



## THE FUNCTIONS AND PROPERTIES OF MANY ENGINEERING MICROORGANISMS USING IN PRODUCTION OF BIOFUELS

<sup>1\*</sup>hsan EK N, <sup>2</sup>Ridvan E EN

<sup>a</sup>Sirnak University, Engineering Faculty, Energy System Engineering Department, Sirnak, Turkey.

<sup>b</sup>Dicle University, Science Faculty, Department of Biology, 21280 Diyarbakır, Turkey.

\*Corresponding Author's E-mail: ekinihsan@gmail.com

### ABSTRACT

Bacteria, algae, fungi, yeast and archeabacter are among the most potentially substantial sources of sustainable biofuels in the future of renewable energy. These engineering microorganisms have a wide variety of functions related to biofuels such as producing acetone, butanol, ethanol by fermenting sugar sources, secreting cellulase for facilitating biofuel production, containing high potential of energy rich oil used for biodiesel, converting lignocellulosic biomass directly into ethanol, producing enzymes that convert biomass to biofuel, consuming sugar and secretes engine-grade biodiesel, producing lipase directly used in transesterification, producing CH<sub>4</sub>, H<sub>2</sub> and CO<sub>2</sub> gasses, making up long chained hydrocarbons that can be converted conventional fuels like fossil oil, containing resistance genes that recombinant to other biofuel producing organisms, taking role in producing biofuels by help of electricity (not yet in practice). Shortly, the study aims to be useful for creating awareness by bringing together various microorganisms that are beneficial to biofuel production.

**KEYWORDS:** Bacteria, Algae, Fungi, Yeast, Archeabacter, Biofuels

### INTRODUCTION

Recently, several studies have been done on bacteria, yeast, algae, fungi, archeabacter and other microorganisms that are involved in the production of biofuels. Recombination of genetic structures on these microorganisms aims to increase the production of more hydrocarbons and other compounds which are mainly used as energy source like ethanol, methanol, butanol, acetone, hydrogen, methane and stored lipid. Researchers try to develop new technologies that use newly manipulated engineered microorganisms that can convert cellulose to ethanol directly, synthesize hydrogen rapidly, store fat in high amount and have some other properties contributed to biofuels. Addition to genetic studies on microorganisms, by many new researches and techniques to be performed in the future, perhaps, different type of biofuels will be discovered by newly recombinant microorganisms.

The mechanisms of biofuel production differ from species to species. For instance, some species only use CO<sub>2</sub> and water as a source of raw materials, while most of the others require a biomass. Conscious and accurate biomass formation will have many benefits to the environment, the use of biomass materials, especially waste materials for supplying biofuels will guarantee a secure and prosperous future; and will also play an important role in reducing global warming by generating fewer pollutants that are the cause of acid rain and other effects. Sustainable energy production has been the most important issue for national economic and security. The use of fuels derived from renewable biological sources is purposed to be one remedy to up to a point replace petroleum based products. Biofuels

can be produced by two main pathways, biochemical (fermentation, digestion, etc.) or chemical - thermochemical (transesterification or direct hydrogenation of oils, gasification, pyrolysis of whole biomass etc.) (Demirbas, 2009; Yan, 2009; Tang *et al.*, 2011).

This review study is included important features of many microorganisms used directly or indirectly in biofuel technology as well as provided an awareness and information about biofuels varieties. Furthermore, it brings together several microorganisms, which are principally used in biofuels synthesis such as *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Thermoanaerobacter ethanolicus*, *Escherichia coli*, *Methanosarcina barkeri*, *Methanothrix* and many others newly realized species that have significant role in biofuels production like *Mariprofundus ferrooxydans*, *Deinococcus radiodurans* and *Botryococcus braunii*.

