacteria to algae can establish specific nutrient fluxes that can benefit  $O_2$  production and/or growth. Some of the metabolites that can potentially be exchanged between algae and other microorganisms are shown in Figure 8.

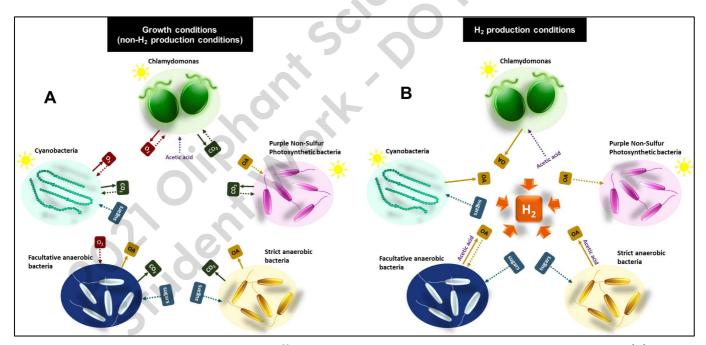


Figure 7. Potential metabolites exchanged among different H<sub>2</sub> producing microorganisms during growth conditions (A) and H<sub>2</sub>-producing conditions (B) (Organic acids (OA) mainly include ethanol, lactate, succinate, butyrate and glycerol.) (Fakhimi, N., Gonzalez-Ballester, D., Fernández, E., Galván, A. and Dubini, A., 2020. Algae-Bacteria Consortia as a Strategy to Enhance H2 Production. Cells, 9(6), p.1353.)

<sup>&</sup>lt;sup>23</sup>Fakhimi, N. and Tavakoli, O., 2019. Improving hydrogen production using co-cultivation of bacteria with Chlamydomonas reinhardtii microalga. *Materials Science for Energy Technologies*, 2(1), pp.1-7.

<sup>&</sup>lt;sup>24</sup>Hwang, J., Church, J., Lim, J. and Lee, W., 2018. Photosynthetic biohydrogen production in a wastewater environment and its potential as renewable energy. *Energy*, 149, pp.222-229.

<sup>&</sup>lt;sup>25</sup> Fakhimi, N., Gonzalez-Ballester, D., Fernández, E., Galván, A. and Dubini, A., 2020. *Op.cit* .p.1353.

<sup>&</sup>lt;sup>26</sup>Bayro-Kaiser, V. and Nelson, N., 2017. Microalgal hydrogen production: prospects of an essential technology for a clean and sustainable energy economy. *Photosynthesis Research*, 133(1-3), pp.49-62.

<sup>&</sup>lt;sup>27</sup> Seibert, M. and Torzillo, G., 2018. Microalgal Hydrogen Production: Achievements And Perspectives. Royal Society of Chemistry.

<sup>&</sup>lt;sup>28</sup>Ibid

#### **DISCUSSION**

Applications to reduce greenhouse gas emissions and extract  $CO_2$  from the atmosphere are desperately sought to help reduce attributed climate change and environment impact. Fossil fuel combustion for energy production produces more than 24 gigatons of  $CO_2$  annually.<sup>29</sup> As a result, atmospheric  $CO_2$  concentrations have risen from 295 parts per million (ppm) to 380 ppm over the last 100 years, and have been the cause of climate change.<sup>30</sup> The Intergovernmental Panel on Climate Change of the United Nation has therefore set the aim to reduce  $CO_2$  emission levels by 25% from 2010 until 2030 and reach net zero emissions by 2050.<sup>31</sup> However,  $CO_2$  emissions continue to rise, and the greatest concentration of  $CO_2$  in the atmosphere in human history was measured in May 2020 at Hawaii's Mount Loa.<sup>32</sup>

