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HISTORICAL DEVELOPMENTS IN CARBON SOURCES, BIOMASS, FOSSILS, BIOFUELS AND BIOTECHNOLOGY REVIEW ARTICLE

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ABSTRACT

Since early human history and existence energy rich plants, wood and forest cellulosic material have been used for fire, light, heating, cooking and other daily activities. Fossil energy was the foundation of our modern society and industrialization since last two centuries, while exploration and exploitation of oil reserves and petrochemistry have largely shaped 20th century. Increasing concerns on environmental pollution, accelerated global warming, and global climate changes, continuing world's crude oil (fossil fuels) consumption and depletion, as well as energy security and energy crisis caused by daily burning large amounts of fossil fuels, led to the attraction, search and development of renewable, carbon-neutral, economically viable alternative energy sources, such as biofuels, slowly displacing petroleum fuels. In continuously growing human population reaching about 10 billion in 2050, various renewable energy sources are promoted and developed, to ensure rising energy demands in a world running out of fossil energy sources. Biofuels are produced from any kind of available biomass and categorized based on utilized carbon resources into first-, second- and third-generation. Nevertheless, biofuels' future outlook is though beset by uncertainty. Hereby, various issues and concerns related to fossils and renewable biofuels are described and analyzed in present review article.

Key word: Carbon sources, biomass, bioenergy, fossil fuels, biofuels, bioethanol, biodiesel, atmospheric carbon dioxide, potential future scenarios of biofuels, fermentation, biotechnology, industrial microbiology.

INTRODUCTION

Energy enables global economic growth and social progress around the world (Exxonmobil.com/energy outlook, 2010). During the last 120 years, societal technological progress has moved from horse and buggy to space flight (Letcher, 2013; Mohagheghi *et al.*, 2015), while fossil energy was the basis of our modern civilization, from moving automobiles to light bulbs (Li *et al.*, 2013). Exploration and exploitation of oil reserves and petro chemistry have largely shaped 20th century's industrial and societal developments (Van Maris *et al.*, 2006). Expanding population and greater living standard expectations have increased world's energy consumption and the detrimental environmental impact of fossil fuels, and of carbon dioxide on climate, reanalyzing the potential of sustainable and renewable plant-based biofuels (Jones and Mayfield, 2012; Sherkhanov *et al.*, 2016). Energy and food demand had steadily increased over the 20th century in parallel with the rapidly growing world population and industrialization, demanding high energy that majorly based on crude oil. Consequently, consumption of renewable biofuels rose exaggerating food and fuel shortages (Pimentel *et al.*, 2009; Tesfaw and Assefa, 2014). We consume daily large amounts of fossil fuels, in spite of increasing environmental problems (Sakuragi *et al.*, 2011) and climate changes. The rate of new fossil resource discovery to satisfy global oil consumption rate and to meet the energy needs has however ceased (Greene *et al.*, 2002), whereas natural resources and environmental quality are constantly declined, simultaneously with the rapidly growing world's population and energy demand (Abramson *et al.*, 2010). Following 20th century's dominating era of fossil fuels, world approached a new epoch of global alternative, renewable green energy resources, biofuels and bio products in ongoing 21st century and forth on, gradually substituting fossil fuels, to accomplish semantic issues, regarding energy security and independence, sustainability, climate and environmental protection, and rural development (Di Lucia *et al.*, 2012; Anastassiadis, 2016). With the advent of 21st century, an urgent need emerged for alternatives and clean sustainable bio production to an economy, predominantly depending on fossil resources (Van Maris *et al.*, 2006). World's politically and economically unstable hydrocarbon-based fossil fuels run inevitably out, steadily destabilizing and fluctuating energy demand and petroleum prices, revealing an intense worldwide interest in advanced, alternative, renewable and maintainable energy resources, carbon neutral biofuels, and clean technologies, in order to satisfy the rising global energy demand and to achieve environmental and economic sustainability (Pimentel and Pimentel, 2007; Anastassiadis, 2016). Apparently, a shift from main fossil-based toward a bio-based and carbon neutral economy is unavoidable.

Earth's population is continuously rising, challenging biotechnology to supplement mankind with commodity products and energy from renewable resources, instead of fossil resources. Humanity lives in an unprecedented historical turn point of slowly ending petroleum-based economy. An energy crisis appeared neglecting alternative energy sources for years, including biodiesel and bio hydrocarbons, bioethanol, methanol and bio butanol, methane, microbial fuel cells generating electricity, and microbial photosynthetic hydrogen production (Demain, 2009). World is facing a crisis of energy due to global economy and population growth, causing the rapid fossil fuel depletion (Ogawa *et al.*, 2015). Depletion of fossil fuels requests numerous small, simple and diverse fuel-free renewable energy resources like wind and solar, and biofuel referred to as soft energy path, continuously grown at high speed from a very low marginal base of total global renewable energy supply, to supply environmentally benign and politically and economically sustainable electric power systems (Popp *et al.*, 2014; Taylor *et al.*, 2016). Diminishing fossil fuels and increasing environmental concerns have stimulated the development of novel efficient and sustainable biofuels (Schuhmann *et al.*, 2012). Bioenergy was the major power and heat source prior industrialization, while economic expansion has largely been depending on fossil fuels since then, releasing large greenhouse gas quantities into atmosphere together with other human actions (Popp *et al.*, 2014). Bioenergy and biomass-derived renewable biofuels reemerged substituting fossil fuels, to expectably supply CO_2-neutral energy over time, to mitigate greenhouse gas emissions, effectively decrease atmospheric pollution and manage to treat tons of biomass waste (Johansson, 1993; Goldemberg, 2000; Faaij, 2006; Berndes, 2008; De Souza, 2013). Microbial production from renewable biomass by engineered microorganisms is a favorable alternative to petroleum-based fuels and chemicals (Sherkhanov *et al.*, 2016). Microorganisms have been broadly investigated for the manufacturing of ecological fuels and chemicals (Saini *et al.*, 2016). The future outlook of biofuels is though beset by uncertainty (Cadenas and Cabezudo, 1998). Historical developments of fuels and biofuels as well as existing microbial and chemical process technologies are described in this review to understand the needs and requirements for future developments as a reference to global energy and environmental issues.

Energy and bioenergy: Energy is a basic need of humanity and inevitable for human existence, as well as the lifeblood of modern societies, and socioeconomic and sustainable human development. World energy consumption has increased by 17 times last century, causing vast and serious atmospheric pollution owing to fossil-fuel combustion. Surprisingly, half of fossil fuel CO_2 emissions of last 200 years has been emitted within the last 30 years. Therefore, the interest in developing and use of alternative, renewable and potentially carbon neutral energy and bioenergy resources has greatly increased for net societal benefits. They are vigorously necessary to replace conventional petroleum transport fuels, including solid, liquid and gaseous fuels (Türe *et al.*, 1997; Hill *et al.*, 2006; Chisti, 2007; Van Vuuren *et al.*, 2009; Brennan and Owende, 2010; Gude *et al.*, 2013; Letcher, 2013; Talebian-Kiakalaieh *et al.*, 2013; Anastassiadis, 2016), which are depleting at an alarming rate (Ghimire *et al.*, 2015). Expanding world population, industrialization, and industrial prosperity have gradually risen global energy consumption and demand dramatically, assuming another 53% increase from 2008 to 2035, while conventional fossil fuels such as coal, oil and natural gas will continue to majorly supply the necessary energy (Conti *et al.*, 2011; Li *et al.*, 2014; Ghimire *et al.*, 2015). Global annual consumption of energy reached 4.1×10^{20} J in 2005, namely equal to an instant-annual average rate of consumption of 13×1012 W (≈13 trillion watts, or 13 terawatts), which will more than double in the middle of 21st century and more than triple by 2100, according to the intended growth of population and economy (Nault, 2005). Climatic alterations, increase of population and the depletion of fossil fuels would therefore require a larger playing role of renewable energy sources in the future. One third of global energy will essentially originate from wind, solar, and other renewable energy sources by 2050, according to two of the biggest oil companies in the world, the British Petroleum and Dutch Shell Royal (Li *et al.*, 2014).