### MICROORGANISMS INVOLVED IN THE PRODUCTION OF BIOFUELS

#### *Saccharomyces cerevisiae*

*S. cerevisiae* is generally known for its role in food production, for example, in the fermentation process that converts sugar into alcohol, an ingredient shared in beer, wine and distilled beverages and also used in the baking process as a leavening agent. The sugar either homogeneous like sucrose and glucose from sugarcane, and starch, or heterogeneous when originating from lignocellulosic biomass, can be broken down to ethanol by traditional *S. cerevisiae* for many years and ethanol

fermentation is principally done by the standard glycolysis pathway. This yeast has many advantages over other known ethanol producing microorganisms, such as high ethanol yields, ethanol tolerance, high robustness and high resistance to toxic inhibitors (Jeffries, 2006). Ethanol productivity have been documented in some studies with different ratios such as in mineral kissiris (74.3 g/d), in delignified cellulosic materials (69.3 - 51.5 g/d), in gluten pellets (63.2 - 118 g/d), in quince pieces (72 g/d), in orange peel (128 - 110.4 g/d), in corn ground tissue (39.6 g/d) (Vuaurovia *et al.*, 2009).

#### ***Sulfolobus solfataricus***

*S. solfataricus* is known as a thermoacidophilic extremophile because of its preference to high temperatures and low pH levels and a constant presence of environmental sulfur, for this reason it grows in volcanic hot springs. It produces cellulase breaking down the cellulose molecule into monosaccharides or shorter polysaccharides and oligosaccharides. The enzymes of this genus are significant in the conversion of lignocellulosic biomass into fermentable sugars, which is a key step in the production of second-generation biofuels. Researchers are looking at ways of genetically modifying this microbe to see if they can get it to improve its performance and produce more cellulase (Girfoglio *et al.*, 2012).

#### ***Yarrowia lipolytica***

Some oleaginous yeast strains have the ability to accumulate lipids to high levels, up to more than 20% of their biomass (Beopoulos *et al.*, 2009). Among these oleaginous yeasts, *Y. lipolytica* is a unique host for biochemical production due to its abilities to accumulate high levels of lipids and utilize hydrophobic and waste carbon sources. For this reason, this species has attracted great attention as a potential biofuels producing host. Recently, *Y. lipolytica* have attracted great attention regarding the production of fatty acid derived biofuels, including fatty acids, fatty acid ethyl esters, fatty alcohols, and fatty alkanes. However, the native yeast strains cannot produce fatty acids and fatty acid derived biofuels in large quantities (Sheng and Feng, 2015).

#### ***Zymomonas mobilis***

*Z. mobilis* is a mesophilic bacterium which is highly efficient in ethanol producing. This homoethanogenic bacterium tolerates up to 12% ethanol and grows 2.5 times faster compared to yeasts (Rogers *et al.*, 1982). Unlike both *S. cerevisiae* and *E. coli*, *Z. mobilis* utilizes the Entner-Doudoroff pathway to produce ethanol (Gunasekaran and Chandra, 1999). In addition, it produces ethanol more exclusively than *S. cerevisiae*, and has capability of withstanding certain stresses, including a higher concentration of environmental ethanol. These combined differences allow *Z. mobilis* to convert glucose to ethanol at as much as 97% of the theoretical yield, and do so at a much faster rate than *S. cerevisiae* (Bai *et al.*, 2008).

#### ***Clostridium thermocellum* and *Clostridium thermohydrosulfuricum***

*Thermophilic C. thermocellum* and *C. thermohydrosulfuricum* are attracting the most attention and the cellulosome of *C. thermocellum* has been characterized extensively. These three species have several advantages that make them suitable for an industrial conversion of lignocellulosic biomass to ethanol such as

converting lignocellulosic biomass directly into ethanol and the fermenters do not need aeration. On the other hand, *C. thermocellum* lacks the ability to convert pentose sugars. On account of this, the use of mixed culture of *C. thermocellum* with another thermophilic anaerobe that is capable of producing ethanol out of pentoses is of great interest (Demain *et al.*, 2005).

