Microorganisms as New Sources of Energy

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Abstract: The use of fossil energy sources has a negative impact on the economic and socio-political stability of specific regions and countries, causing environmental changes due to the emission of greenhouse gases. Moreover, the stocks of mineral energy are limited, causing the demand for new types and forms of energy. Biomass is a renewable energy source and represents an alternative to fossil energy sources. Microorganisms produce energy from the substrate and biomass, i.e., from substances in the microenvironment, to maintain their metabolism and life. However, specialized microorganisms also produce specific metabolites under almost abiotic circumstances that often do not have the immediate task of sustaining their own lives. This paper presents the action of biogenic and biogenic–thermogenic microorganisms, which produce methane, alcohols, lipids, triglycerides, and hydrogen, thus often creating renewable energy from waste biomass. Furthermore, some microorganisms acquire new or improved properties through genetic interventions for producing significant amounts of energy. In this way, they clean the environment and can consume greenhouse gases. Particularly suitable are blue-green algae or cyanobacteria but also some other pathogenic microorganisms (E. coli, Klebsiella, and others), as well as many other specialized microorganisms that show an incredible ability to adapt. Microorganisms can change the current paradigm, energy–environment, and open up countless opportunities for producing new energy sources, especially hydrogen, which is an ideal energy source for all systems (biological, physical, technological). Developing such energy production technologies can significantly change the already achieved critical level of greenhouse gases that significantly affect the climate.

Keywords: bioenergy; biomass waste; hydrogen; microorganisms; renewable energy sources

1. Introduction

The basic feature of life is oxidoreduction, which creates energy from matter [1]. However, some microorganisms can embed solar energy in very complex mechanisms of production of low-energy compounds from so-called nature pollutants caused by natural pollutants created by the technology of processing oil, sugar cane, and natural oils (harmful technologies) [2]. In addition to photosynthesis, some microorganisms, such as cyanobacteria, can decompose water into the desired oxygen and even more desirable hydrogen, and some can directly produce hydrogen via anaerobic processes [3]. Some, in turn, can convert classic environmental pollutants into very highly potent energy compounds (methane, alcohol) [4]. Thus, the genotypic and phenotypic traits of many species of microorganisms can direct the production of energy products to more perfect and efficient technologies and environmental purifiers [5].

Current technologies of energy production (energy) are a big problem (technical, environmental, and financial), because in addition to environmental pollution, they require significant investment (initial research, adaptation of new technologies, remediation as the final stage of production) [6]. However, natural pollutants (in terms of quantities, environmental impact, and permanent need for disposal) significantly reduce the benefits of conventional energy from fossil fuels (oil, gas, coal) and represent a subsequent often unsolvable problem of the remediation of CO₂, NO₂, SO₂, and other oxides [7]. Technologies
are being developed to use waste products (biorefinery) to produce renewable energy, as they permanently pollute the environment in the repeated energy production cycle [8]. Thus, microorganisms are undoubtedly crucial in developing waste purification and use strategies [9]. Bioenergy research is the center of scientific and technological research in the strategy of finding cost-effective biorefineries [10] as a way out of the current high level of air, water, and soil pollution to find photosynthetic and non-photosynthetic microorganisms that can produce clean energy (directly) or clean hydrogen [11].

An increasingly common research target of potential raw materials for biofuel production is microalgae obtained from adjusted wastewater. However, this may also significantly impact the environment, especially when compared with other renewable energy sources [12]. This can be particularly important when disposing of farm wastewater, representing an increasing environmental issue [13].

The present review aims to demonstrate the activity of biogenic and biogenic–thermogenic microorganisms that produce methane, alcohols, lipids, triglycerides, and hydrogen and contribute to creating renewable energy sources from waste biomass.

2. Microbial Technologies for Biofuel Production

The main reason for the increased interest in biomass as an energy source is the application contribution to the sustainable development paradigm. In addition, biomass sources are often present at the local level, and conversion into biofuel is possible with low initial costs [14]. Per the Renewable Energy Directive (EU Directive 2018/2001) [15], a common framework for the promotion of energy from renewable sources in the EU was established, setting a binding target for final gross consumption in the EU, with the total share of energy from renewable sources having to be 32% by 2030. This directive also promotes using non-food crops to produce biofuels and limits the number of biofuels and bioliquids produced from food or animal feed [15,16]. Methane is the so-called greenhouse gas produced indirectly by organic waste landfills (mainly in anaerobic processes) [17] and directly produced by all living beings (especially ruminants) [18]. There are two known sources of methane production: biological and non-biological. Non-biological methane is formed as a result of some geological processes. However, most methane (over 90%) is produced by the action of microorganisms and is a biological way (source) of methane production. This process of biological methane production is called methanogenesis, and microorganisms that carry out the same process are called methanogens [19,20]. Methanogens belong to the Archaea domain, which differs from bacteria because they do not possess peptidoglycan in the cell wall. Still, in Methanosarcina, it is a protein; in Methanosarcina, it is a heteropolysaccharide; and in Methanobacterium and Methanobrevibacter, it is replaced by pseudomurein [21]. The most crucial methanogen in the rumen is Methanobacteriales ruminantium, which contains pseudomurein in its cell wall. It needs formate, coenzyme M, hydrogen, and carbon dioxide for methane production [22]. For the process of methanogenesis, coenzymes F420, M, and HSHTP and lipids that methanogens have as cofactors are essential [23]. Cofactor F420 is necessary for the activity of hydrogenase as well as the formation of dehydrogenase enzymes, while coenzyme M acts as a terminal methyl carrier in the process of methanogenesis [24].