Energy originates from many diverse sources and in many different forms, which are categorized in two principal types, namely the potential energy (mechanical, electrical, nuclear, chemical, and gravitational) that is stored in an object, and the kinetic energy (light, heat, motion, and sound) carrying out work (Energy4me, 2006-2014). Energy conservation is the alternate statement of “First Thermodynamics Law”, claiming that “energy can neither be created nor destroyed, but only transformed from one type to another” (Letcher, 2013). Primary sources of energy, directly generated from real resources, can be categorized into two different groups, namely renewable or nonrenewable. Secondary sources of energy are generated from primary sources, and serve to deliver, move, or store energy in a simply utilizable form, like hydrogen or electricity (Energy4me, 2006-2014). Current known sources of energy are divided into three broad types, namely fossil fuels (non-renewable), nuclear energy (non-renewable alternative) and renewables such as biodiesel, bioethanol (Demirbas, 2009). Less than 15% of global supply of primary energy is renewable, mainly wood fuel and hydropower in developing nations, whereas technologically advanced renewables like geothermal, solar and wind energy, share only a very low quantity of the entire supply (Lund, 2007). Being like a fusion reactor which burns since more than 4 billion years, the average star sun emits an incredible, inexhaustible stream of free solar energy onto Earth. Sun delivers sufficient energy in one minute to provide enough energy to the world for one year, and within one day more than present global inhabitants would need in 27 years, while three days of solar emission to the earth would provide so much energy, as has been stored inside of all of existing earth's fossil energy sources (Li *et al.*, 2014). Hence, sun energy necessary for the production of biofuels is abundantly available (Schenk *et al.*, 2008). Bioenergy, referring to the energy produced by photosynthetic organisms, is presently the sole source of renewable energy capable of providing fluid transportation fuels (Bilgen *et al.*, 2015). Lund (2007) referred in case of Denmark to the possibility of covering present energy requirements to 100% by a renewable energy system, crucially integrating flexible energy technologies, expanding amount of intermittent renewable energy, and designing solutions in existing energy supply system. Global potential to produce bioenergy from dedicated biomass plantations and all biomass sources in 21st century will cover only 15–25% of World's energy in 2050, providing between 130 and 270 EJ yr^{-1}, under sustainability constrains. An intensive cultivation of energy crops on large-scale will however threaten the biodiversity as well as the habitats of numerous endemic and endangered species (Beringer *et al.*, 2011). Denmark's dependence on oil decreased substantially from 92% in 1972 to 41% in 2007, whereas transportation sector will expectably account for almost all oil consumption by 2020 (Lund, 2007).

Fossils and fossil fuels: During the pass of millions of years, a part of global biomass has been captured and immobilized inside the Earth in form of fossils. Industrial revolution, industrialization, modernization and technological development have enormously increased the demand for energy and transportation fuels, and consequently CO_2 emissions on global level. The discovery, availability and use of fossil energy resources brought initially technological superiority, prosperity, wealth and wellness, as also strong historical, political and socio-economic conflicts to the world, increasingly causing ecological disasters and climate catastrophes, as well as serious socio-economic, political and ecological emigration and expatriation, semantic conflicts and strong confrontations on global level in historically recent times. Burning fossils and fossil fuels, strongly influenced and disturbed continuity, climate, as well as carbon and oxygen balances (CO_2, ozone), which has been harmonically established during the pass of millions of years without any human impact, influence and presence (Anastassiadis, 2016).

Since the industrialization, the steadily growing energy requirement has been mainly covered by unrenewable fossils, such as natural gas, oil and coal, which do not regenerate at sustainable rates (Wei *et al.*, 2013; Devarapalli and Atiyeh, 2015). Rapidly growing population, continuously increasing lifestyle ambitions and living standards have increasingly driven humanity’s insatiable desire for fossil fuels, while numerous of modern daily live materials are derived from petrochemicals, mainly fractions of crude oil, such as heavier oils and naphtha, beside natural gas (Roddy, 2013). Fossil fuels, comprising coal, natural gas and crude oil, play a critical role in today's world economy (Shafiee and Topal, 2008). World energy consumption of petroleum for fuel electricity, automobiles, and industrial processes has been estimated to 4.4 billion tons per year (Roddy, 2013). Road, sea and air transport, as well various industries including agriculture, civil engineering and construction depend almost completely on fossil fuels, while many railways, power generation systems and pumping units are using fossils and fossil fuels as well (Onion and Bodo, 1983). 30-year projections from 1990 to 2020 indicated a triple increase of automobile travel and therefore demand on fossil fuel, posing various serious energy and environmental problems. Half a billion of existing motor vehicles circulating on the roads in 2007 was more than 10 times higher compared with 1950 (Agarwal, 2007). Natural energy resources, fossil fuels, being a very important and integrated part of our civilization, culture, technologies, progression, lives and daily life, are continuingly shortened, depleted and significantly expensive, because of steadily and ever increasing human population and demand of fossil fuels. Therefore, energy requirement is continuously growing along with the industrial development and increasing population around the world (Demirbas, 2009). Fossils are also used to some extent to produce plastics, inks, bulk chemicals, synthetic fertilizers, and steel (Berndes, 2008; Anastassiadis, 2016). The rising price, insecure supply, and environmental worries about fossil fuels have overshadowed the industries based on oil (Saini *et al.*, 2016). World had consumed only about one eighth of its endowment of readily accessible conventional crude oil by 1973, however its supply has been unable to keep up with demand beyond, contradicting oil industry reports (Campbell and Laherrère, 1998). Excessive use of fossils cannot deal anymore with the growing demand of energy worldwide and ecological measures preventing Earth’s overheating (Ledesma-Amaro *et al.*, 2015). Independent estimates pointed to a steady drop of the production of petroleum which started in 2010, being unable keeping up with demand thereafter. Oil deposits are believed to completely finite by 2050 (Campbell and Laherrère, 1998), contradicting oil-industry reports that are strongly influenced by economic and political motives, which suggest that there will have been another 50 years' worth of cheap oil to sustain us. According to Shafiee and Topal (2008), fossil fuel reserves did not diminish during the last decades and predictions of running out did not substantiate.

Human activities have significantly increased atmosphere's carbon dioxide by about 40% over pre-industrial levels and of last 800,000 years and more than doubled the available to ecosystems biosphere's nitrogen, causing global warming and climate change (Brown *et al.*, 2014; Zhang *et al.*, 2016). Since millions of years must pass for the conversion of biomass into fossil fuels and coal, they aren’t renewable to emerge within a reasonable time frame and chronic scale, over which mankind can observe and use them. Hence, time lag between instantaneous CO_2 release from burning fossil fuels and its eventual incorporation into fossil biomass fuels can take millions of years (Aristidou and Penttilä, 2000; McKendry, 2002). Burning “old geological biomass” of fossil fuels produces “new CO_2”, depleting a non-renewable resource, accumulatively contributing to “greenhouse effect”. Alternatively, burning new biomass does not add new CO_2 to atmospheric balance, since CO_2 is reabsorbed by replanting harvested biomass, ensuring so a new growth-CO_2 cycle (McKendry, 2002).