#### ***Clostridium acetobutylicum***

*C. acetobutylicum* can ferment sugar from various sources including corn, molasses, sugar cane, plant, woody biomass ect., and produce acetone, butanol, ethanol by fermenting sugar sources. The fermentation products are then converted into a mix of hydrocarbons that are similar to those in diesel fuel. The resulting fuel burns as well as petroleum-based fuel and has more energy per gallon than ethanol. Researchers have studied on the metabolic pathways of this species in order to improve industrial fermentation operations. The metabolic pathways which produce industrial useful solvents such as acetone, acetate, butanol, butyrate, and ethanol, all derived from the common precursor, acetyl-CoA are most notable in *C. acetobutylicum* (Nölling *et al.*, 2001).

#### ***Dunaliella tertiolecta***

*D. tertiolecta* is a genetically engineered the marine alga producing five different enzymes that could be used to convert biomass to fuel. The results suggest that algal biofuels can also be produced in the ocean, in the brackish water of tidelands, or even on otherwise unusable agricultural land with high salt content in the soil. Methyl linolenate and methyl palmitate were found to be the major components of fatty acid methyl esters produced from *D. tertiolecta* oil. *D. tertiolecta* and its derived oils should be a suitable feedstock for biofuel production (Tang *et al.*, 2011).

#### ***Thermoanaerobacter thermohydrosulfuricus***

*T. thermohydrosulfuricus* is a good ethanol synthesizing bacteria. High ethanol quantity was indicated in this species, varied from 0.9 to 1.9 mol ethanol mol glucose-1 (Lovitt *et al.*, 1984, 1988). Furthermore, in some other studies, the level of ethanol was found to be between 1.5 and 1.9 mol ethanol/mol glucose in *T. ethanolicus*, *T. thermohydrosulfuricus* and *T. finnii* (Wiegel and Ljungdahl, 1981; Avci and Dönmez, 2006; Carreira *et al.*, 1983).

#### ***Thermoanaerobacter ethanolicus***

As its name implies, *T. ethanolicus* ferment sugars into ethanol and carbon dioxide more than other anaerobes. *T. ethanolicus* ferment a variety of substrates found in plants, including cellobiose, starch, glucose, maltose, sucrose, pectin, and xylose oligomers with the exception of cellulose (Wiegel and Ljungdahl, 1981). It was showing extremely good yields of ethanol from glucose (1.9 mol ethanol mol glucose-1). This species showed also very good ethanol yields from xylose and glucose at low (10 g L-1) substrate concentrations, or 1.45 and 1.95 mol, respectively (Carreira *et al.*, 1983).

#### ***Thermotoga neapolitana***

*T. neapolitana* is hyperthermophilic bacterium and used for producing H<sub>2</sub> from green algal biomass. There are some methods that yield good effect on H<sub>2</sub> production but the highest H<sub>2</sub> yield (2.5 mol H<sub>2</sub> mol glucose-1) is obtained with enzymatic hydrolysis (Nguyen *et al.*, 2010c). Significant studies have been present about *T.*

*neapolitana* particularly on production yields of H<sub>2</sub> with enzymatic hydrolysis. On the other hand, the species can yield substantial amounts of lactic acid without affecting H<sub>2</sub> synthesis, thus offering novel applications for the fermentative process (Pradhan *et al.*, 2015).

#### ***Trichoderma reesei***

Another common wood digester is the fungus *T. reesei*. It has been found in nearly all soils and secretes huge quantities of cellulase. This mesophilic and filamentous fungus is widely known for its ability to efficiently decompose the non-edible parts of plants by secreting cellulolytic enzymes such as cellulases and hemicellulases in collaboration with a specially engineered strain of the bacteria *E. coli*. In an experiment, the fungi degraded the husks into sugars, and then *E. coli* finished the job. The result was isobutanol, flammable liquid that researchers hope could one day replace gasoline (Kumar *et al.*, 2008).