Bio-sequestration of CO<sub>2</sub> with algae via photosynthesis has emerged as a promising application to reduce the emission of CO<sub>2</sub>. Data shows that algae when used in conjunction with novel photobioreactors are up to 400 times more efficient in removing CO<sub>2</sub> from the atmosphere than trees.<sup>33</sup> Moreover, unlike terrestrial plants algae can be cultivated in vertical and compact systems, thus providing the opportunity for algae CO<sub>2</sub> sequestration to occur in densely populated cities.<sup>34</sup> This was shown last year when the company EcoLogicStudio installed 'urban curtains' in London.<sup>35,36</sup> The curtains consist of thin vertical arrays of algae and have been shown to be as effective as seven fully grown trees.<sup>37,38</sup> This is due to the uncomplicated cellular structure and rapid growth of microalgae resulting in a CO<sub>2</sub> fixation efficiency which is 10–50 fold higher than that of terrestrial plants.<sup>39</sup> Therefore, carbon-capture with algal bioreactors in the urban environment would potentially enable cities to become CO<sub>2</sub> neutral or even negative.<sup>40</sup> Furthermore, algae are known to grow in high CO<sub>2</sub> concentration environments and methods have been developed for the integration of flue-gas from power plants with algae production for the capture and utilization of CO<sub>2</sub>.<sup>41,42</sup>

Scientific research has also shown that algae can represent a key substitute to current conventional biofuels, reducing negative environmental impacts and solving ethical concerns.<sup>43</sup> Currently, many of the leading sources of biofuel, such as corn, require large areas of arable land for their production and therefore compete with food production which is concerning in developing countries which are struggling to keep up with agricultural demand. Moreover, conventional biofuel production entails the removal of native vegetation, thus frequently being carbon positive. In contrast, algae can be grown on unproductive land and are much more efficient in their production than conventional biofuels (algae culture produces about 19,000 L of biodiesel per equivalent acre compared to soy which produces only about 220 L per acre).<sup>44</sup> Additionally, researchers from Exxon Mobil Australia have shown that algae fuel production is carbon neutral with the same amount of CO<sub>2</sub> being sequestered as is released.<sup>45</sup>

Biohydrogen from algae would be the perfect energy source against climate change as it only produces water vapor when combusted, thus being carbon neutral.<sup>46</sup> Although large-scale hydrogen production from algae has not been applicable yet due to technical issues (such as the high sensitivity of the process towards oxygen), significant progress has been made due to advances in culture strategies. Recently published research by N. Fakhimi, A. Dubini and D. Gonzalez-Ballester from May 2020 has shown an increase of hydrogen yield by 60% when combining the unicellular

<sup>&</sup>lt;sup>29</sup>United Nations Sustainable Development. 2020. Climate Change. [online] Available at: <a href="https://www.un.org/sustainabledevelopment/climate-change/">https://www.un.org/sustainabledevelopment/climate-change/</a> [Accessed 28 June 2021].

<sup>&</sup>lt;sup>30</sup>lbid

<sup>&</sup>lt;sup>31</sup> Steg, L., 2018. Limiting climate change requires research on climate action. Nature Climate Change, 8(9), pp.759-761.

<sup>&</sup>lt;sup>32</sup> Concas, A., Pisu, M. and Cao, G., 2018. Novel simulation model of the solar collector of BIOCOIL photobioreactors for CO2 sequestration with microalgae. *Chemical Engineering Journal*, 157(2-3), pp.297-303.

<sup>&</sup>lt;sup>33</sup>Hypergiant. 2020. *Green R&D -HYPERGIANT EOS BIOREACTOR*. [online] Available at: <a href="https://www.hypergiant.com/green/">https://www.hypergiant.com/green/</a> [Accessed 1 July 2021].

<sup>&</sup>lt;sup>34</sup>Bonifield, J., 2020. How This Aussie Brewery Incorporates Algae Into Beer Production. [online] Greenbiz.com. Available at: <a href="https://www.greenbiz.com/article/how-aussie-brewery-incorporates-algae-beer-production">https://www.greenbiz.com/article/how-aussie-brewery-incorporates-algae-beer-production</a> [Accessed 3 August 2021].