Two kinds of important global issues and concerns have risen in recent years, specifically environmental and energy crisis. Environmental issue is the global warming, induced by intensively using fossil fuels resulting in increasing atmospheric concentrations of greenhouse gases, smog, and acid rain, ozone depletion, climate change world-over, steadily changing earth's heat balance, etc. (Pearson and Palmer, 2000). Early 1970s, OPEC, managing to corner 36% of market, decreased oil output, which resulted in an energy and oil crisis and a dramatic rise of oil prices, significantly sparking world's renewed eager interest in finding new energy resources as well as in the synthesis of biofuels and biomaterials and search for alternative fuels to replace petroleum, as a hot topic worldwide (Galbe and Zacchi, 2002; Ragauskas *et al.*, 2006; Huang *et al.*, 2010). From there onwards, steadily and ever increasing global crude petroleum oil prices have semantically affected domestic energy situations, as well as local society life, resulting into energy crisis. Therefore, renewable sustainable clean energy replacing fossil fuels with renewable biomass is necessary, to reduce CO_2 emission and atmospheric CO_2, the major driving force of global warming and climatic changes (Watanabe and Hall, 1996; Agarwal, 2007; Amin, 2009; Brennan and Owende, 2010). 99 million barrels of oil will be daily necessary in 2015 and 116 million in 2030, compared with only 84 million in 2005 (Leblond, 2006). Declining as also becoming more difficult to extract and process, the remaining global fossils, crude oil deposits, and petroleum fuels-derived fuels such as gasoline, diesel, or kerosene will become rarer and expensive, arising therefore the need for alternative replacement liquid fuels of whatsoever origin, consequently creating a strong market for biofuels, and other renewable energy resources. Nature and origin of potential alternative fuels are obviously of great importance, until cheap and abundant power becomes available, for example electricity generated by nuclear fusion and other means (Onion and Bodo, 1983; Gallagher, 2011; Lee and Lavoie, 2013; Srivastav *et al.*, 2014). Current energetic power structure is unreasonable, unmaintainable and uncertain for equity concerns, as well as due ecological, socioeconomic, geopolitical and strategic worries, and furthermore future outlook of biofuels is though beset by uncertainty. This has semantic and dramatic implications on global economy which is literally running on energy, and on development into near and far future (Cadenas and Cabezudo, 1998; Demirbas, 2009). Not only fossil carbon sources will become limited in near future, but there is also a growing skepticism, concern and pressure to renounce exploitation of environmental issues, a tendency which is also motivated by oil disaster accidents, happened in Gulf of Mexico and other areas.

Biomass, carbon resources, bioenergy and biofuels: Food, chemicals, and industry are confronted with a fast growing world population, increasing longevity and quality of life (Golberg *et al.*, 2016). Independently whose viewpoint on the future we decide to accept, the supply of fossil oil, natural gas and coal will be ultimately insufficient (Sheehan *et al.*, 2000). Petroleum sourced fuels as well as reliance on energy resources originated from fossils (millions of years to evolve) is politically (concerns about petroleum supplies, increasing energy imports, conflicts), environmentally (environmental consequences of fossil fuels, climate policy) and economically (high-energy prices, concerns about energy security and agricultural income, land use and land change) unsustainable. Moreover, it is yet a depleting source (e.g. depleting world reserves), strongly contributing to environment's greenhouse gas emissions, mainly CO_2, consequently resulting in global warming and dramatic climatic changes (Anastassiadis, 2016). A free from fossils maintainable progress can only be obtained with the changeover to the bio economy, which necessitates the discovery of novel low energy requiring technologies and practices, for sustainably converting biomass into useful products such as biofuels, biochemical, biomaterials, food, and feed, a collective term called bio refinery (Golberg *et al.*, 2016). A truly from oil independent society, utilizing renewables, would develop a maintainable industrial society and operative ecological managing (Ragauskas *et al.*, 2006). Biomass is biosphere's alternative carbon resource to million years' old fossil carbon, suitable for the production of necessary chemical intermediates by thermochemical (often dry feedstock) or biochemical (often wet feedstock) conversion processes (Roddy, 2013). Photosynthetic biomass formed by fixing atmospheric carbon dioxide is one of most abundant renewable and carbon neutral resources for biomaterials, including solvents, bio-derived plastics (e.g. polylactic acid, poly-trimethylene terephthalate), lubricants and fragrances, and of bioenergy, addressing several societal needs and supplying nearly 12% of world's energy by thermochemical and biological processes, especially production of hydrogen via dark fermentation (Ni *et al.*, 2006; Ragauskas *et al.*, 2006). Renewable agricultural residues and wastes, can be converted into liquid biofuels (Nigam and Singh, 2011). Microalgae are third generation biofuel feedstock, which grow on non-arable land and fix CO_2 very fast, and overproduce lipids, without competing with food or feed crops (Méndez-Vilas, 2010). Unprocessed primary biofuels like wood fuel are mainly used for the production of electricity, cooking or heating, while secondary biofuels like biodiesel and bioethanol used in vehicles and industry are categorized into first, second and third generation, depending on feedstock origin. Biofuels refer to liquid, gaseous or solid fuels generated from any organic matter (Méndez-Vilas, 2010; Anastassiadis, 2016).

Humans use biomass since very long times, nevertheless, the production of synthetic chemicals and energy often prefers fossils sources, because biomass processing and mostly traditional converting technologies are inefficient in terms of performance and power consumption (Golberg *et al.*, 2016). The development of alternative to fossil fuels energy sources is an urgent global priority (Rubin, 2008). Biofuels are derived from present-day renewable biological plant, microbial, animal and waste material (Aro, 2016). Photosynthetic plants and autotrophic microorganisms convert solar energy into chemical energy, setting biofuels apart from fossil fuels, which were created by ancient photosynthesis (Aro, 2016). Cell walls store sun energy in form of polymeric lignin, hemicellulose and cellulose (Rubin, 2008). Alternative to petroleum and chemicals renewable resources have been intensively investigated for decades (Martin, 2014). As fossil resources are diminished or depleted and unsustainable in ongoing 21st century, the need for discovering, developing, managing and using alternative renewable and sustainable energy and biofuels, and concomitantly carbon resources for biotechnological applications (fermentation technology), without affecting food and feed availability and accessibility, grows rapidly. Semantic rise in demand for conventional transport fuels, as well as unsustainability, uncertainty, and semantic interconnected ecological worries, together with lessening and depleted deposits of crude oil, emphasized the development of alternative renewable sources of energy. Following the era of fossil fuels' 20th century, the world entered a new era of global green energy, alternative energy resources, renewables and biofuels in the 21st century and forth on, gradually replacing fossil fuels (Anastassiadis, 2016). Biofuels reached unprecedented volumes over the last 15 years and became the biggest renewables that have been manufactured and used worldwide to replace fossil fuels, thus lowering releases of greenhouse gases and alleviating climatic alterations (Popp *et al.*, 2014; Aro, 2016). Biomass provides increasingly further renewables, heating energy, electric power, fuels, pharmaceuticals and feed stocks for green chemicals, while cellulosic biomass obtained from bioenergy crops will expectably have a considerable role in future's energy infrastructures (Popp *et al.*, 2014). Nevertheless, the effectiveness of renewable biomass generation is insufficient for fully replacing fossils (Aro, 2016). Sustainable and environmentally friendly renewable energy can be obtained from wind, water, geothermal sources, sunlight, and biomass (Devarapalli and Atiyeh, 2015). Despite enormous advances in solar photovoltaic generation of power, biofuels are yet of major significance in current societies (Aro, 2016). Bioenergy and biomass-derived renewable biofuels are emerging as an excellent new alternative source of energy, to substitute traditional fossil fuel-derived energy sources, produced from abundant renewable biomass. In the future, they are expectably supposed to offer huge quantities of CO_2 neutral energy and considerably over time, to mitigate greenhouse gas emissions, effectively decrease atmospheric pollution and additionally assist in the management of tons of biomass waste (Johansson, 1993; Goldemberg, 2000; Faaij, 2006; Berndes, 2008; De Souza, 2013; Wei *et al.*, 2013). Modern bioenergy, comprising all biomass and biofuel types, is the commercial production of energy from biomass for the production of power, heat and industry, or transportation fuels. The term green energy is alternatively used for renewable energy which is generated from environmentally friendly sources. Locally obtainable and accessible, sustainable and reliable as well as not polluting biofuels are liquid or gaseous fuels. They include various alcohols including bioethanol like sugar, cellulosic and grain ethanol, biobutanol and bio methanol, as well as bio crude, vegetable and pyrolysis bio-oils (thermal biomass conversion processes), biogas (methane), bio hydrogen, algal diesel and biodiesel, algal jet fuel, syngas liquids, hydrocarbons as well as green diesel (renewable petroleum-based diesel replacement) which is synthesized from biomass through the Fischer-Tropsch process. World's fluid transport fuels bioethanol and biodiesel which are derived from various types of biomass have the potential to substitute diesel and gasoline fuel. Renewable biomass sources, made from plant matter and residues, are converted through biochemical or thermochemical processes, such as pyrolysis, dilution with hydrocarbons (blending), emulsification, and transesterification (Balat, 2007; Demirbas, 2009). Biogas is another alternative clean form of energy derived from easily available raw materials by efficient technologies, reducing indoor air pollution and conserving forest resources, wildlife habitat and natural balanced ecosystem (Ghimire *et al.*, 2015). Bioenergy competes not only with fossil fuels but also with other renewable energy sources such as wind, solar and wave power, reducing the impact of energy production and use on global environment (McKendry, 2002). In contrary to other renewable energy forms, such as sun, tidal and wind, fluid biofuels can store solar energy in a directly usable form of mater for existing engines and transport infrastructure (Scott *et al.*, 2010).

