

Microbial bio-fuels: a solution to carbon emissions and energy crisis

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1. ABSTRACT

Increasing energy demand, limited fossil fuel resources and climate change have prompted development of alternative sustainable and economical fuel resources such as crop-based bio-ethanol and bio-diesel. However, there is concern over use of arable land that is used for food agriculture for creation of biofuel. Thus, there is a renewed interest in the use of microbes particularly microalgae for bio-fuel production. Microbes such as micro-algae and cyanobacteria that are used for biofuel production also produce other bioactive compounds under stressed conditions. Microbial agents used for biofuel production also produce bioactive compounds with antimicrobial, antiviral, anticoagulant, antioxidant, antifungal, anti-inflammatory and anticancer activity. Because of importance of such high-value compounds in aquaculture and bioremediation, and the potential to reduce carbon emissions and energy security, the biofuels produced by microbial biotechnology might substitute the crop-based bio-ethanol and bio-diesel production.

2. INTRODUCTION

During 1990-2013, the primary energy demand of the world was increased by 55% to

13,560 million tons and is projected to grow about 45% by 2040 (1). According to US EIA (2), worldwide consumption of energy derived from fossil fuels will grow about 177 quadrillions British thermal units (BTUs) in the year 2040. If the fossil fuels had kept the same share the CO₂ emissions will increase from 32.3 billion metric tons in 2012 to 35.6 billion metric tons in 2020. To cope up this energy-related CO₂ emissions, the dependence on fossil fuel should be reduce before arriving vulnerable consequences of climate change. Recently in 2016, all the nations gathered and agreed to keep a global temperature this century well below 2.0 °C. Some largest carbon emission nations such as China, US and India have also started to promote renewable energy in great amount. This can be considered as a better approach to reduce the fossil fuel consumption to minimize the carbon emissions.

The crop-based biofuels could also be another option which can help to provide us a clean-green environment as well as energy security. Currently, crop-based biofuels such as ethanol and biodiesel, successfully used in many countries, helps in the reduction of carbon emissions. However, crop-based biofuels have their limitations as they may influence

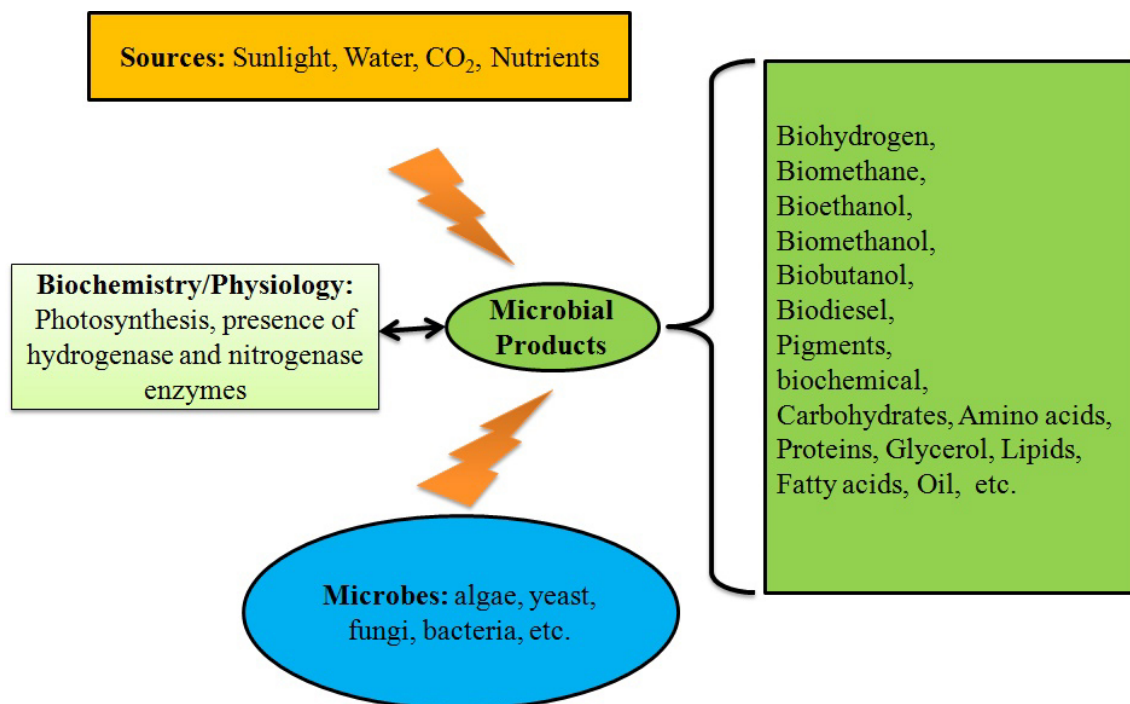


Figure 1. Microbes in biofuels and bioenergy production.

farmers to produce oleaginous crops instead of cereals that could create a risk for food security. There is a less surety that the crop-based biofuels can really help in reduction of carbon emissions, and at the same time it is assumed that they can contribute more carbon emissions by promoting large scale mechanized monocultures (9). This requires agrochemical inputs and machinery, an overall increase in CO₂ emissions is more likely to be end of biofuel producing crop cultivation. Microbial biofuels could provide us greater advantage over crop-based biofuels in terms of less requirement of arable land for food production and increasing harvesting related carbon emissions (4). This review highlights the need of microalgae as alternate energy resource that can fulfill the current and future energy demand and food security in a sustainable manner to the common people.