It is estimated that microorganisms annually produce and consume about one billion tons of methane [25]. However, the methane removal process can also occur in biological and non-biological ways. The most significant is the non-biological pathway in the Earth’s atmosphere (specifically, the troposphere and stratosphere), where various chemical reactions under ultraviolet radiation decompose methane. In chemical reactions, the issue is associated with the breaking of the covalent bond in methane–carbon-hydrogen, which is one of the strongest bonds among all hydrocarbons [26]. Regardless, methane is used in a process called the catalytic steam reforming of methane, where methane is first converted into synthetic gas, i.e., into a mixture of hydrogen and carbon monoxide. Then, it serves as a raw material for producing hydrogen, methanol, and other chemicals, where the catalyst is nickel, and the reaction takes place at temperatures from 700 °C to 1100 °C [27].
Pyrolysis is the process of burning methane, in which formaldehyde (HCHO or H₂CO) is formed in the first step, to which the HCO radical is added, after which carbon monoxide (CO) is formed [28].

The photocatalytic oxidation of methane is similar to the natural atmospheric process that oxidizes CH₄ to CO₂ [29]. Ultraviolet light is used to split the oxygen molecule into two free radicals that react with methane to produce products such as CH₃OOH, CH₃OH, HCOOH, CO₂HOCH₂OOH, and water. In photocatalytic reactors, catalysts increase the formation of free radicals and thus the rate of the methane reaction [30].

\[
\text{CH}_4 + \text{O}_2 \rightarrow \text{CO} + \text{H}_2 + \text{H}_2\text{O}_4 + \text{H}_2\text{O} \rightarrow \text{700-1100 °C NiCO + 3H}_2
\]

Unlike non-biological methods, biological methods of methane decomposition are carried out by the action of microorganisms called methanotrophs, and the process is named methanotrophy [19] (Figure 1). Methanotrophs can use methane to produce methanol, and Geobacter sulfurreducens and Shewanella oneidensis can use the mechanism of specific electron transfer from the membrane’s outer surface to visible surfaces. This phenomenon can be used in bioelectrochemical devices to produce biohydrogen [31]. In addition, methanotrophs have a significant role in reducing the production of large amounts of greenhouse gases via their formation below the surface of the Earth (below the ground) and the utilization of methane produced in the soil conversion of methane emissions into the atmosphere [32,33]. Methane oxidation begins with reducing oxygen to peroxide and then to methanol with the action of the monooxygenase enzyme (MMO) [34].

![Figure 1](image-url)  
Figure 1. The process of formation and decomposition of methane via biological and non-biological means.

3. Production of Ethanol and Butanol

One of the most immediate and vital applications of biomass is the fermentation of biomass and the production of bioethanol, the most common renewable fuel today. Various microorganisms can be involved in the fermentation process for bioethanol production.
Bioethanol is the leading liquid biofuel, with a global production of 29 billion gallons in 2019. The top producers are the United States and Brazil, which account for 84% of global production [36].

Table 1. Bioethanol yield from different microorganisms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Substrate</th>
<th>%</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Zymomonas mobilis</td>
<td>Corn steep liquor</td>
<td>98%</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces cerevisiae</td>
<td>Barley straw</td>
<td>82%</td>
</tr>
<tr>
<td></td>
<td>S. cerevisiae</td>
<td>Coffee grounds</td>
<td>87.2%</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Kluyveromyces marxianus SUB-80-S</td>
<td>Poplar and eucalyptus biomass</td>
<td>50–72%</td>
</tr>
<tr>
<td></td>
<td>K. marxianus IMB3</td>
<td>Wild millet</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>Candida shehatae</td>
<td>YNB (nitrogen base without yeast) without amino acid</td>
<td>71.6%</td>
</tr>
<tr>
<td>Mold</td>
<td>Fusarium oxysporum</td>
<td>Beer trope</td>
<td>60% of the theoretical yield</td>
</tr>
</tbody>
</table>

Yeast can produce ethanol via the direct decarboxylation of pyruvate formed via biomass oxidation [44]. At the same time, bacteria (E.coli) with coenzyme A activate the acyl group during the decarboxylation of pyruvate and convert it to ethanol (reduction) (Figure 2) [45].