Several threats, driving forces and opportunities have increased the emphasis on renewable energy, triggered the interest in biofuels, and have driven the worldwide efforts on search, development, investment and application of alternate, renewable clean resources of energy like biofuels, as an elegant outstanding unconventional solution to traditional fossil fuel-based energies, creating new opportunities for agriculture, worldwide, which can be produced from abundant supplies of renewable biomass. These include increasing ecological worries, coupled with shrinking crude oil deposits, the steadily and ever increasing global demand for energy and transportation fuels, instability of world oil prices and security of energy supply, rising and fluctuating prices of crude oil and natural gas, pressuring national and household budgets. Further reasons are dependency on fossil fuel reserves, predicted fossil fuel's shortage and rapid depletion in near future, as well as their unequally distribution and supply across the world. Other reasons are global political and economic dependencies, instability and commitment, the negative environmental like global warming, socioeconomic effects associated with that, and environmental issues and concerns about global climatic alteration due to the creation and emission of greenhouse gases, especially CO_2, resulting from burning fossils and reliance on fossil petroleum. Opportunities emergence for agrarians in developing as well as developed countries, opening new markets for existing (cassava maize, oil palm, soybean and sugarcane), or novel oily crops, vegetable oils and other sources (Jatropha, castor, rapeseed, candlenut oil, bintaro oil, nyamplung oil) (Budianto *et al.*, 2006; Ruth, 2008; Satyanarayana *et al.*, 2011; Yang *et al.*, 2012; Berni *et al.*, 2013; Wei *et al.*, 2013; Slingerland and Schut, 2014; Anastassiadis, 2016). New technologies are necessary to reduce oil dependency and to substitute diesel, gasoline, heavy distillates, jet fuel, and a series of bio-derived products and chemicals. World has steadily searched for alternative renewable resources of energy, gradually developed along with growing global demand and consumption, to substitute fossil fuels after 2030 (Leblond, 2006). Meanwhile, world's investigation and commercial technology progress of fluid transport biofuels run at a fast rate (Walker, 2011). Bioenergy supplies nearly 10% of world's energy (50 EJ/year) as major renewable energy source, while bioenergy will potentially deploy between 100 to 300 EJ in 2050 (Horn *et al.*, 2012). Projected global demand of primary energy by 2050 will expectably reach between 600 and 1000 EJ, in comparison to about 500 EJ in 2008 (Balat and Kırtay, 2010).

Unconventional fuels like bitumen and oil shale, fluid fuels derived from coal, methane obtained from methane hydrates, as well as secondary fuel hydrogen and biofuels can replace traditional fossil fuels like mineral oil and natural gas (Reijnders, 2009). Renewable and sustainable biodiesel derived from animal fat, crop and waste cooking oil, as well as bioethanol might replace fossil fuels for socioeconomic and ecological sustainability (Chisti, 2007). Moreover, biofuels like ethanol are biodegradable, less toxic, and less polluting compared with fossil fuels (Wei *et al.*, 2013). Replacing fossils with renewable biofuels may considerably accomplish future purposes, like security and independence of energy, environmental and climatic protection, as well as rural development (Di Lucia *et al.*, 2012). Economic and geopolitical factors, as well as uncertainty for uninterrupted supply and supply instability, rapidly increasing and fluctuating oil prices, as well as ecological worries emphasized and compelled the interest and urgent action of researchers, economists, politicians and policy-makers to look for technically feasible alternative renewable energy sources and indigenous substitutes, for example ethanol and biodiesel (Stephanopoulos, 2007; Srinivasan, 2009). Much attention has been given to biofuels by regional and national governments around the world, being promoted through policy decisions, especially in increasingly energy-hungry OECD nations, including US, Brazil, Colombia, EU and the Australian state of Queensland (Charles *et al.*, 2007). The need to support, promote, regulate and enhance generation and usage of cheap renewable biofuels derived from biomass feed stocks, replacing fossil fuels for economical and energy security reasons, has been reflected and set up in the political agendas and bioenergy policies of many countries (e.g. US, European Union, Brazil, China, India). They are aiming in developing reliable renewable energy sources, to ensure fuel security, to promote rural development, and to address climatic changes by reducing greenhouse gases emission. Emitted carbon during biofuel combustion has been recently removed from atmospheric carbon dioxide by newly developing plants (Granda *et al.*, 2007; Havlík *et al.*, 2011; Macrelli *et al.*, 2014). Major reasoning for such policies is declining of dependency on traditional fuels, particularly in oil importing and specifically developing nations (Havlík *et al.*, 2011), converting vegetable oils into biodiesel, to meet domestic demand or for export, in order to improve trade balances and save foreign currency (Srinivasan, 2009). Energy security and economics influence decisions and driving forces regarding biofuels (Granda *et al.*, 2007). Renewable energy like sun and wind energy was almost 50% of new added power capacity first half of 2014, which was more than twice of first half of 2013 (Huaman and Jun, 2014). Huaman and Jun (2014) projected a 2.1% growth of total demand for renewable sources for heat and electricity generation in 2014, while hydropower will sink by 4.2% and non-hydropower grow by 5.5%. Wind was projected to generate 4.6% of entire electricity in 2015. Furthermore, ethanol was predicted to produce 929,000 bbl/d in 2014 and 934,000 bbl/d in 2015, while biodiesel averaged 87,000 bbl/d in 2013 and was estimated to reach 80,000 bbl/d in 2014 and 84,000 bbl/d in 2015 (Huaman and Jun, 2014) (Figure 1 and Table 1). Countries like Thailand, Uruguay and Ghana could potentially lead the estimated 51-billion-liter industry of biodiesel, displacing about 4-5% of global petroleum-diesel (Srinivasan, 2009).