#### ***Escherichia coli***

First report of de novo biosynthetic pathway of biodiesel is indicated in *E. coli* by the simultaneous overexpression of the ethanol production genes from *Z. mobilis* and the wax ester synthase/acyl-CoA-diacylglycerol acyltransferase gene from the *Acinetobacter baylyi* strain ADP1 (Kalscheuer *et al.*, 2006). Strains of the *E. coli* can naturally utilize multifarious carbon sources like sugars and sugar alcohols, and is the best suited for a variety of industrial products in addition to biofuels such as proteins, hormones, amino acids, and diverse high-volume chemicals (Liu and Khosla, 2010; Becker and Wittmann, 2015; Koppolu and Vasigala, 2016). The hemicellulose component of lignocellulosic biomass hydrolyzes into hexose sugars including galactose, mannose and glucose, and pentose sugars including xylose and arabinose, which are at last converted into ethanol by the fermentation process. Organisms such as *S. cerevisiae* and *Z. mobilis* are currently used as front runners to produce ethanol through fermentation. However, these organisms cannot use pentose sugars and thus limit our ability to harness maximum productivity, whereas *E. coli* has the ability to use both pentose and hexose sugars for ethanol production (Koppolu and Vasigala, 2016).

#### ***Rhodobacter sphaeroides***

*R. sphaeroides* has been isolated from deep lakes and stagnant waters. It is purple non sulfur bacterium that can produce H<sub>2</sub> from organic acids by the nitrogenase-hydrogenase enzyme system under anaerobic conditions. Additionally, the activity of three different *R. sphaeroides* strains has been investigated in the production of photohydrophilic H<sub>2</sub> with acid hydrolyzed wheat starch (Kapdan *et al.*, 2009). *R. sphaeroides* has been investigated to evaluate its potential for biofuel and bioplastic production and it is able to produce polyhydroxybutyrate, a polymer of 3-hydroxybutyrate which is a potential bioplastic. As described before, *R. sphaeroides* is capable of H<sub>2</sub> production by way of its nitrogenase. Scientists attempts to develop a strain of *R. sphaeroides* that is able to produce H<sub>2</sub>, a potential biofuel, using light as its energy source (Bai *et al.*, 2006).

#### ***Chlamydomonas reinhardtii***

*C. reinhardtii* is a particularly well studied biological model green algae, partly because of its ease of culturing and the ability to manipulate its genetics. It attracts interest for producing biopharmaceuticals and biofuel, as well being a valuable research tool in making H<sub>2</sub> gas. Under anaerobic conditions, it can produce either H<sub>2</sub> or use H<sub>2</sub> as an electron donor. The hydrogen ions produced are converted into H<sub>2</sub> gas in the electron presence with the help of hydrogenase enzyme (Das and Veziro lu, 2008).

#### ***Dekkera bruxellensis***

*D. bruxellensis* has been shown in an ethanol facility with yeast cells recirculation that it is a good production organism (Passoth *et al.*, 2007) which is producing ethanol, aldehydes and other by-products (Dato *et al.*, 2005). It out competed the original inoculant *S. cerevisiae* strain without affecting the productivity or ethanol yield. Additionally, it has also been noticed in bioethanol industrial processes in Brazil, which run continuously with cell recycling similar to the Swedish ethanol plant (Blomqvist *et al.*, 2010).

#### ***Klebsiella ozaenae* and *Pseudomonas aeruginosa***

In a study, it is stated that among 35 cellulose degradation bacteria isolated from water and sediment samples; *K. ozaenae*, and *P. aeruginosa* created largest zone of lyses. In the experiment, ethanol production was done by using wood powder, paddy straw and millet, ragi, corn, sugarcane stalks. This two species were tried as a consortium, 11.99 % of ethanol content observed. When wood and agricultural residues used with the consortium of bacteria, the yeast yielded 11.0% of ethanol (Kalaiselvi *et al.*, 2013).

#### ***Metarhizium anisopliae*, *Aspergillus oryzae* and *Chromobacterium viscosum***

Researchers have identified that lipase made from *M. anisopliae*, *A. oryzae* and *C. viscosum* can cause transesterification at room temperature (Adachi *et al.*, 2011; Fiametti *et al.*, 2011; Talukder *et al.*, 2011). This cuts the energy expenditure of biodiesel production, making the process more energy efficient and less sensitive to process problems than the standard process (Lam and Lee, 2011).