Ethanol production via direct decarboxylation (Saccharomyces cerevisiae) is more efficient than that of E. coli. Butanol can be commercially similarly produced from sugar–starch biomass [46] (Figure 3). In addition, it can be made from so-called polysaccharides; from acetone–butanol fermentation (anaerobic process); and from and and acetone, CO₂ and hydrogen (Clostridium acetobutylicum) [47].

Some microorganisms are used in the gasification process. They can partially oxidize biomass by means of air or oxygen at about 800–1000 °C, whereby microalgae biomass is converted into a gaseous product—syngas—which means that microalgae are a suitable raw material for gasification [48,49]. Syngas is a mixture of hydrogen, CO, CO₂, methane, and nitrogen [50]. It is used as a turbine fuel but primarily as a feedstock for producing methanol, ethanol and synthetic hydrocarbons, butanol, methane, butyric, and acetic acid [51]. Microalgal bio-oils also contain metals (Fe, Mg, Ni, Zn), which can be removed via heat treatment [52]. Microbial oil can be obtained from microalgae, yeasts, and molds, and triglycerides (oleic, linoleic, and palmitic acid) can also be obtained [53]. These raw materials can be used to produce biodiesel. Some rare yeast and fungus species can
yield various substrates (lignocellulosic biomass, industrial waste glycerol, whey fat, and molasses) [54]. Some microorganisms can grow in sewage sludge and wastewater [55].

![Figure 3. Ethanol formation from glucose in bacteria. AdhE—bifunctional CoA-dependent ethanol/aldehyde dehydrogenase.](image)

**Main Metabolic Pathways for Ethanol in the Most Prominent Microorganisms**

The most common microorganisms used in ethanol production are yeast *Saccharomyces cerevisiae* and bacterium *Escherichia coli*. They possess specific metabolic pathways and different types of catalytic enzymes for producing biofuels [56]. For example, *Saccharomyces cerevisiae* produces ethanol via the direct decarboxylation of pyruvate, while *E. coli* activates the acyl group during the decarboxylation of CoA, which is then reduced to ethanol [57,58].

A more efficient route in the production of ethanol is considered to be a route without the use of CoA. This pathway can also be achieved in other microorganisms by means of genetic engineering techniques. However, this method represents a significant challenge because synthesizing an artificial metabolic pathway requires highly sophisticated tools to control mRNA and protein levels for the synthetic pathway to be functional [59].

*Zymomonas mobilis* is another well-studied strain with a known genome that produces ethanol. It is important to note that it has a significantly higher ethanol yield than *Saccharomyces cerevisiae*. Furthermore, ethanol yield considerably increases after genetic manipulation, i.e., after introducing genes encoding catalytic enzymes mannose and xylose, so the theoretical yield of ethanol within 72 h can reach 89.8% [60].

The costs of bioethanol production from lignocellulosic raw materials are high, so for commercial reasons, this production method is still not used [61,62]. However, the production of second-generation bioethanol is in the development phase. For this production, microbial strains that can produce ethanol from glucose and xylose, the main fermentable sugars, are necessary [63].

**4. Biodiesel Production**

Biodiesel is the first alternative fuel and, at the same time, the most widespread biofuel in Europe. It is obtained from oil and fat through the transesterification process and is very similar in composition to mineral diesel fuel [64]. Biodiesel production is an inherently complex system that requires optimization, keeping profitability and environmental sustainability in mind [65]. Recent studies suggest an unquestionable benefit for the environment. Economic profitability depends on feedstock sources and choice, technological process and production capacities, and transport to the consumer [66,67]. Sources of third-generation feedstock, microalgae, have an unquestionable advantage over other sources [68].
Regarding the need for increasing the amounts of energy (due to direct use in internal-combustion engines or the production of heat and electricity), guided by the imperative to reduce CO$_2$, modern science and technology are giving the first positive results [69]. These are the so-called biofuels produced as a product of microorganisms from biological materials and even from organic waste biomass [70]. In addition to the already considered bioenergy agents (methane, methanol, ethanol), we especially highlight the importance of biodiesel production. The European Union is at the forefront of applying such technologies in biodiesel consumption, which is about 105 billion liters—about 53% of the total world biodiesel consumption [71].

One of the most promising biodiesel production methods is the production of lipids, triglycerides, and other oil molecules from rapeseed, soybeans, and some other specialized plants, which incorporate this otherwise undesirable greenhouse gas into lipid molecules via photosynthesis [72]. At the same time, these plants have different types of fixatives (nitrofixatives, Azotobacter sp. and Azospirillum sp.; phosphofixatives, Acinetobacter junii and Pseudomonas fluorescens) in the soil, from which they benefit via the rhizome system [73]. Rhizome nodules fix nitrogen and phosphorus, conducive to plant growth and the formation of products (lipids, triglycerides) [74].