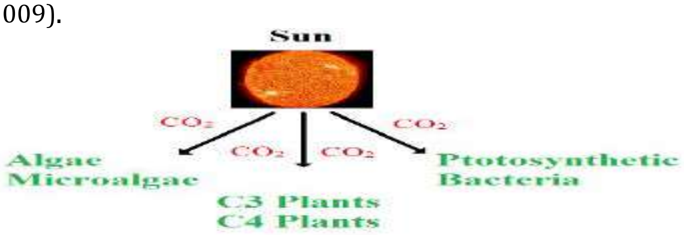


Fig. 1. Solar energy and biomass formation (Anastassiadis, 2016)

1st generation biofuels	2nd-generation biofuels	3rd –generation biofuels
Biodiesel	Lignocellulosic bioethanol	Biodiesel from algae, microalgae, algal fatty acids Cellulosic material from algae (Pythia Institute of Biotechnology)
Bioethanol		
Renewable	Renewable	Renewable

Table 1: Photosynthetic biomass-based biofuels and bio products

Increasing concerns on environmental pollution and concern, accelerated global warming, and global climate changes caused by burning fossil fuels, energy security and insecure supply, and increasing crude oil price, as well as world's crude oil (fossil fuels) consumption and depletion overshadowed those industries and led to the attraction and attention, search and development of renewable, carbon-neutral, economically viable alternative energy sources, such as biofuels displacing petroleum. Selected or genetically and metabolically engineered autotrophic algal and micro algal strains are most promising sustainable bio resources for lipid-based biodiesel production, supposedly appropriate to cover entire US oil demand, using 3-5% of country's area (Aristidou and Penttilä, 2000; Lin and Tanaka, 2006; Méndez-Vilas, 2010; Schuhmann *et al.*, 2012; Levitan *et al.*, 2014; Saini *et al.*, 2016). New semantic technologies developed for new and renewable energy resources in industrialized as well as developing nations, will have a significant impact on world's future (Demirbas, 2009). Biofuels exist since the discovery of fire and have been intensively used daily for ages, majorly in cooking and heating (e.g. solid wood), and later liquid oil (e.g. olive and whale oils) to light up homes and paths for a very long period, until they have been replaced by kerosene. Historically, the use of ethanol (called spirit oil) for lamp oil and cooking has been reported for decades, replacing whale oil before being substituted by petroleum distillate (starting with kerosene for lighting), while oil-derived products replaced ethanol for most of 20th century (Lee and Lavoie, 2013). Biofuels are by definition convenient energy containing fuels, which are generated by the conversion of biomass from any biological material, derived photo-synthetically by geologically recent carbon fixation, mostly meaning microalgae and plants or plant-derived materials. Three different ways are applied, namely thermal, chemical and biochemical conversion, a concept called renewable sources of carbon (Ruth, 2008; Lee and Lavoie, 2013; Anastassiadis, 2016). Biofuels refers to as liquid such as biodiesel, bioethanol, or gaseous transport fuels, including biogas and hydrogen, or also propanols and butanol, as well as propane and butane diols, predominantly produced from plant residues and matter, like agricultural crops and residues and forestry byproducts or municipal wastes (Balat and Kırtay, 2010; Elshahed, 2010). Unlike crude oil, biomass feed stocks have diverse and complex composition, so that diverse transformation procedures have been established for the production of a biofuel variety (Devarapalli and Atiyeh, 2015). Multiple approaches apply microorganisms for the conversion of multiple feed stocks to various biofuels, such as hydrogen, biogas, alcohols and biodiesel (Elshahed, 2010). Renewable, environmentally friendly biomass-derived biofuels reintegrate carbon dioxide that has been released from their combustion into photosynthetic cycle, avoiding net CO_2 buildup into atmosphere (Inui *et al.*, 2005). Traditionally, scientists consider CO_2 emissions derived from biomass combustion as climate neutral in a carbon flux neutral bioenergy system, underestimating climate impact of bioenergy. However, CO_2 molecules from C flux neutral systems spend some time in atmosphere before their capturing by biomass regrowth, contributing to global warming as well (Cherubini *et al.*, 2011). Comparatively, biodiesel especially derived from vegetable oils, animal fats and other sources, can replace petroleum-based diesel in Diesel engine motors, like ethanol which replaces in Otto cycle motors gasoline. Worldwide raising demand and production of renewable biofuels and the use of biomass in recent times may stop the rigorous and reckless exploitation of earth's resources. Biofuels, like biodiesel and bioethanol, as well as biomass-based diesel manufactured by the Fischer-Tropsch process, are among most presently promising used as transport fuels, as well as for heat, power and chemicals, to displace, substitute and replace fossil fuels (Demirbas and Balat, 2006; Balat, 2007; Demirbas, 2007; Granda *et al.*, 2007; Da Silva *et al.*, 2009). Worldwide biofuels industry has been rapidly extended to about 2% of world's demand of transportation fuels in 2008 (Di Lucia *et al.*, 2012). A few main routes generate various biofuels with very distinguished properties, comprising hydrogen, biodiesel, ethanol, methanol, synthetic diesel, and bio-oil from biomass, such as extraction of vegetable oils, fermentation of sugars to alcohol, gasification as well as direct liquefaction and chemical synthesis and (Faaij, 2006; Huber *et al.*, 2006). Pyrolysis is the most important process among thermal biomass conversion processes (Demirbas and Balat, 2006; Balat, 2007; Demirbas, 2007). Advanced biofuels have an impact on land-use, depending on assumptions about availability of land and feedstock (Havlík *et al.*, 2011). The department of Agriculture and of Energy (DOE) of US prioritized the development of resources and transformation technologies for the generation of chemicals, power and fuels from biomass, the only renewable feedstock of liquid transportation fuel reducing oil imports (USDA, 2005). Biomass resources, comprising the whole plant related materials, can potentially supply America's renewable future energy. A broad diversity of forestry residues, fuelwood and agricultural residues, including grains such corn, cotton, sugarcane, rice, fruit and nut orchards, as well as small grains like wheat straw, crop residues as well as animal manures and residues can serve as biomass resources for biofuel production. Municipal and industrial solid and urban wood remains, along with oil crops already used for food and energy, starch and sugar are another source. About 1 billion tons of dry biomass feedstock is annually necessary to replace 30 or more of present country's petroleum demand. Forestland with slightly more than 75% (about 142 million dry tons) and agricultural land (about 48 million dry tons) are the largest potential biomass sources used for the production of biobased products and biofuels in US (USDA, 2005). Biofuels provided only around 2% of total transport fuel in 2011 offering a considerable potential for growth over coming decades (IEA, 2011) or about 2.7% of global fuels for road transport in 2014, largely involving ethanol and biodiesel (Wikipedia, 2014). World's biofuel capacity was about 68 billion liter in 2007 (Demirbas, 2009), 90 billion liters in 2009 and increased by 17% to touch an unsurpassed amount of 105 billion in 2010, corresponding to 28 billion US gallons. EU bioenergy plans targeted a 5.75% share for the year 2010 and 10% for 2020, while Japan has targeted a bioenergy share of total energy supply of 20% in 2030 (Ruth, 2008). World's manufacturing of biofuel increased in 2013 at about 7%, reaching above 115 billion liters, while manufacturing of biofuels corresponded to 3.5% of worldwide oil needs for road transportation in 2013, against 3.4% in 2012 and only 2.0% in 2007 (IEA, 2014). International production of renewable electricity is predicted to raise by nearly 45% in 2020 at an annual increase of 5.4%, whereas bioenergy grows less faster in China, while renewable sources are foreseen meanwhile as the greatest new resources of non-OECD production up to 2020 (IEA, 2014). According to Demirbas (2009), worldwide manufacture of biofuel in 2007 approached 68 billion liter. Since millennium start, biofuels production jumped from 2000's 16 billion liters to just under 115 billion in 2013 (Fig. 2), while about 50 countries introduced legislation mandating the use of renewables, according to the IEA. In 2006 ethanol production approximated 46 billion liters (Jørgensen *et al.*, 2007), while worldwide ethanol manufacture approached 86 billion liters in 2010, corresponding to 23 billion US gallons, while the global highest producers US and Brazil produced together 90% of worldwide capacity (Wikipedia, 2014) (Worldwatch, 2011). Sugarcane bioethanol supplied in Brazil 41.5 % (corresponds to 48% capacity) of energy for light duty transport, planning to construct 103 sugarcane mills until 2019 to increase the capacity by 66% (Worldwatch, 2011). EU is worldwide largest biodiesel manufacturer, accounting for 53% total capacity in 2010. Though, some of the European nations switched from biodiesel to ethanol production, since ethanol crops are more efficient sources carrying more energy compared with crops for biodiesel (Worldwatch, 2011). Europe and United States have promoted large-scale commercial biodiesel manufacturing, being greatly increased in US accounting 75 in 2005, 250 in 2006 and 450 million gallons in 2007, expecting to surpass 1 billion gallons in coming years (Gude *et al.*, 2013). Asia created 12% of global biodiesel in 2010, being 20% higher compared with 2009, mainly from palm oil in Thailand and Indonesia, while whole Argentina's biodiesel exports of 1.5 billion liters, accounting for 71% of entire capacity, was exported to Europe (Worldwatch, 2011). Brazil produced 12 million m^3 ethanol and United States about half of this in 2002 (Galbe and Zacchi, 2002). EU is after US and Brazil world's third larger biofuel producer, whereas Germany is the biggest and France second biggest European manufacturer (Balat, 2007; Demirbas, 2009). Brazil's electric power is primarily supplied with renewables (89%), whilst diesel oil (48.6%), gasoline (28.2%), and natural gas (2.2%) yet lead the transport area, although sugarcane based ethanol provides over 14.5% (Flórez-Orrego *et al.*, 2015). Renewable fuels will predictably supply 8.5% of worldwide energy, while bioethanol will replace about 20% of gasoline by 2030 (Walker, 2011). Fluid hydrocarbons are suitable for transportation due to extraordinary dense energy and convenient usability (Koonin, 2006), while transportation area accounts around 20 % of world's principal energy needs (Popp *et al.*, 2014). Liquid biofuels have been generating greatest consideration, even though only a minor portion of biomass is globally utilized for biofuel manufacture at the present, representing about 3–4% total's transportation fuel and 5% total's bioenergy demand (Popp *et al.*, 2014). Bioenergy was the source of approximately 7.5% of energy used in the EU in 2010, which is foreseen by the European Environment Agency (EEA) to rise to around 10% by 2020 (Popp *et al.*, 2014). in 2010, which is foreseen by the European Environment Agency (EEA) to rise to around 10% by 2020 (Popp *et al.*, 2014). International Energy Agency is aiming to replace >25% of world's needs for road transport with biofuels until 2050, to decline dependency on coal and petroleum (IEA, 2011).