3. MICROBES IN BIOFUELS PRODUCTION

Microbes are tiny architects, play a crucial role in recycling and nurturing the life of Earth (5, 9). They produce, accumulate and secrete some biologically active molecules such as sugars, lipids, amino acids and phytohormones that maintain the soil viability and plant growth (10, 18). They could be used in the production of next-generation biofuels such as bioethanol, biodiesel and biomethane (19, 20) (Figure 1). This could be achieved through conversion of lignocelluloses into ethanol by bacteria and fungi; alternatively, through CO₂ conversion into

biomass by microalgae; or through the use of methane generated from landfill in to biofuels production. These substrates can be directed to the biosynthetic pathways of various fuel compounds and optimize biofuels production by engineering fuel pathways and central metabolism (21).

Microbes like yeast are the efficient fermenting organisms that can be used to produce ethanol from biomass for long duration. Some microbes like cyanobacteria can accumulate large amount of lipids that could be useful in production of biodiesel. Furthermore, though microbes may also release gases as by-products such as hydrogen, which could be very useful to use as gaseous biofuels and may be alternate to natural gas. Further, several microbes can be used as fuel cell to produce electricity; that can be useful in the development of batteries. The electrons are produced during microbial oxidation of various substrates; which then transfers to other electrode to produce electricity.

3.1. Bioethanol

Currently cane molasses or enzymatically hydrolyzed starch (22, 23) is primarily used for industrial ethanol production. Yeasts like *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, etc. are known as preferred microbes for large-scale ethanol production. Bacterium *Zymomonas mobilis* is used in ethanol production and has more capability to take more

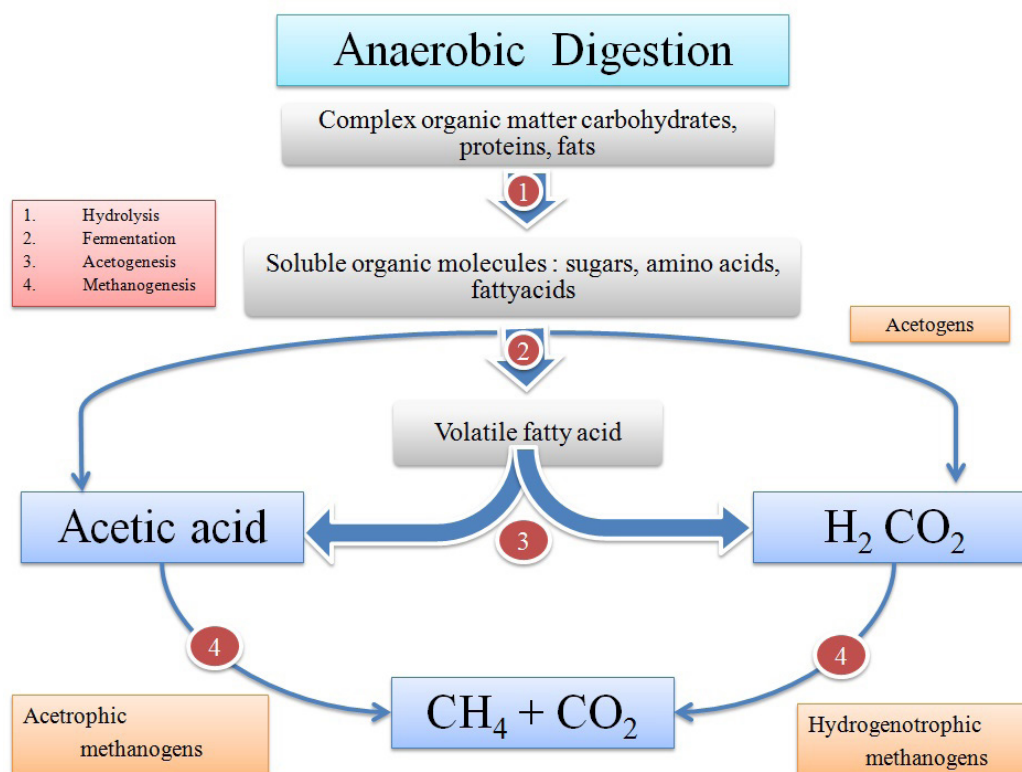


Figure 2. Ethanol production from various biomass feed-stocks.