Biodiesel can then be produced directly from the vegetable oils of the above-mentioned plants [75] (Figure 4). Likewise, a biodiesel composition similar to vegetable oils can be obtained via the transesterification of Rhodotorula glutinis oil, and Yarrowia lipolytica can be used to produce microbial oil [76]. Genetic engineering can increase the tolerance of lipids or fatty acids in microorganisms, and some types of bacteria and fungi can tolerate higher amounts of accumulated triacylglycerol [77]. In terms of efficiency, bacteria show significantly more favorable properties than fungi (higher growth rate, easier maintenance, and the possibility of genetic adaptation) [78], because it is known that bacteria are subject to genetic improvement and possess the property of rapid growth, which can be used for the highly efficient production of microbial oil. Because of this, even Escherichia coli, under certain circumstances, can directly produce biodiesel in the form of fatty acid esters and can ferment biomass from renewable carbon sources (specialized or waste biomass) [79,80]. Thus, microbial oils are becoming a very likely source (raw material) in biodiesel production mainly due to the faster growth of microorganisms, easier maintenance, and the possibility of genetic adaptations [79,81].

The possibilities of direct electricity production by means of biochemical treatment systems are also being studied very intensively, and microbial fuel cell (MFC) devices convert chemical energy into electricity (without the Carnot cycle) from biodegradable raw materials and even from wastewater [82,83]. Furthermore, potentially electrogenic bacteria can be identified in the MFC device; in it, microorganisms feed on organic compounds, releasing electrons to the electrode, thus generating electricity [84]. In summary, it is clear that modern science and technology have efficient responses to increasing environmental pollution (especially greenhouse gases—CO$_2$ and methane) [85,86].

**Main Metabolic Pathways for Biodiesel in the Most Prominent Microorganisms**

Biodiesel belongs to the group of renewable energy sources and represents an ideal replacement for petroleum-based diesel fuels. It is produced using transesterifying fatty acid sources with short-chain alcohols, giving monoalkyl esters of long-chain fatty acids [87]. For this process to be realized, it is essential that microorganisms can produce fatty acids and short-chain alcohols that are available for transesterification and that they possess acyltransferases with more significant activity for short-chain alcohols. Genetic engineering significantly transforms microorganisms into forms that produce biodiesel with high efficiency [88].
Microalgae attract particular attention as a raw material for biodiesel production (Figure 5). Namely, it is an economically profitable raw material for oil. They are characterized by easy cultivation, diverse metabolic activities, and a high content of fatty acids [89,90]. The results of the research study by Huang et al. suggest that the problems with fossil energy could be alleviated by the additional processing of microalgae residues after the lipid extraction process, using the pyrolysis process [91]. A moderately fast pyrolysis temperature (~700 °C) is essential for higher bio-oil production and a lower limit of pollutants [92].
Among the numerous autotrophic algae are Botryococcus braunii, Chlorella vulgaris, Cryptothecodinium cohnii, Dunaliella primolecta, Navicula pelliculosa, Scenedesmus acutus, Cryptothecodinium cohnii, Monallanthus primolecta, Monallanthusornussel olia, and Teallanthus chloridea sul. The oil content in microalgae varies from 1 to 70% (Table 2).

Table 2. Lipid yields from different microalgae.

<table>
<thead>
<tr>
<th>Microalgae</th>
<th>Substrate</th>
<th>Mass Proportion of Oil (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botryococcus braunii</td>
<td>dry matter</td>
<td>25–75</td>
<td>[94]</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>dry matter</td>
<td>28–32</td>
<td>[94]</td>
</tr>
<tr>
<td>Cryptothecodinium cohnii</td>
<td>dry matter</td>
<td>20</td>
<td>[94]</td>
</tr>
<tr>
<td>Nannochloropsis sp.</td>
<td>dry matter</td>
<td>31–68</td>
<td>[94]</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>dry matter</td>
<td>20–30</td>
<td>[94]</td>
</tr>
<tr>
<td>Schizochytrium sp.</td>
<td>dry matter</td>
<td>50–77</td>
<td>[94]</td>
</tr>
<tr>
<td>Cylindrotheca sp.</td>
<td>dry matter</td>
<td>16–37</td>
<td>[95]</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>dry matter</td>
<td>45–47</td>
<td>[95]</td>
</tr>
</tbody>
</table>

The cultivation conditions include the composition of the nutrient medium, pH, temperature, the efficiency of light delivery to the cells of microalgae, the intensity and wavelength of light, the speed and method of mixing the nutrient medium in the bioreactor, and the ratio of the concentration of dissolved oxygen and CO₂ in the nutrient medium [96,97]. Accordingly, the biotechnological production of microalgal lipids is determined by the physiological potential of the microalga (Table 3), that is, the conditions and procedure of conducting the bioprocess in the bioreactor system [98]. Therefore, it is considered that microalgae are an inevitable trend in the development of future biodiesel, provided that for the industrial production of biomass, i.e., lipids of microalgae, the optimal conditions, and procedures for running the bioprocess in the bioreactor system are chosen so that an ecologically and economically sustainable bioprocess of the production of biomass, i.e., lipids of microalgae, is obtained for the production of biofuels (biodiesel) [99].