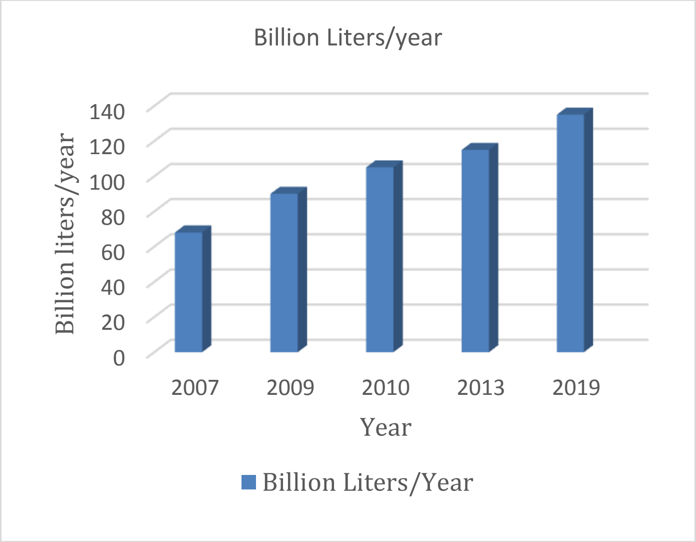


Fig. 2. Global annual biofuel production (Ruth, 2008; Demirbas, 2009).

Renewable investment from a variety of financing sources has risen to high levels, while yearly venture in novel renewable power volume will average at a rather lower point through 2020, at more than 230 billion US Dollar (IEA, 2014). Sustainability of biofuel feeds tocks has to be viewed holistically, taking into account economic, environment and social aspects. World's ability to generate bioenergy is restricted, since cultivable land is multiply used and needed to produce timber, feed, food, and fiber, as well as for nature's conservation and climate stability. Large-scale farming of devoted biomass is likely to influence bioenergy capabilities, worldwide food costs and water shortage, requiring combined policies for energy, land usage and managing of water, as also changes in land management (e.g. intensification), and indirect land-use change (ILUC) (Popp *et al.*, 2014). Worldwide food demand is rapidly growing along with the ecological influences of agricultural enlargement, and the worldwide needs for agricultural crops, driven by a growth of human population of 2.3 billion and bigger per capita earnings, expected until middle of century (Tilman *et al.*, 2011), and will increase for at least another 40 years. To satisfy expected needs due to growing human population, nutritional changes, and increasing use of biofuels, global agricultural production must double by 2050, preferably boosting crop yields at 2.4% per year rate, mainly of the four main worldwide crops rice, maize, soybean and wheat, presently producing closely 2/3 of world's agrarian calories, instead of eliminating more land for agricultural uses (Ray *et al.*, 2013).