sugars and produce more ethanol rather than yeasts. But while using food crops for ethanol production could be ethically unfair when billions of peoples in developing countries facing hunger problem creates a negative impact on agricultural sector in respect to crops which are harvested solely for their energy value (24, 25). So future microbial biofuels trends in ethanol production from lignocellulosic (non-food) materials like straw, husk, saw dust and wood chips which abundantly available as agricultural waste products (26) (Figure 2). The conversion of cellulosic biomass into ethanol requires additional processes like pre-treatment, hydrolysis, saccharification and fermentation. However, as the enzymatic hydrolysis reaction of cellulose is about two times slower than the average ethanol fermentation rate with yeast, which depicts a theoretical gap in simultaneous saccharification of cellulosic biomass and ethanol fermentation (27). Hahn-Hagerdahl *et al.* (27) also was concerned about that total cellulosic biomass to be fermented, not only all sugars in cellulosic biomass. Lynd *et al.* (28) and Demain *et al.* (29) proposed that thermophilic bacterium *Clostridium thermocellum* would be better option regarding hydrolysis and fermentation of cellulosic and hemicelluloses biomass. Algal (microalgae and cyanobacteria) biomass could be sustainable option for ethanol production (30) because it consists of high amount of polysaccharides i.e. starch, sugar and cellulose that could generate

greater amount of ethanol. It has been found that in case of microalgae, their carbohydrate content could be reached up to 70 % in specific conditions (31, 32).

3.2. Biodiesel

Biodiesel is a mono-alkyl ester of fatty acids and produced by the trans-esterification of vegetable oil (sunflower, rape, soybean and palm) in the presence of a catalyst by petrochemically derived methanol (33). Instead of using vegetable oil, microbe such as cyanobacteria could be used for biodiesel production due to their high lipid contents. Another economical advantage using cyanobacteria to biodiesel production is that they do not need arable land for cultivation as required for oleaginous crops (34).

Other microbes such as *Mucor circillienous*, *Mortierella isabellina*, etc. are also capable to store high lipid contents (35, 36). A number of study has been carried out regarding lipid accumulation by oleaginous yeasts on different substrates such as industrial glycerol, sewage sludge, whey permeate, sugar cane molasses and rice straw hydrolysate (37,40). In biodiesel production from vegetable oil, some waste products are also released such as glycerol; which could be useful for producing other chemicals including alcohols through microbes (41, 42). Some bacteria such as *Klebsiella*, *Enterobacter*, *Clostridium*, etc.

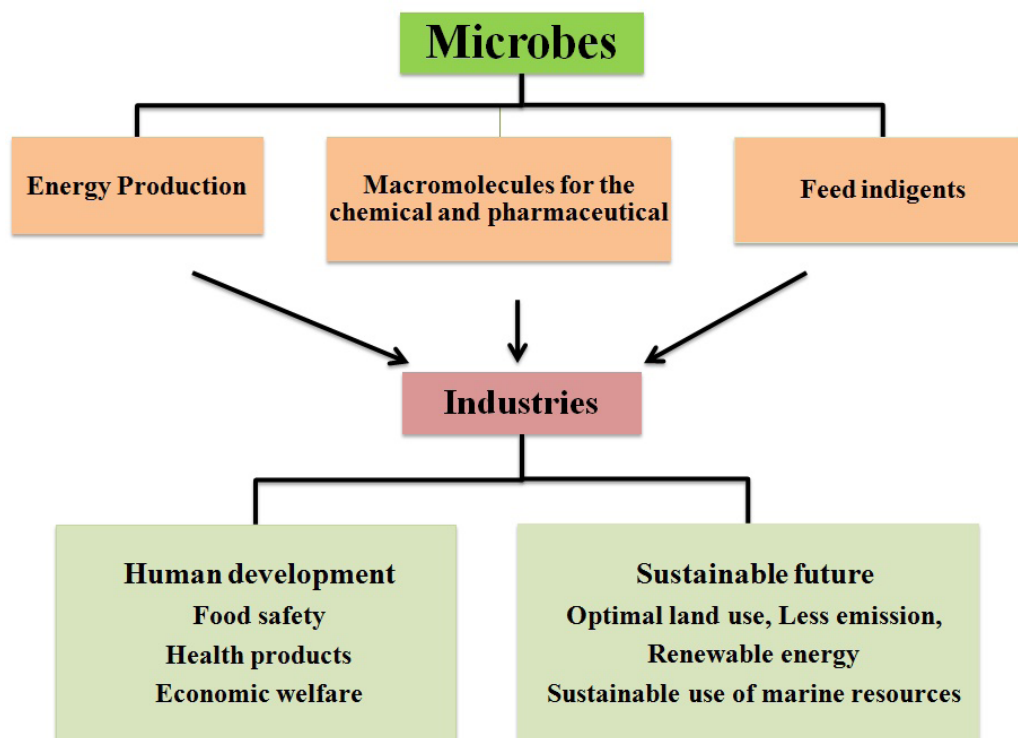


Figure 3. Microbial processes involved in bio-methane/biogas production.