#### ***Chlorella protothecoides***

Microalgae generally have been cultivated for their energy rich oils. Single-cell green microalgae *C. protothecoides* has high potential for the energy rich oil and food production. Heterotrophic growth of *C. protothecoides* supplied with acetate, glucose, or other organic compounds as carbon source, results in high biomass and high content of lipid in cells (Zhang and Hu, 2011).

#### ***Chlorella vulgaris***

*C. vulgaris* has the potential to serve as a food and energy source due to its high photosynthetic efficiency as well as great potential as a resource for biodiesel production due to easier cultivation and rapid growth. It can grow with both autotrophic and heterotrophic modes, and its mixotrophic growth rate is the sum of its autotrophic and heterotrophic growth rate, separately (Zhang and Hu, 2011). Lipids are produced in high amount by photosynthesis in *C. vulgaris* this makes the algae more viable source for biofuel. Lipid content of *C. vulgaris* per biomass is approximately 42%. This is more than

soybeans, sugarcane, and corn; making it an alternative for biodiesel (Feng *et al.*, 2011).

#### ***Spirulina platensis***

Many algae are grown for their high content of protein and some of them contain up to 60% protein. Mostly *Spirulina* is produced for its high protein content, health benefits and high nutritional value (Spolaore *et al.*, 2006). *Spirulina* is a well-known alga that is usually cultivated for its protein content (Vonshak, 1990). *S. platensis* is the most popular *Spirulina* species used for biofuels with its high content of oils. Additionally, the biomass oil content of microalgae is highly dependent on the specific growth conditions not only influenced by the microalgae species (Ehimen *et al.*, 2010). The microalgae culture conditions, nutrients and light intensity can be optimized to increase the oil content of the biomass, and therefore increases in the biodiesel production (Hu *et al.*, 2008).

#### ***Botryococcus braunii***

*Botryococcus* genus does not produce the lipids for biofuels, however can produce long chain hydrocarbons, which are not suitable for biodiesel production. Instead, they can be converted to biofuels, via a process similar to the production of conventional fuels from fossil oil. *Botryococcus* is a freshwater species but can also grow in saline water and it can produce certain carotenoids. *B. braunii* is a potentially good renewable source of useful hydrocarbons, polysaccharides, and other chemicals. It is a major interest in the fuel production industry, because 40-75% of its dry mass is made up of hydrocarbons. Catalytically cracked algal hydrocarbons have sufficiently high octane ratings for use as motor fuel (Banerjee *et al.*, 2002).

#### ***Methanosarcina barkeri* and *Methanotherix***

These bacteria are very sensitive to environmental variations because they are obligatory anaerobic. The methane producing bacteria take advantages of H<sub>2</sub>, CO<sub>2</sub> and acetic acid in order to form methane and CO<sub>2</sub>. The methanogenic bacteria are included in the archaeobacter genus in contrast to acidogenic and acetogenic bacteria. Three types of methanogenic bacteria are presented in terms of methane producing process; *Methanosarcina*, *Methanotherix* genus and furfural and sulfates catabolized bacteria (Kossmann *et al.*, 2007). *M. barkeri* is the most popular species of *Methanosarcina* genus and produce methane anaerobically through different metabolic pathways.

#### ***Deinococcus radiodurans***

*D. radiodurans* is (known polyextremophile) an extremely tolerant bacterium isolated in highly radioactive and extreme environmental conditions such as cold,

dehydrative, vacuum, acidic, alkaline, high radioactive. By using *IrrE* gene from *D. radiodurans*, researchers are creating more durable recombinant species to use in biofuel production. Although the *E. coli* and *D. radiodurans* are quite different organisms, the *IrrE* gene protects *E. coli* from against oxidative, osmotic and thermal shocks stresses (Pan *et al.*, 2009). Recently, researches have shown that the *IrrE* gene also confers improved *Z. mobilis* cell viability, abiotic stress tolerance and ethanol production (Zhang and Lynd, 2010). Although it is not used directly in the synthesis of biofuels, with its resistance genes that transferred to other organisms, contribute to more efficient biofuel synthesis.