However, they have averaged annual harvest enhancements of only among 0.9 to 1.6% (Ray *et al.*, 2013). Neither biofuels can substitute petrol without affecting nutritional demands, because even offering whole US soybean and corn to biofuel manufacturing would only substitute 12% of gasoline and 6% of diesel (Hill *et al.*, 2006). Biofuels may supply environmentally responsibly about 30% of global demand without affecting food production (Koonin, 2006), whilst lesser than 3% of worldwide agrarian land is currently applied for the cultivation of energy crops (Popp *et al.*, 2014). Compliance with global fuel quality criteria is a crucial prerequisite for entire biofuels to access market (Popp *et al.*, 2014). Lignocellulosic trade market will possibly grow speedily in the long term (Popp *et al.*, 2014). Cellulosic ethanol derived from low value biomass, growing on marginal agricultural land or from woody leftover biomass residues, might deliver considerably larger supplies and ecological benefits than food-based biofuels (Hill *et al.*, 2006). In addition to be a maintainable substitute to petrochemicals and relevant to plant physiology, lignocellulosic feedstock originated from cell walls of plants is an abundant resource of saccharides, chemicals, biopolymers and sugars, and a persistent basis of inspiration for biotechnological, biomaterial and bioenergy industry (Guerriero *et al.*, 2016). Advanced high yielding biofuel feedstock, e.g. genetically improved by combining modern breeding and transgenic techniques energetic crops like switch poplar, jatropha and grass, which would be produced independently and differently from nutriment in large environmental range on low value agricultural land with little agrarian effort, e.g. fewer energy pesticide and fertilizer, would provide greater benefits, and require low-input energy for their conversion to biofuels, enhance energy security (locally sustainable production), reduce GHG emissions by recycling atmospheric/ocean/biomass carbon dioxide, provide economical transport fuels and support agriculture (Hill *et al.*, 2006; Koonin, 2006). Main multidisciplinary challenging technological groups might fulfill those objectives, involving chemical engineers, biologists, fuel specialists, agronomists, and social scientists, importantly reducing agricultural efforts. Plant growth, chemical structure, toughness and resistance to abiotic and biotic pressures, and nutritional necessities are significant characteristics to manipulate, resulting in greater achievements in food crops and in much less period than in Green Revolution (Koonin, 2006). Contemporary biotechnology can overcome chemical recalcitrance and utilization of lignocellulosic biomass for optimally manufacturing fuels and by-products (Koonin, 2006). Our living is strongly related to petroleum founded manufacturing (Saini *et al.*, 2016), since today's fossil oil refinery creates manifold fuels and goods from petrol, whereas alternate feedstock for the chemical production have got extensive attention and renewables and their derivative byproducts are of specific concern in recent years (Przystałowska *et al.*, 2015). Analogously, bio refinery established by developments in genetic engineering, biotechnology, process chemistry and engineering is a facility integrating biomass transformation procedures and necessary apparatus. It offers the potential to produce bio power, valuable biofuels and biomaterials, as well as value-added chemicals from renewable biomass resources, including agro energy crops like corn crops, and lignocellulosic biomass such as agricultural remains, forestry trashes and thinning, sludge paper and energy grains, leading to a new manufacturing paradigm (Ragauskas *et al.*, 2006; Himmel *et al.*, 2007; Demirbas, 2009). Similarly, to an oil refinery, numerous opportunities exist for the integration of taking away heat in thermochemical or biochemical processing facilities, within the overall bio refinery as part of cooling, to provide heat somewhere else in the facility. Leftover heat might also be provided in order to maximize total effectiveness and efficacy of bio refinery facility (Roddy, 2013). Renewable, carbon neutral transport biofuels may replace fossil fuels for ecological and financial maintainability, like biodiesel based on edible food grains (1st generation) or non-edible oil crops (2nd generation), animal fats, waste cooking oil, waste oil, grease and animal fat, and bioethanol (gasoline additive/substitute) (Chisti, 2007; Ruth, 2008; Demirbas, 2009; Mata *et al.*, 2010). Scientists categorize and classify biofuels into three categories, according to biomass origin used, namely, 1) 1st generation biofuels, directly connected to an edible biomass, derived from crop, 2) 2nd generation biofuels originated from a broad range of diverse feedstock, fluctuating from lignocellulosic cultivation and forestry remains and inedible grain substrates to municipal solid wastes, and (3) 3rd generation biofuels originated from microalgae or algae, identified as one of oldest existing creatures producing 15 to 300 times more oil than conventional grains, and other microorganisms able to a certain grade to the consumption of carbon dioxide (CO_2) as substrate (Brennan and Owende, 2010; Méndez-Vilas, 2010; Lee and Lavoie, 2013; Srivastav *et al.*, 2014). Oleaginous microorganisms are favorable due to short time growth cycles, high lipid concentrations and easiness of biotechnological modifications (Huang *et al.*, 2010). Besides, 4th generation biofuels–photo-biological solar fuels and electro fuels derived by direct alteration of sun energy into fuel consuming inexhaustible, cheap and widely available raw materials, exhaust the possibilities of novel synthetic biology tools and are just emergent at the level of elementary research, expectably to supply important advances in the arena of biofuels (Aro, 2016). A combination of photovoltaics or inorganic water-splitting catalysts with metabolically engineered pathways forming microbial fuels (electro biofuels) is another powerful emergent knowhow for effective manufacture of fluid fuels (Aro, 2016).

1st generation biofuels (Biodiesel, bioethanol, pure plant oil): The so-called "first generation biofuels", including three different types of biofuels such as (1) fatty acid methyl ester (FAME or biodiesel), (2) ethanol or methanol and (3) pure plant oil (PPO), have a key role in international biofuel scene (Havlík *et al.*, 2011) and are more than often directly related to an edible biomass (Lee and Lavoie, 2013). Crops generating starch, sugars primarily coming from sugarcane, beet or corn (ethanol), or vegetable plant oil germs like rape, soybeans or coconut, palm and additional plant oils, or animal fats (biodiesel) are the basis for the production of transport biofuels bioethanol and biodiesel. Numerous further plants can be utilized to manufacture fuels, depending on numerous aspects, including yield, agrarian performances, ecological thoughts and global trade agreements (Ruth, 2008; González-Delgado and Kafarov, 2011; Anastassiadis, 2016). Global oil production from plant germs, like rape, palm and soy, amounted about 160 Tkg per year in 2014 (Donot *et al.*, 2014). Biodiesel, requiring a smaller amount of fossil energy of 0.31 units to create 1 fuel unit, offers a tremendous potential for semantically lowering dependency on petroleum oil and for lessening fossil fuel usage. In contrary, 1.2 units of fossils are consumed to generate 1 unit of petrol diesel, confirming the renewable characteristics of biodiesel. Furthermore, soybean oil, the biggest feedstock of biodiesel, is renewable and it diminishes net emissions of CO_2 by 78.45% compared to petro-diesel (Sheehan *et al.*, 2000). Food based 1st generation biofuels, lowly providing greenhouse gas reductions, while putting agriculture, food and natural ecosystems at risk, will be probably essentially expelled in European Union (Aro, 2016).

First generation biodiesel: Biodiesel mentions any renewable replacement of diesel which is originated from renewable biomass, made chemically joining every kind of natural biomass like fat and oil, mostly originated from edible vegetable oils, with a molecule of alcohol like ethanol and methanol, whereby methanol is furthermost usually utilized alcohol in industrial biodiesel manufacturing (Sheehan *et al.*, 2000; Huang *et al.*, 2010). It might be locally synthesized from various agrarian oils as well as from leftover oils or fats and might be applied unaltered in diesel machines, directly offering the possibility to lower the needs for petroleum biodiesel (Sheehan *et al.*, 2000). Biodiesel is a harmless, ecofriendly biologically degradable, substitute to fossil diesel, referring to from diesel-equivalent lower alkyl esters (mono-alkyl ester) made, oxygen containing fuel of long chain fatty acids, derived from edible and nonedible crop oils, animal fats and other triacylglycerol-containing feedstock, which are synthesized either by transesterification with lower alcohols or by esterification of fatty acids (Demirbas, 2009; Murugesan *et al.*, 2009; Knothe, 2012). The carbon number of diesel molecule is around 15, similarly to the oils of the plants containing on average 14 to 18 carbons (Huang *et al.*, 2010). High oxygenated biodiesel doesn't contain any aromatic composites and further environmentally harmful chemical constituents, and it generates less emissions of particles, carbon monoxide and sulphur dioxide in comparison to diesel, while applying biodiesel may reduce the toxicity of air by 90 % and cancers by 95 % in comparison to usual diesel. Contrarily, biodiesel increases NOx emissions (Sheehan *et al.*, 2000; Huang *et al.*, 2010). Raw materials (fats and oil) accounting for 60% to 75% of total cost and processing determine final production cost of biodiesel fuel, whereas inexpensive oleaginous resources and advanced transesterification procedures are essential for successful biodiesel production (Huang *et al.*, 2010). Feedstock price is the most crucial factor affecting biodiesel production, therefore seeking less expensive sources such as used waste oils and oils from non-edible plants grown in marginal land like *Jatropha curcas* L. (Lee and Lavoie, 2013), being often considered as a magical biodiesel plant with multiple environmental benefits (Edrisi *et al.*, 2015).