Numerous studies have established that residual biomass contains carbohydrates from which ethanol can be produced through fermentation. It is the specific share and yield during biodiesel production [100].

Table 3. Yields of lipids from different microorganisms.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Substrate</th>
<th>Mass Proportion of Oil (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Acinetobacter calcoaceticus</td>
<td>dry matter</td>
<td>27–38</td>
</tr>
<tr>
<td></td>
<td>Arthrobacter sp.</td>
<td>dry matter</td>
<td>&gt;40</td>
</tr>
<tr>
<td></td>
<td>Bacillus alcalophilus</td>
<td>dry matter</td>
<td>18–24</td>
</tr>
<tr>
<td></td>
<td>Rhodococcus opacus</td>
<td>dry matter</td>
<td>24–24</td>
</tr>
<tr>
<td>Yeasts</td>
<td>Candida curvata</td>
<td>dry matter</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Cryptococcus albidus</td>
<td>dry matter</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Lipomyces starkeyi</td>
<td>dry matter</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>Rhodotorula glutinis</td>
<td>dry matter</td>
<td>72</td>
</tr>
<tr>
<td>Trichosporon</td>
<td>ocelligerous</td>
<td>Lignocellulosic substrate</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molds</td>
<td>Aspergillus oryae</td>
<td>dry matter</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Humicola lanuginosa</td>
<td>dry matter</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Mortierella isabellina</td>
<td>dry matter</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Mortierella vinacea</td>
<td>dry matter</td>
<td>66</td>
</tr>
</tbody>
</table>

5. Hydrogen

Biohydrogen represents an essential factor in solving global energy problems [102]. It is a substitute for primary fossil fuels and their derivatives. Its main advantage is that the product of its combustion with oxygen is water, and not CO and CO₂, which are greenhouse gases [103]. Therefore, it is expected to play a crucial role in the future energy infrastructure. Biohydrogen has a gross energy or heat value of 142 MJ/kg, which is significantly higher
than those of natural gas or crude oil, whose values are 52 and 45 MJ/kg [102], while petrol has a value of 44 MJ/kg [103]. The global demand for hydrogen is predicted to increase from 70 million tons in 2019 to 120 million tons by 2024. Hydrogen development should also fulfill the seventh United Nations goal on affordable and clean energy [104].

Hydrogen is the first atom from which everything in the universe was created. The energy produced by fusion reactions (stars) was sufficient for forming all other elements, which created the conditions for evolution and the creation of life [105–108]. Traces of the life of the most primitive microorganisms (recognized today through the simplest microorganisms such as prions and viruses) used oxidation–reduction processes in which, under anaerobic conditions, hydride was oxidized to sulfides, nitrides, and phosphides and generated enough energy to start the process (which is still insufficiently explained) that could constitute life [109].

Modern life requires unimaginable amounts of energy [110,111], and fire is the simplest form of clean energy. In the same way, hydrogen is slowly and rapidly introduced into our daily lives [112]. Moreover, all mineral fuels provide energy by burning hydrogen (wood, coal, oil) [113], while nuclear processes, such as fusion, use hydrogen as fuel [114,115]. However, other products of hydrogen combustion (organic hydrocarbons, oxides) are today putting in question the continuation of life as we know it [116]. In addition, so-called greenhouse gases threaten civilization to such an extent, arousing the necessity for the creation of new mechanisms for increasing amounts of needed energy [117].

Microbial universality through life-saving adaptations has created natural reactors for producing biofuels and future fuels, i.e., hydrogen [118,119]. Moreover, they can produce it (extract it) from hydrocarbons, thus launching a more certain perspective for civilization [120]. The development and selection of microbial biorefineries are the result of the creation of syntrophic communities (a symbiotic form of joint metabolism) [121,122]. One example of syntrophy is methanogenic communities in which reducing equivalents, e.g., hydrogen and formate, are transferred among syntrophic partners [20,123]. In the coal seam, the anaerobic fermentation of organic matter includes hydrolysis, acidogenesis, acetogenesis, and methanogenesis [22,124]. In the first phase, bacteria hydrolyze macromolecules into simple sugars, amino acids, and fatty acids [125,126]. Then, acido- genic bacteria decompose them into propionic acid, butyric acid, and alcohol [127,128]. Microorganisms capable of acetogenesis then convert them to acetic acid, hydrogen, and CO₂ and ultimately methanogenic microorganisms can produce methane [129]. Thus, the production of carbon-based biogas significantly improves protection against the formation of unwanted gases (primarily sulfur). As noted, coal conversion into methane requires the synergistic action of three groups of microorganisms (syntrophic community) [130,131]. They are mainly from genera *Clostridium*, *Enterobacter*, *Klebsiella*, and *Citrobacter* [118,132]. Methanogenic bacteria, based on mcrA and the phylogeny of ribosomal genes, are classified into seven orders, among which *Methanopyrales*, *Methanococcales*, *Methanobacteriales*, *Methanomicrobiales*, and *Methanocellales* comprise hydrogenotrophic methanogens. At the same time, the *Methanomasiliicoccales* guild is obligated to perform methylogenotropic respiration [20].