were investigated for the production of alcohols 1, 3-propanediol, 2, 3-butanediol, butanol and others from glycerol (43, 45). Dharmadi *et al.* (46) and Durnin *et al.* (47) demonstrated that *E. coli* can convert glycerol to ethanol anaerobically as well as aerobically. In case of conventional biodiesel production, presently microbial processes are not involved; however, alcohols from microbial fermentations such as ethanol, propanol and butanol can be used instead of methanol needed for trans-esterification of vegetable oil (22). Even a mixture of alcohols characteristic of acetone–butanol fermentation could be used (48). The inclusion of biologically fermented ethanol and butanol will not pose technical problems. The use of enzymes or biological systems in trans-esterification is to be developed.

3.3. Biohydrogen

Biohydrogen can be produced by cyanobacterial bio-photolysis of water or by photo-fermentation of organic substrates from photosynthetic bacteria (49). *Cyanothece* sp. ATCC 51142- a cyanobacterium has been capable to generate high levels of hydrogen under aerobic conditions (50) and has been reported that a wild-type *Cyanothece* sp. 51142 can produce hydrogen at rates as high as 4651 mol/mg of chlorophyll/h in the presence of glycerol. Wu *et al.* (51) stated that biohydrogen also produced

by anaerobic organisms such as acidogenic bacteria through dark fermentation from organic substances and which could be the better strategy for reducing excess organic waste. Thermophilic microorganisms such as *Caldicelluliruptor saccharolyticus* or *Thermotoga elfii* are also known for high hydrogen yields (52, 55). These fermentations can be operated in liquid phase with immobilised cells or by enabling the formation of self-flocculated granular cells or sludge to prevent washout of the hydrogen-producing cells. However, microbial biohydrogen production is not yet reached to that level, which it could be an economically viable approach as well as there is a concern also regarding amount of hydrogen production that results far more behind expectations.

3.4. Biomethane or Biogas

Biomethane or biogas constituted about 50-75% CH₄; 25-45 % CO₂ and it are produced through anaerobic digestion and fermentation of variety of feed stocks mainly lignocellulosic (51) (Figure 3). This process completed in four steps (22) as listed:

1. Hydrolysis: The insoluble organic compounds like polysaccharides, proteins and fats hydrolyzed in to monosaccharides, amino and fatty acids. In this process, anaerobic bacteria

such as *Streptococcus* and *Enterobacterium* are involved.

2. Acidogenesis: The fermentation of these products into mainly acetic, propionic and butyric acid, carbon dioxide and hydrogen, alcohols and other minor compounds.
3. Acetogenesis: The production of acetic acid and carbon dioxide by Acetogens such as *Syntrophomonas*, *Syntrophobacter*.
4. Methanogenesis: Up to 70% (v/v) CH₄ and 30% CO₂ and the by-products NH₃ and H₂S by methanogens such as *Methanobacterium*, *Methanococcus*, *Methanosarcina*, etc.

Currently, about 70% of the organic matter in biomass is converted to CH₄ and CO₂ and which can be further improved through improvement in biogas technology to increase production efficiency. To increase production efficiency, maximum breakdown of biomass would be enhanced at hydrolysis stage. The steps like hydrolysis and acetogenesis/methanogenesis could be allowed at different optimized pH and temperature conditions. Thermophilic bacterium can be used to enhance biomass hydrolysis over traditional mesophilic bacteria used in biogas production.

3.5. Microbial fuel cell (MFC)

The microbial fuel cell uses an active microbe as catalyst in an anaerobic anode compartment instead of metal as a catalyst in a typical fuel cell (56). In MFCs, there are two chambers, i.e. anode and cathode, which are separated by a proton exchange membrane (PEM) (57). The active microbe (biocatalyst) in the anode chamber generates electrons and protons through oxidation of organic substrates (58); the protons moved to the cathode chamber through the PEM; while the electrons are transported through the external circuit (59). Sharma and Li (60) stated that protons and electrons are reacted in the cathode chamber along with parallel reduction of oxygen to water. There are some microbes such as *Geobacter*, *Shewanella*, *Pseudomonas*, *Clostridium*, *Desulfuromonas*, etc. are commonly used into MFCs for electricity production and they are able to oxidize acetate, ethanol, lactate and butyrate or propionate as substrate (61).

To exploit MFCs, a number of aspects such as electron transfer mechanisms, enhancing power outputs, reactor developments and applications are needed to study in more details for the further improvements. However, MFCs are facing challenges such as low levels of power density, scale-up feasibility, the high cost of component materials and large internal resistance which could be a sustainable approach for renewable energy production without energy loss (62).