#### ***Mariprofundus ferrooxydans***

*M. ferrooxydans* acquires energy by oxidizing reduced iron, resulting in the formation of iron oxides, in the presence of oxygen. Reduced iron serves as the electron donor and can be oxidized from several compounds, such as FeCl<sub>2</sub>, FeS, FeSO<sub>4</sub>, FeCO<sub>3</sub>, (SO<sub>4</sub>)<sub>2</sub> and Fe(NH<sub>4</sub>)<sub>2</sub> (Emerson *et al.*, 2007). The University of Minnesota team has studied on *M. ferrooxydans* in developing project called "electrochemical cultivation". If the process could be advanced and controlled, it could be used in the future as a way of storing electricity generated by wind or solar power through using *M. ferrooxydans* to make biofuel.

#### ***Dunaliella salina***

*D. salina* is a unicellular green alga found in environments with high salt concentration and has a high tolerance to temperature and light. This microorganism is quite easy to cultivate and has a relatively high growth rate and lipid content (Tang *et al.*, 2011). In a study on *D. salina*, the properties of biodiesel fuel described by its kinematic viscosity, cetane number, oil stability and oil density were discussed. As a result of discussion, it is assumed that this species is suitable for biodiesel production in terms of its lipid structures (Fakhry and El Maghraby, 2013).

#### ***Rhodospseudomonas palustris***

*R. palustris* has the potential to be very useful because it can degrade and recycle several different aromatic compounds that make up lignin, the main constituent of wood and the second most abundant polymer on earth. For this reason, the bacteria may be useful in removing these types of waste from the environment. In addition, *R. palustris* converts N<sub>2</sub> into NH<sub>4</sub> and H<sub>2</sub>, which can be used as a biofuel (Larimer *et al.*, 2004). Genetically manipulated *R. palustris* purple non-sulfur bacterium is capable of producing 7.5 ml of hydrogen/liter of culture has been obtained, and initial engineering designs have been proposed (Gosse *et al.*, 2007).