A more complex form of microorganisms can be considered as the factory of electrochemical devices for producing electricity and biohydrogen [133,134]. The mechanism of biohydrogen generation can start from wastewater and some other types of organic matter [135–137]. By creating an electro-biofilm, the mechanism of electron transfer to conductive surfaces is triggered [138,139]. These electrons can then be used to produce electricity and hydrogen [140], similar to the so-called electrochemical cells (BECs), molecular machines that transfer electrons from a microbial membrane [141,142]. Microalgae can further produce hydrogen via the reaction of the photolysis of water, i.e., by converting water into hydrogen ions and oxygen, after which they convert these hydrogen ions into hydrogen, all under anaerobic conditions [143,144]. Likewise, photosynthesis can produce hydrogen through two stages [145]. The first stage is created via photosynthesis, in which acid production is separated from hydrogen production. In the second stage, microalgae
are denied access to sulfur and are forced to change their cellular metabolism for survival by forming starch, from which they produce hydrogen [146,147]. As a result, the amount of hydrogen produced gradually reduces, but this process does not create undesirable and harmful by-products [148]. Biological hydrogen can also be made via the fermentation of lignocellulosic raw materials and cotton-sludge hydrolates. It can be produced by bacteria isolated from higher organisms (such as fish and termites) [149]. These are predominantly Enterobacter, Klebsiella, Clostridium, and Citrobacter [150]. Several metabolic pathways exist for biohydrogen production, and anaerobic fermentation is the most efficient and rapid way to produce it [151,152] (Table 4).

Table 4. Methods for hydrogen production and their efficiency.

<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Production Method</th>
<th>Energy Efficiency (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Bioelectrolysis (microbial electrolysis)</td>
<td>70–80</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Biothermolysis (co-fermentation hydrolysis)</td>
<td>35–45</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Thermolysis (pyrolysis)</td>
<td>35–50</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Thermolysis (gasification)</td>
<td>35–50</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Thermolysis (partial oxidation)</td>
<td>60–75</td>
<td>[103]</td>
</tr>
<tr>
<td></td>
<td>Biophotolysis (photofermentation)</td>
<td>&lt;1</td>
<td>[103]</td>
</tr>
<tr>
<td>Microalgae</td>
<td>Biophotolysis (photofermentation)</td>
<td>&lt;1</td>
<td>[103]</td>
</tr>
<tr>
<td>Microorganism</td>
<td>Biolysis (dark fermentation)</td>
<td>60–80</td>
<td>[103]</td>
</tr>
</tbody>
</table>

The production of hydrogen using distinct methods and using various feedstock implies different capital investment costs and the costs of hydrogen production itself, as shown in Table 5. The level of production-technology innovation, the accessibility of existing infrastructure, and the feedstock cost significantly impact this cost.

Table 5. Hydrogen production costs by different methods.

<table>
<thead>
<tr>
<th>Process</th>
<th>Energy Source</th>
<th>Feedstock</th>
<th>Capital Cost (M USD)</th>
<th>Hydrogen Cost (USD/kg)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass pyrolysis</td>
<td>Generated steam</td>
<td>Biomass</td>
<td>53.4–3.1</td>
<td>1.25–2.20</td>
<td>[102]</td>
</tr>
<tr>
<td>Biomass gasification</td>
<td>Generated steam</td>
<td>Biomass</td>
<td>149.3–6.4</td>
<td>1.77–2.05</td>
<td>[102]</td>
</tr>
<tr>
<td>Direct biophotolysis</td>
<td>Solar</td>
<td>Water + algae</td>
<td>50 USD/m²</td>
<td>2.13</td>
<td>[102]</td>
</tr>
<tr>
<td>Indirect biophotolysis</td>
<td>Solar</td>
<td>Water + algae</td>
<td>135 USD/m²</td>
<td>1.42</td>
<td>[102]</td>
</tr>
<tr>
<td>Dark fermentation</td>
<td>-</td>
<td>Biomass</td>
<td>-</td>
<td>2.57</td>
<td>[102]</td>
</tr>
<tr>
<td>Photo-fermentation</td>
<td>Solar</td>
<td>Biomass</td>
<td>-</td>
<td>2.63</td>
<td>[102]</td>
</tr>
</tbody>
</table>

5.1. Main Metabolic Pathways for Hydrogen in the Most Prominent Microorganisms

For bacteria that participate in the production of biohydrogen, such as Geobacter sulfurreducens and Shewanella oneidensis, it is significant that they possess specific molecular mechanisms that facilitate the transfer of electrons from the outer membrane of the microorganism to visible surfaces, after which this feature can be used to produce biohydrogen and, accordingly, bioelectric energy [156]. Thus, such bioelectrochemical cells (BECs) represent an exceptional significance in the potential production of bioenergy from wastewater and organic biomass [157].