4. CHALLENGES AND TRENDS IN COMMERCIALISATION OF MICROBIAL BIOFUELS

The production of microbial biofuels is alternative sources to fossil fuels as biofuels derived from microbes are not economically competitive. One way to overcome this bottleneck is the use of microorganisms to transform substrates into biofuels and high value-added products. At the same time taking advantage of an integrated process, the various microbial biomass components can be exploited for the production of products. In this way, it is possible to maximize the economic value of the whole process with the desired reduction of the waste stream produced. It is expected that this integrated system will make the biofuels production more economically sustainable and competitive in the near future. So, investigation on integrated microbial processes (based on bacteria, yeast and microalgal cultivations) and developing innovative experimental tools are required for microbial biofuels production (63). The main commercial challenges are concerned with integration into existing value chains and funding difficulties. To overcome these challenges, multiple stakeholders need to play an active and important role in promoting integrated microbial based technologies in order to develop an economical and more viable option. The sustainability challenges of must be ensured to the implementation of integrated microbial processes that may fulfill the environmental–socio–economic criteria and affordable biomass production. If all these targets are achieved, the future of integrated microbial processes seems to be promising and is expected to contribute to the satisfy the growing demand of energy sources, the replacement of fossil fuels and the production of a wide range of bio-based products (Figure 4) of much more of commercial interest (63).

5. ECONOMIC VIABILITY OF MICROBIAL BIOFUELS

Biofuels like biodiesel as well as bio-ethanol represent a secure, renewable and environmentally safe alternative to fossil fuels and their economic viability is a major concern. Bio-refinery model are advantageous if the conversion of by-products or waste streams generated during biofuels production. The implementation of bio-refineries has been proposed as a means to increase the economic viability of the biofuels industry (64). The higher revenue from the co-product, which benefits itself from the economies of scale available in a large biofuels plant improve the economics of biofuels production. The use of anaerobic fermentation to convert abundant and low-priced glycerol streams generated in the production of biodiesel into higher value products represents a promising route to achieve economic viability in the biofuels industry.

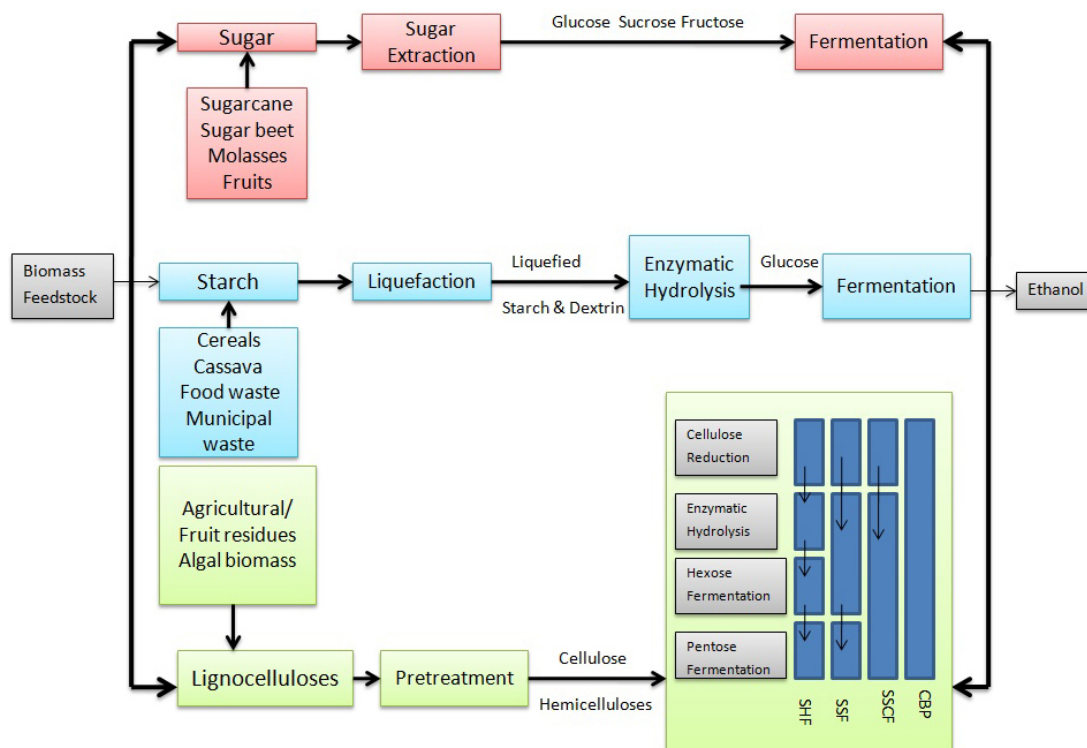


Figure 4. Microbial mediated biofuels production and its contribution in sustainable industrial productions.