**Table 1.** The list of microorganisms that contribute directly or indirectly to biofuel production.

Species	Group	Contribution to energy
<i>Saccharomyces cerevisiae</i>	Yeast	Producing bioethanol
<i>Sulfolobus solfataricus</i>	Archeabacter	Secreting cellulase for facilitating biofuel formation
<i>Yarrowia lipolytica</i>	Yeast	Accumulating high levels of lipids for biodiesel
<i>Zymomonas mobilis</i>	Bacterium	Producing bioethanol
<i>Clostridium thermocellum</i>	Bacterium	Converting lignocellulosic biomass directly into ethanol
<i>Clostridium thermohydrosulfuricum</i>	Bacterium	Converting lignocellulosic biomass directly into ethanol
<i>Clostridium acetobutylicum</i>	Bacterium	Producing acetone, butanol and ethanol by fermenting sugar sources
<i>Dunaliella tertiolecta</i>	Algae	Producing enzymes that convert biomass to biofuel
<i>Thermoanaerobacter thermohydrosulfuricus</i>	Bacterium	Producing bioethanol
<i>Thermoanaerobacter ethanolicus</i>	Bacterium	Producing bioethanol
<i>Thermotoga neapolitana</i>	Bacterium	Producing H <sub>2</sub>
<i>Trichoderma reesei</i>	Fungus	Secreting cellulase and hemicellulase facilitating biofuel formation
<i>Escherichia coli</i>	Bacterium	Consuming sugar and secretes engine-grade biodiesel
<i>Rhodobacter sphaeroides</i>	Bacterium	Producing H <sub>2</sub>
<i>Chlamydomonas reinhardtii</i>	Algae	Producing H <sub>2</sub>
<i>Dekkera bruxellensis</i>	Yeast	Producing bioethanol
<i>Klebsiella ozaenae</i>	Bacterium	Breaking down cellulose for facilitating bioethanol formation
<i>Pseudomonas aeruginosa</i>	Bacterium	Breaking down cellulose for facilitating bioethanol formation
<i>Metarhizium anisopliae</i>	Fungus	Producing lipase directly used in transesterification
<i>Aspergillus oryzae</i>	Fungus	Producing lipase directly used in transesterification
<i>Chromobacterium viscosum</i>	Bacterium	Producing lipase directly used in transesterification
<i>Chlorella protothecoides</i>	Algae	Containing high potential of energy rich oil used for biodiesel
<i>Chlorella vulgaris</i>	Algae	Containing high potential of energy rich oil used for biodiesel
<i>Spirulina platensis</i>	Algae	Containing high potential of energy rich oil used for biodiesel
<i>Botryococcus braunii</i>	Algae	Producing long chain hydrocarbons that can be converted to conventional fuels like fossil oil
<i>Methanosarcina barkeri</i>	Archeabacter	Producing CH <sub>4</sub> and CO <sub>2</sub>
<i>Methanotherix</i>	Archeabacter	Producing CH <sub>4</sub> and CO <sub>2</sub>
<i>Deinococcus radiodurans</i>	Bacterium	Containing resistance genes that transferred to other biofuel producing organisms, contribute to more efficient biofuel synthesis
<i>Mariprofundus ferrooxydans</i>	Bacterium	By using this bacteria to make biofuel from electricity (not yet in practice)
<i>Dunaliella salina</i>	Algae	Containing energy rich oil used for biodiesel
<i>Rhodospseudomonas palustris</i>	Bacterium	Producing H <sub>2</sub>

**CONCLUSION**

Due to the fact that fossil fuels are environmentally harmful, expensive, difficult to obtain and exhausted, in recent times, biofuels including bioethanol, hydrogen,

biogas, biodiesel, biometanol, biobutanol, attract more attention. As highlighted in the above table, there are many microorganisms directly or indirectly involved in the synthesis of biofuels (Table 1). Most of the listed bacteria,

algae, fungi and yeasts in the table, for example, *S. cerevisiae*, *Z. mobilis*, *T. ethanolicus*, *E. coli*, *M. barkeri*, *Methanotrix*, *T. neapolitana*, *C. reinhardtii*, *C. vulgaris*, *S. platensis* are very commonly used in the biofuels facilities. The functions of these microorganisms are usually producing acetone, butanol, ethanol by fermentation, stored high potential of energy rich lipid used for biodiesel, producing cellulase for degradation of starch to facilitate biofuel production, converting lignocellulosic materials directly to ethanol, producing enzymes that breaking down biomass to biofuel, consuming sugar and secretes engine-grade biodiesel, secreting lipase directly used in transesterification, producing CH<sub>4</sub>, H<sub>2</sub> and CO<sub>2</sub> gasses. On the other hand, some of them such as *M. ferrooxydans*, *D. radiodurans*, *B. braunii* are very unusual with their interesting contributions to biofuel formations, making biofuel from electricity (not yet in practice), containing resistance genes that transferred to other biofuel producing organisms, contribute to more efficient biofuel synthesis, producing long chain hydrocarbons that can be converted to conventional fuels like fossil oil, respectively.

This collection work summarizes functions of some of the engineering microorganisms and gives a brief overview of how they can be functioned in biofuels fields and which biofuels can be produced (Table 1). In recent times, there seems to be more studies ongoing in the field of genetic engineering of microorganisms, however there are not enough and noteworthy studies on yeast, bacteria, alga which are involving in biofuels production. In spite of the large efforts that have been made in genetic engineering, there are still no recombinant microorganisms that achieve high biofuels yields. However, we also have the belief that there will be a superior recombinant strains with genes to be taken from microorganisms resistant to difficult conditions and genes derived from microorganisms with high biofuel production. So that biofuel synthesis will come to a much better extent.

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