Microbial electrolysis cells (MECs) and microbial fuel cells (MFCs) are primarily used to produce biohydrogen and bioelectricity. Based on a biological perspective, both species function in a similar manner, and accordingly, common microorganisms can be used for bioenergy production. These microorganisms are called electrogenic or exoelectrogenic [158]. However, it is essential to note that the output energy from MECs and MFCs is insufficient for practical application and commercialization [159].

5.2. Hydrogen Production via Photofermentation with Photofermenting Bacteria

Biological methods for producing hydrogen are in increasing focus because they can use renewable raw materials such as the remains of plant biomass, organic waste, and sunlight [160]. There are two main ways in which microorganisms produce hydrogen:
photosynthesis and fermentation. The process of photosynthesis is dependent on light and includes direct biophotolysis, indirect biophotolysis, and photofermentation. On the other hand, dark photofermentation is essentially anaerobic fermentation, and this process is not dependent on light [161]. Microorganisms produce hydrogen at room temperature and pressure, significantly reducing the need for additional energy. Photosynthetic hydrogen is produced by microorganisms, such as photosynthetic bacteria, algae, and cyanobacteria. Fermentative hydrogen production is carried out by fermentative microorganisms, such as strict anaerobes, e.g., strains of *Clostridium* sp. thermophilic rumen bacteria and methanogenic facultative anaerobes or mixed cultures [162].

Based on the available data, the conclusion is that fermentative hydrogen production has more potential for practical application than photosynthetic hydrogen production. Hydrogen production via fermentation is currently more profitable in energy gain than photosynthesis [163]. This is supported by the facts that fermentation bacteria have fast growth and that oxygen does not affect the anaerobic process to a large extent; they do not need light; and they have a higher level of hydrogen production. It is also important to note that there is a large selection of substrates and that the methods (techniques) of setting up bioreactors are simple [164–166]. The production of hydrogen by means of dark fermentation can be carried out under different thermodynamic conditions so that it can be carried out under mesophilic, thermophilic, and hyperthermophilic conditions. However, the production degree is still more favorable at higher temperatures [167].

It is known that there is a significant difference between theoretical and practical energy yield, which can be seen from the following example:

**Theoretical yield**—12 moles of H2 can be produced from each mole of glucose.

**Practical yield**—a maximum of 3.8 moles of H2 can be produced from each mole of glucose.

Yields can be increased by combining two metabolic pathways and using compatible mixed bacterial cultures [79].

### 6. Cyanobacteria

Hydrogen from bacteria is also produced via photosynthesis, because bacteria do not consume the created hydrogen but rather retain it [168]. Namely, the process of photosynthesis of cyanobacteria begins with the fixation of solar energy due to water splitting, so this energy is stored by hydrogenase in sugars [169,170]. Electrons undergo a series of reactions produced by the ATP energy carrier and the reduction equivalents of NADPH, which are required for CO\(_2\) fixation or sugar production [171]. These electrons and protons (energy) can be diverted to primarily produce hydrogen, that is, by linking the photosystem to hydrogenase. Modified cyanobacteria produce less sugar than unmodified ones at the expense of greater hydrogen production [169,172]. This fusion can function indefinitely. It is also transmitted during cell division, and since the oxygen created is blocking the processes, metabolism is transferred to anoxygenic photosynthesis. All these processes offer a new concept for the production of green hydrogen [173].

In addition to genetic modifications that create unique and desirable traits in some bacteria, some bacteria are also subject to natural phenomena that can still produce everything necessary for growth and reproduction under unfavorable conditions [174]. Extremophiles, which live where there is no liquid water and no solar energy, can use hydrogen from the air as a fuel to create water for their needs, specifically, by capturing hydrogen and oxygen from the air [175]. Thus, hydrogen drives chemosynthesis with enzyme RuBisCo, which otherwise uses sunlight to capture CO\(_2\) [176].

Thus, about 400 species of evolutionarily adapted microorganisms live without free water and can use inorganic energy sources (hydrogen, CO) as sources that drive metabolism (chemosynthesis) [177,178]. Some marine bacteria work similarly. Seawater has sufficient amounts of hydrogen and CO, which can be sources of energy for bacteria from families *Rhodobacteraceae*, *Flavobacteriaceae*, and *Sphingomonodaceae* [179,180]. A representative of this
group of ultramicrobacteria, *Sfinopyxis alascensis*, grows mycotrophically on hydrogen by expressing NiFe hydrogenase [181,182].

Thus, one of the possible solutions for obtaining new renewable energy sources is hydrogen (H\textsubscript{2}), which could be obtained via photosynthesis, which requires sunlight, water, and cyanobacteria. However, in doing so, it is necessary to consider the development of cost-effective production technologies [183].