6. MICROBIAL BIOFUELS PRODUCTION: OPPORTUNITY FOR SUSTAINABLE PRODUCTION OF BIOACTIVE COMPOUNDS

Microbes in general, are found all over the globe and in every ecological niche conceivable. They, therefore, have unique properties to help them survive even in adverse conditions of various extreme ecosystems. These unique attributes are brought about by changes in their macro- and micro-molecular constituents in the microbial cell which are formed under the stressed situations. These unique metabolites often have special properties and are considered as bioactive compounds in addition to the macro-molecules the microbes generally have numerous species out of which only few of them have been identified and studied. Hence, there is a huge unexplored microbial resource available to be exploited by the pharmaceutical industry. Microbes are known to produce various therapeutically effective bio-compounds that can be obtained from the biomass or released extracellular into the medium (65, 66). These microorganisms contain many bioactive compounds such as proteins, polysaccharides, lipids, vitamins, enzymes, sterols and other high-value compounds with pharmaceutical and nutritional importance that can be employed for commercial use (67).

Bioactive compounds from microbes can be obtained directly from primary metabolisms, such as

proteins, fatty acids, vitamins and pigments or can be synthesized from secondary metabolism. Such compounds can be used for the purpose of anti-fungal, anti-viral, anti-algal, anti-enzymatic or antibiotic actions (68). Many of these compounds (cyanovirin, oleic acid, linolenic acid, palmitoleic acid, vitamin E, B12, β -carotene, PC, lutein and zeaxanthin) have been antimicrobial, antioxidant and anti-inflammatory properties, with the potential for the reduction and prevention of diseases (69, 72). In most of the microbes, the bioactive compounds are accumulated as biomass however, in some cases; these metabolites are excreted into the medium, known as exometabolites. Microbial extracts contain compounds such as carbohydrates, proteins, minerals, oil, fats, polyunsaturated fatty acids as well as bioactive compounds such as antioxidants (polyphenols, tocopherols, vitamin C and mycosporine-like amino acids) and pigments, such as carotenoids, chlorophylls and phycobilins. These compounds possess antibacterial, antiviral, antifungal, antioxidative, anti-inflammatory and antitumor properties.

A number of investigations on bioactive compounds from various microbial agents have led to the identification of anti-microbial, anti-viral, anti-coagulant anti-enzymatic, antioxidant, anti-fungal, anti-inflammatory and anti-cancer activity (73, 74, 67). Recent studies are based on the extraction of bioactive compounds from these microbes that produce

intracellular and extracellular metabolites with potential biological activities, such as anti-bacterial, anti-fungal, anti-viral, anti-tumor, anti-inflammatory, antioxidant, anti-malarial, herbicidal and immunosuppressant effects (75, 76). The pharmaceutical importance of microbes is attributed to its medicinal properties and reflects ample experimental evidence of its anti-tumor, anti-coagulant, anti-bacterial, anti-oxidant and anti-hyperlipidemia effects in addition to a hepatoprotective property and the immune-stimulatory activity of enzymatic protein hydrolyzate (77, 81). Many antioxidant compounds are thought to be responsible for microbial functional activities. Antioxidants such as lutein, α -carotene, β -carotene, ascorbic acid and α -tocopherol have been identified active against free radicals. In general, several microbial species are considered as rich source of antioxidants with potential applications in pharmaceuticals, food and cosmetics (80). Antioxidant compounds, such as dimethylsulfoniopropionate and mycosporine amino acids have been isolated from microbes and are potent chemical blockers of UV radiation (82). In addition to these compounds, pigments, lipids and polysaccharides with antioxidant activity are also being found in microbes.

Bioactive metabolites of microbial origin are of special interest in the development of new products for pharmaceutical, cosmetic and food industries. Further research should be conducted to isolate and identify the new bioactive compounds to and to verify their beneficial effects for humans, their degradability when released into the environment and their effects. Recently, certain scientists have initiated to tap the enormous biological resource and physiological potential of microbial species growing in all ecological niches. In recent years, innovative processes and products have been introduced in microbial biotechnology. One can expect that the future trends in the involvement of microbial utilization in the pharmaceutical industry adapted to the autecological demands of microbial species and in application for microbial biomass, valuable substances and ecological services.

7. INTEGRATED MICROBIAL BIOFUELS PRODUCTION USING WASTEWATER TREATMENT PLANTS FOR ALGAL BIOMASS

Integration of algae cultivation with wastewater treatment is considered as one of the most promising routes to produce bio-energy and bio-based by-products in an economically and environmentally sustainable way (Figure 5). Wastewater is rich in nitrogen and phosphorous, which can use for the large scale cultivation of algal biomass (including microalgae and cyanobacteria) for sustainable biofuels production (83).