<sup>&</sup>lt;sup>36</sup> Nandan, S., 2020. Biofuel Production Technology from Bioenergy Crop-Algae Biofuel. *SSRN Electronic Journal*.

<sup>&</sup>lt;sup>37</sup> BBC News. 2020. Could These Plastic 'Trees' Help Save The Planet?. [online] Available at: <a href="https://www.bbc.com/news/av/business-49044832/algae-bio-curtains-architects-radical-solution-to-capture-carbon">https://www.bbc.com/news/av/business-49044832/algae-bio-curtains-architects-radical-solution-to-capture-carbon</a> [Accessed 21 July 2021].

<sup>38</sup>BBC News. 2020. Op.cit.

<sup>&</sup>lt;sup>39</sup> Nandan, S., 2020. *Op.cit*.

<sup>&</sup>lt;sup>40</sup> Jalilian, N., Najafpour, G. and Khajouei, M., 2020. Macro and Micro Algae in Pollution Control and Biofuel Production – A Review. ChemBioEng Reviews, 7(1), pp.18-33.

<sup>&</sup>lt;sup>41</sup> Energy Procedia, Volume 37, 2013, Pages 6687-6695, Energy Procedia, New Methodologies for the Integration of Power Plants with algae ponds, KiraSchipperSvenvan der GijpRobvan der StelEarlGoetheer

<sup>&</sup>lt;sup>42</sup>BBC News. 2020. Co Op.cit.

<sup>&</sup>lt;sup>43</sup> Phillips, B., 2018. *Algae As Energy: A Look To The Future - Climate, Energy, And Society - College Of Liberal Arts - Auburn University*. [online] Cla.auburn.edu. Available at: <a href="https://cla.auburn.edu/ces/energy/algae-as-energy-a-look-to-the-future/">https://cla.auburn.edu/ces/energy/algae-as-energy-a-look-to-the-future/</a> [Accessed 21 July 2021].

<sup>&</sup>lt;sup>44</sup> Tsai, D., Chen, P. and Ramaraj, R., 2017. The potential of carbon dioxide capture and sequestration with algae. *Ecological Engineering*, 98, pp.17-23.

<sup>&</sup>lt;sup>45</sup> Nandan, S., 2020. *Op.cit*.

<sup>&</sup>lt;sup>46</sup> Tsai, D., Chen, P. and Ramaraj, R., 2017. *Op.cit.* pp.17-23.

algae Chlamydomonas reinhardii with E. coli bacteria. <sup>47</sup> The increased hydrogen production caused by higher respiration rates in the bioreactors during symbiotic growth, has led to the potential application of this system as a hydrogen-based fuel source. <sup>48,49</sup> Kristina Libby, the chief scientific officer at Hypergiant, stated "We see right now as a turning point in awareness to the potential that algae can play in the larger climate change conversation." <sup>50</sup> Next generation companies are recognising this opportunity and investing now. Testing and innovations are ongoing to further improve the efficiency of algae cultivation, reduce the inhibition of hydrogenase and allow the mass capture of algal biohydrogen. <sup>51</sup>

While the application of algae and photobioreactors for carbon sequestration could be the basis of a sustainable green future, there are still significant obstacles to overcome. An important factor currently limiting the widespread implementation are the costs. For example, the overall sum of biohydrogen production costs with algae were evaluated at 15 USD/GJ (in comparison, the production costs for natural gas are about 4 USD/GJ). <sup>52</sup> For photobioreactors to become more competitive, carbon taxes will have to be imposed by governments. Furthermore, to make the algae industry economically feasible, the production of algae will need to be combined with a biorefinery approach that provides a range of commercial algae products, such as biofuels, animal feeds and human dietary supplements. This would create an income stream and reduce the attributed cost of the algae production.