7. Future Perspective

The biggest challenge in employing microorganisms to produce biofuels is producing a considerable amount of fuel more cheaply and efficiently than traditional fossil fuels. To replace petrol with bioethanol should be cheaper, which is very demanding to cover the necessary daily quantities. For example, in the USA, about 19 million barrels of petrol are consumed daily, and producing this amount on an industrial scale is challenging. Therefore, future biofuel productivity should be prioritized to increase microbial biofuel’s acceptability [184]. Some of the most common advantages and disadvantages of the biofuel production process are shown in Table 6. Due to the politically increasingly unstable oil market, many countries are turning to renewable energy sources. Biofuels (bioethanol and biodiesel) are a sustainable energy source due to their high chemical similarity, carbon neutrality, and comparable energy content, and microorganisms are crucial to their synthesis. Depending on the feedstock’s evolutionary hierarchy and the manufacturing technique, biofuels are divided into four generations. Biofuel production advances with each generation toward achieving sustainability and financial success in energy production. They are created to most effectively address the issues of the energy crisis, pollution, global warming, and waste management. Microorganisms used to be mere biomass decomposers, but because of advancements in biotechnology, gene editing, and synthetic biology, they now produce biofuel [185].

Table 6. Advantages and disadvantages of biofuel production.

<table>
<thead>
<tr>
<th>Biofuel</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioethanol</td>
<td>Renewable sources; low cost; algae can rapidly absorb carbon dioxide; accumulate high concentrations of lipid and carbohydrates, be easily cultivated, and require less land than terrestrial plants</td>
<td>High costs of lignocellulosic feedstock; inputs of energy and water; requirements for large volume bioreactors and distillation columns; generation of large volumes of waste or low-value coproducts</td>
<td>[35]</td>
</tr>
<tr>
<td>Biodiesel</td>
<td>Renewable, sustainable, environmentally friendly, and biodegradable sources; low cost and high conversion rate; ideal replacement for petrol; reducing greenhouse gases; less harmful carbon emission; ecologically and economically sustainable bioproduct; use of existing engines without changes</td>
<td>High energy consumption; environmentally unfriendly processing including chemical catalysts, high cost, and limited supply of feedstocks; complex production processes; downstream technology; simultaneously produced waste; production is dependent on large quantities of water and oil</td>
<td>[79]</td>
</tr>
<tr>
<td>Biohydrogen</td>
<td>Renewable sources; cleanliness; low greenhouse gas emissions; biohydrogen has the advantage of being able to use a wide range of substrates to produce hydrogen; the first stage of the waste treatment and valorization process uses mild temperatures and does not need the external addition of metal catalysts; clear environmental benefits</td>
<td>Low performance; high capital cost investment; expensive materials; complex maintenance; variable energy loss; decreasing hydrogen production with the increase in the volume of the reactor; hydrogen storage; global-warming potential; land use; terrestrial- and freshwater-ecotoxicity potential; ecotoxicity potential; human-toxicity potential</td>
<td>[103,186,187]</td>
</tr>
</tbody>
</table>
The production processes for the second and third generations of biofuels are quite complex, which results in high energy costs. Additionally, the feedstock for the third generation has very complex requirements for structure, storage, and content. The expenses mentioned above explain the capital intensity of manufacturing second- and third-generation fuels, respectively, and the decision by most nations to choose first-generation biofuels [188]. The microbial lipids produced by microorganisms are the ideal feedstock for biodiesel synthesis due to their high production rate and independence from environmental conditions such as soil and climate. In the study by Wang et al., several ideas for generating biodiesel using microbes from inexpensive lignocellulosic biomass are addressed [79]. A country that intends to develop alternative fuels must have enough land to prevent a food shortage and enact stringent controls limiting the proportions of raw materials provided to the food and fuel markets. The ratios in which a blend of biodiesel with diesel and bioethanol with petrol can be created must also be governed by state standards. Developing second- and third-generation biofuel production, which uses significantly less land and is mostly not arable, despite having a higher capital during production, needs help and subsidies [188].

8. Conclusions

Numerous studies are being conducted based on the growing need to find new renewable energy sources that could replace gas and oil and reduce harmful effects to the ecosystem. As a result, scientists are increasingly turning to biofuels based on microorganisms. In doing so, it is necessary to use increasingly advanced genetic engineering technologies. The imperative of preserving and surviving civilization can be met—using enough species of microorganisms that we can find in our immediate environment. In this way, microorganisms are ready to lead us into new human–environmental relationships to be our companions in a more confident and secure future.

Author Contributions: Conceptualization, J.T. and D.T.; writing of the manuscript, J.T., D.T., A.M. and I.Š.; updating of the text, J.T. and I.Š.; literature searches, J.T., D.T., A.M. and I.Š.; figure drawings, A.M.; critical reviewing of the manuscript, J.T. and I.Š.; organization and editing of the manuscript, J.T. and I.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research study was funded by a grant from the Croatian Ministry of Science and Education and dedicated to multi-year institutional financing of scientific activity at Josip Juraj Strossmayer University of Osijek, Faculty of Dental Medicine and Health, Osijek, Croatia, grant number IP11-FDMZ-2021.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Energies 2022, 15, 6365


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