Municipal wastewater possess good amount of nitrogen, phosphorous and other essential nutrients such as trace metals Fe, Cu, Mn and Zn; which may be required to support the algal growth. Zhou *et al.* (84) examined growth and lipid content of *Auxenochlorella protothecoides* UMN280 and their wastewater nutrient removal efficiency using concentrated municipal wastewater as a culture media. *A. protothecoides* removed total nitrogen, total phosphorus, chemical oxygen demand (COD) and total organic carbon (TOC) up to 59%, 81%, 88% and 96%, respectively; with high growth rate, high biomass productivity (269 mg/L/day) and high lipid productivity (78 mg/L/day). It is also reported that the industrial wastewaters are generally considered unsuitable for algae cultivation due to presence of high toxic compounds. Because cyanobacteria and micro-algae are photoautotrophic and some also fix atmospheric nitrogen, their use for decontamination of polluted water systems can be a very effective tool for waste-water treatment. Chinnasamy *et al.* (85) reported that two freshwater microalgae *Botryococcus braunii* and *Chlorella saccharophila* and a marine alga *Pleurochrysis carterae* are capable to grow successfully in carpet mill effluent. Wu *et al.* (86) investigated nitrogen and phosphorus assimilation and lipid production of microalgae *Chlamydomonas* sp. TAI-2 and found that *Chlamydomonas* sp. TAI-2 able to remove 100% $\text{NH}_4^+\text{-N}$ (38.4 mg/L) and $\text{NO}_3^-\text{-N}$ (3.1 mg/L) and 33% $\text{PO}_4^{3-}\text{-P}$ (44.7 mg/L) and accumulate the lipid up to 18.4%. Current systems for introducing micro-organisms for bioremediation of polluted water are restricted to the implementation of bio-degradative microorganisms from reactors. Therefore, water reservoirs contaminated with synthetic chemicals remain largely untreated by remediation programs. On the basis of previous investigations, it may be proposed that the use of cyanobacteria and micro-algae can be considered for low cost, low maintenance remediation of pollutants in water ecosystems and can be used for bio-fuel production.

8. USE OF BIOTECHNOLOGY TO IMPROVE AND ENHANCE MICROBIAL BIOFUEL PRODUCTION EFFICIENCY

Microbial biotechnology appears to possess a high potential for biodiesel production because a significant increase in lipid content of microbes is possible through heterotrophic cultivation and genetic engineering approaches. Heterotrophic cultivation of lipid-rich microbes with fast pyrolysis leads to a high yield of bio-oils on a large scale. Research in genetic engineering coupled with advanced cultivation and downstream technologies benefit the future development of microbial biofuels production (87). The combination of biofuels production by microbial biotechnology with co-products contributes to the sustainability of biofuels results less impact on natural resources and biodiversity.

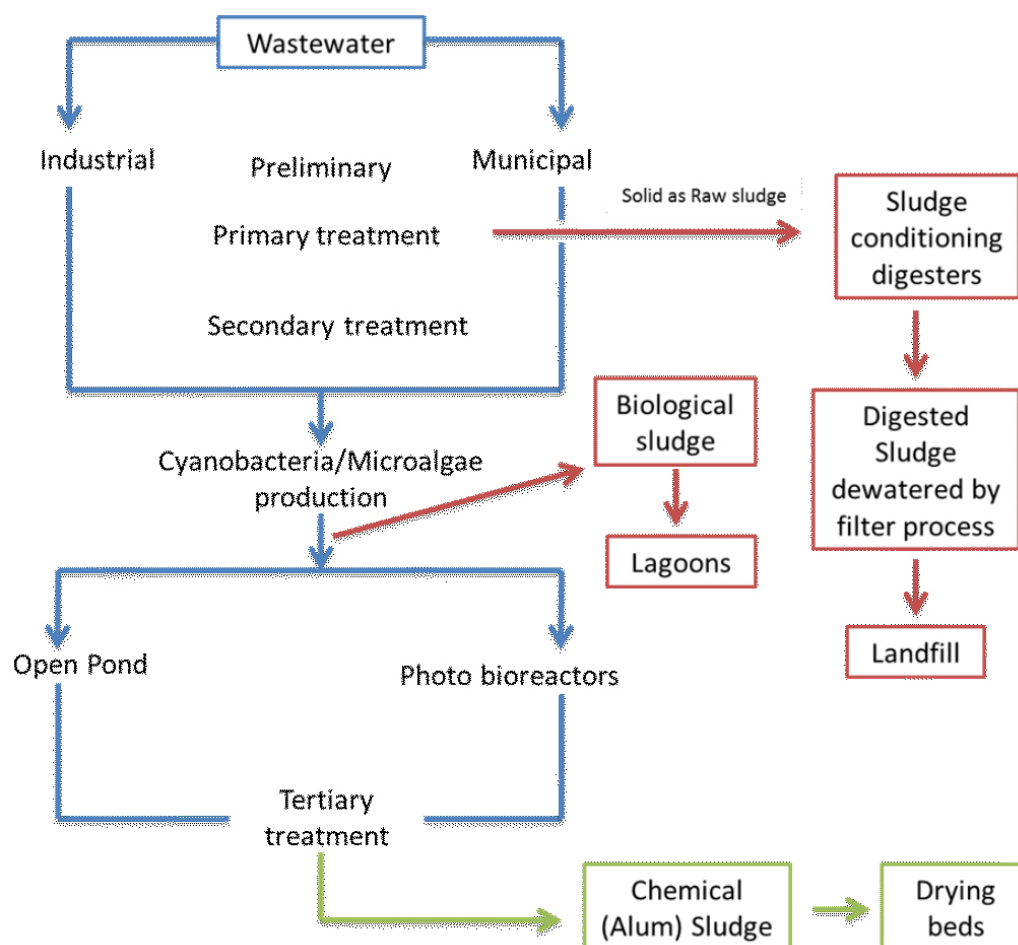


Figure 5. Integrated microbial biofuels production using wastewater treatment plants for algal biomass.