#### **CONCLUSIONS**

Algae absorb large amounts of  $CO_2$  and their biomass can be converted to biofuels. However, their application has been constrained in the past due to technical limitations and costs. Latest scientific developments, such as Al photobioreactors, are now allowing large-scale application of algae culture for carbon capture and production of sustainable ecofriendly fuel. The upscaling of algae culture will make it more economically feasible and advance the development of additional fuel sources from algae, such a biohydrogen. <sup>53</sup> Biohydrogen production for fuel provides an attractive option as a clean fuel for the future, but microbiological obstacles such as  $O_2$  inhibition of hydrogenase function are still preventing practical implementation of the process on an industrial scale. Nevertheless, recent advancements in the use of algal-bacterial co-culture are increasing the efficiency of hydrogen production. Optimization of key elements of the culture process as well as genetic and metabolic engineering of microalgae will make hydrogen production from algae increasingly cost-effective and sustainable.

Future novel applications are paving the way for an innovative future low-emission bioeconomy. Latest examples are the incorporation of algae CO<sub>2</sub> sequestration in the beer brewing process, as performed by the Australian Henry Banks Brewery, and scientific progress such as the research currently being undertaken by the Arizona University into the genetic modification of algae to produce hydrogenase enzymes which are oxygen resistant and would increase hydrogen production by 85%.<sup>54,55</sup> Algae's future, however, will ultimately depend on how seriously nations around the world consider the economic threats of climate change to the world population, compared to their own near-term economic self-interest.

### **WORD COUNT - 1544**

49Ibid

<sup>&</sup>lt;sup>47</sup>Fakhimi, N. and Tavakoli, O., 2019. Improving hydrogen production using co-cultivation of bacteria with Chlamydomonas reinhardtii microalga. *Materials Science for Energy Technologies*, 2(1), pp.1-7.

<sup>&</sup>lt;sup>48</sup> Damayanti, A., Sarto, S. and Sediawan, W., 2020. Biohydrogen Production by Reusing Immobilized Mixed Culture in Batch System. *International Journal of Renewable Energy Development*, 9(1), pp.37-42.

<sup>50</sup>Hypergiant. 2020. Green R&D -HYPERGIANT EOS BIOREACTOR. [online] Available at: <a href="https://www.hypergiant.com/green/">https://www.hypergiant.com/green/</a> [Accessed 1 July 2021].

<sup>&</sup>lt;sup>51</sup>Damayanti, A., Sarto, S. and Sediawan, W., 2020. Biohydrogen Production by Reusing Immobilized Mixed Culture in Batch System. *International Journal of Renewable Energy Development*, 9(1), pp.37-42.

<sup>&</sup>lt;sup>52</sup>Bayro-Kaiser, V. and Nelson, N., 2017. Microalgal hydrogen production: prospects of an essential technology for a clean and sustainable energy economy. *Photosynthesis Research*, 133(1-3), pp.49-62.

<sup>&</sup>lt;sup>53</sup> Dourou, M., Dritsas, P., Baeshen, M., Elazzazy, A., Al-Farga, A. and Aggelis, G., 2020. High-added value products from microalgae and prospects of aquaculture wastewaters as microalgae growth media. *FEMS Microbiology Letters*, 367(12).

<sup>&</sup>lt;sup>54</sup>Holmes, S., 2020. *Photosynthesis Rewired To Generate Hydrogen*. [online] Chemistry World. Available at: <a href="https://www.chemistryworld.com/news/photosynthesis-rewired-to-generate-hydrogen/4011702.article">https://www.chemistryworld.com/news/photosynthesis-rewired-to-generate-hydrogen/4011702.article</a> [Accessed 3 August 2021].

<sup>55</sup> Bonifield, J., 2020. How This Aussie Brewery Incorporates Algae Into Beer Production. [online] Greenbiz.com. Available at: <a href="https://www.greenbiz.com/article/how-aussie-brewery-incorporates-algae-beer-production">https://www.greenbiz.com/article/how-aussie-brewery-incorporates-algae-beer-production</a> [Accessed 3 August 2021].

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