The integration of all the components of the uses of microbes i.e. high-value compounds, aquaculture, and bioremediation coupled to the production of biofuels play an important role in the production of biofuels from microbial sustainability. This review presents harvesting technologies with a focus on microbial biofuels production resulting different microbial metabolisms, cultivation systems, and biomass harvesting methods on microbial biomass and oil production to provide useful information to help future development of efficient and commercially viable technology for microbial biofuels production. Worldwide, considerable research progress has been achieved in the area of microbial and biological mediated bio-fuel technology. It has also been demonstrated and proven that this technology can be the very effective and potential means for alternative source of future energy. However, the technology needs further improvement for its better exploitation under sustainable bio-energy development programs. Cyanobacteria and microalgae are excellent model systems that can provide the biotechnologist with novel genetic constituents and bioactive chemicals with multipurpose use in wastewater treatment and source of green energy. Current

and future progress in our understanding of microalgae and cyanobacterial diversity, colonization ability, mechanisms of interactions, formulation and application could facilitate their development as the reliable components in management of crisis of energy and foods.

9. CONCLUSIONS AND OUTLOOK

Based on current knowledge, the usage of microbes is being considered as an attractive feedstock for biofuels production. Several microorganisms have been discovered to produce biofuels efficiently with the available technologies are:

1. Genetic engineering of cyanobacteria to enhance the hydrogen production (88).
2. Optimization of hydrogen production and metabolic engineering for bio-fuels production in bacteria (89, 90).
3. Dark fermentation by bacteria to convert carbohydrates to bio-hydrogen and other biofuels.

4. Photo-biological methods to enhance the biohydrogen production by micro-algae.
5. Genetic engineering of the yeast to increase ethanol production by tolerating high alcohol concentration.
6. Genetic engineering of microorganisms that can ferment carbohydrates to increase bio-ethanol and bio-butanol production.
7. Screening and selection of efficient micro-algae to produce more lipid contents for bio-diesel production.
8. Fermentation of plant cell wall carbohydrates by yeast or other microorganisms to produce biofuels.

The production of biofuels achieved by several species of microbes and has the highest potential to produce alternative sources of energy. Presently, ethanol, alcohols, biodiesel, triglycerides, fatty acids, lipids, carbohydrates, cellulose and the biomass of organisms are considered as the major biofuels sources (91). Some species of green algae, such as *B. braunii* and *C. protothecoides*, also contain high levels of terpenoid hydrocarbons and glyceryl lipid, which can be converted into shorter hydrocarbons as major crude oil (92). Thus these green algae have great potential for the production of petroleum fuels such as bioethanol, triterpenic hydrocarbons, isobutyraldehyde, and isobutanol (93). Even genetic engineering of many bacteria species such as *Escherichia coli* and *Bacillus subtilis* also produced higher amounts of bioalcohol, isoprenoids and fatty acids derivatives. Additionally, some species of bacteria have unique properties may be exploited as source of biofuels. *C. acetobutylicum* and *C. beijerinckii* are used for the production of biofuels by acetone-butanol-ethanol fermentation (94). Some species of bacteria, such as *Bacillus* and *E. coli*, produce lactic acid and glutamic acids as a source of some chemicals (95) and several species of bacteria have the ability to produce ethanol. Genetic studies have shown higher amount of hydrogen production and lower amount of ethanol production in *Caldicellulosiruptor*, *Thermococcus*, *Pyrococcus* and *Thermotoga* species. This fact indicates the important role of gene regulation in the bacterial cell biochemical pathways and also relationship between these pathways and production of biofuels (88). The co-culture of *Thermoanaerobacter* species with cellulolytic organisms and popular microorganism *S. cerevisiae* found effective for ethanol production as well as third generation of biofuels is the production of biodiesel from microalgae (96, 97). Though the initial push focused on biofuels produced from cellulosic waste, economic and technical production challenges in this approach have led to the emergence of bacteria and algae-based biofuels as a more sustainable alternative. Therefore, further research in the development of novel upstream and downstream technologies in the benefits of commercial bio-fuel

production and environmental sustainability should be focused.

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Abbreviations: U.S. EIA: United States energy information administration; UNFCCC: United Nations framework for climate change; SHF: Separate hydrolysis and fermentation; SSF: Simultaneous saccharification and fermentation; SSCF: Simultaneous saccharification and co-fermentation; CBP: Consolidated bioprocessing

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