Primary biodiesel enterprises have been established in South Africa back in 1981, and thereafter in New Zealand, Germany Austria, and in 1982 (Shay, 1993; Körbitz, 1999; Khanna *et al.*, 2012). Global entire biodiesel manufacture valued in 2003 about 1.8 billion litters (Fulton, 2004). Global capacity of biodiesel is estimated to approach the 37 billion of gallons in 2016 corresponding to 140.06 billion liters, resulting in the by production of around 4 billion of gallons of rough glycerol as a byproduct (Anand and Saxena, 2012). Now todays, about 1.4 million hectares (1 hectare=10 acres) of arable land is devoted to the manufacturing of biodiesel in European Union, annually generating until 3,184.00 metric tons' biodiesel in about 40 factories, which are mainly placed in Germany, Austria, Sweden, Italy and France (Medipally *et al.*, 2015). Factors affecting profitable operation of a biodiesel facility include facility volume, procedure know-how, price of crude feedstock and expenses in chemicals (Demirbas, 2009). Most European biodiesel is made mainly from rapeseed oil, which is a relative of canola oil, while soybean oil is primary feedstock in US, which is the biggest manufacturer of soy bean oil in the world (Sheehan *et al.*, 2000).

Vegetable oils consist of a triglyceride ester composed of one molecule of glycerol and 3 fatty acids varying in the length of carbon skeleton as also in the amount of chemical double bonds, which influences biodiesel properties (Srivastava and Prasad, 2000; Park *et al.*, 2008). Plant oils enclose usually free fatty acids (usually 1 to 5%), phosphatides, sterols, phospholipids, tocopherols, carotenes, Sulphur compounds, as well as water traces, odorants and other impurities. The numbers of cetane range between 32 and 40, while content of iodine varies between zero and 200 in dependence on degree of unsaturation. Values of cloud and pour of plant oils surpass diesel (Srivastava and Prasad, 2000; Murugesan *et al.*, 2009). Physical features of fatty acids, like length of molecule, grade of unsaturation and branching of molecule influence physicochemical biodiesel characteristics (Islam *et al.*, 2013). Biodiesel production is a quite different from chemical ethanol process, although it uses biomass. It relies on oil extraction, after the cleaning, drying and dehulling of oily plants and seeds by hydraulic or expeller pressuring and solvent withdrawal, leaving a dry solid remainder called meal. To convert oils into biodiesel, the bonds of long chain fatty acids are broken into glycerol, replacing it with methanol in a process called transesterification (Huber *et al.*, 2006; Lee and Lavoie, 2013). Microwave applications achieve superior results in biodiesel production compared with conventional techniques (Gude *et al.*, 2013). Biodiesel is a hopeful substituting and renewable fuel with a well-developed and increased production capacity in recent years, considering that more than 18 million tons of biodiesel are being globally produced each year, 11.2 million metric tons in Europe and 6.96 in USA in 2010, generating a tremendous surplus of main low price co-product crude glycerol (~10%). Glycerol (1,2,3-Propanetriol) is one of greatest multipurpose chemicals with an international price between 0.21 and 0.23 US $/lb and projected annual worldwide manufacture of 1.78 million metric tons in 2012. Glycerol is plentifully available to synthesize numerous value-added commodity chemicals and other compounds, via a variety of microbial metabolic pathways (fermentations), biochemical or chemical (catalytic) processes, generating additional revenue for biodiesel industry. Ethanol, butanol, 1,3-propanediol (1,3-PDO) or 1,2-propanediol dihydroxyacetone, polyhydroxyalkanoates, hydrogen and other lower molecule fuels, carbonic acids such as citric, succinic, isocitric, malic, oxalic, hydroxypyruvic, itaconic, mesoxalic and glyceric acid, 3-hydroxypropion-aldehyde, various polyols like erythritol, mannitol and arabitol, propylene glycol, and glyceraldehyde are produced from glycerol. It has also been used for composting and burning, or as an animal feed (Da Silva *et al.*, 2009; Kamzolova *et al.*, 2011; Dobson *et al.*, 2012; Khanna *et al.*, 2012; Yang *et al.*, 2012; Przystałowska *et al.*, 2015; Sen *et al.*, 2015; Dobrowolski *et al.*, 2016). Tremendous amounts of concentrated crude glycerol is annually generated by oleochemical activities (biodiesel production, fat saponification), which's valorization attracts increasingly remarkable interest (Papanikolaou *et al.*, 2016). Pure glycerol is a valuable industrial chemical compound with numerous multiple applications in pharmaceutical, biotechnological, cosmetic, and food industries, whilst utilization of renewable rough glycerol, which is also formed by many other manufacturing units like saponification of fats (10% of soap weight) and manufacture of stearin and alcoholic beverage, offers great opportunities for new applications, defraying and lowering production cost and promoting large scale industry and further development of biodiesel production (Yang *et al.*, 2012; Dobrowolski *et al.*, 2016). Applying adaptive laboratory evolution, increased enormously growth of not genetically engineered *Ustilago trichophora* and production of C4 dicarbonic malic acid, which has great potential as building-block chemical, from glycerol, reaching a final titer of 196 g/liter, yield of 0.82 g mal/g glycerol, and overall production rate of 0.39 g/(h l). Glycerol is also an alternative feedstock for the manufacturing of many petroleum-based chemical products (Dobson *et al.*, 2012), while microbial fermentation of glycerol to various chemicals adds value to biodiesel process economy and avoids waste disposal (Almeida *et al.*, 2012). *Yarrowia lipolytica* has been reported to initially assimilate glycerol before glucose (Papanikolaou *et al.*, 2016). Global annual glycerol demand for manufacturing various goods like tobacco, glycerin triacetate, drugs, toothpaste and cosmetics, paints, food and cellophane attitudes 1.81 million metric tons (Khanna *et al.*, 2012). Rapid development of biofuels like bioethanol and biodiesel sharing less than 1% of total agricultural area has slowed down, accused answerable for the strong food price raise during the second half of 2008, whereas controversial articles have also reported about ecological effects, like greenhouse gas reductions and alterations in land use (Mittelbach, 2009). Global food and crop demand is increasing rapidly, commensurately with the human population, consumption and need of natural resources and serious long-term environmental impacts of agricultural expansion on issues like global biodiversity, forecasting a strong increase in worldwide harvest of 100 to 110% within 2005 and 2050, while Earth's crop harvests stand under its dynamics (Foley *et al.*, 2011; Tilman *et al.*, 2011). Efficient management and strategies can double food production substantially lowering nitrogen use, to minimize environmental impacts (Foley *et al.*, 2011; Tilman *et al.*, 2011). Transferring adapting technologies to under-yielding nations would enhance their soil fertility, employ more efficiently nutrients, minimize land clearing, and provide further ecologically maintainable agrarian strengthening and supplementary fairer global food provisions worldwide (Tilman *et al.*, 2011). Advanced technologies, especially in the fields of industrial microbiology and biotechnology, will be necessary to encounter worse situations that may come in near and far future on Earth, along with the strong increase of human population and demand for necessary goods. They include environmental pollution, extreme global warming and climate changes, natural catastrophes and disasters, as well as soil contamination, neutralization and infertility (misuse of fertilizers and chemicals). A novel advanced biological and ecological soil conditioner (plant antifreeze, stimulator, protector) of microbial origin named EcoPlant© has been developed and is produced since more than 10 years at Pythia Institute of Biotechnology by Dr. Savas G. Anastassiadis (Avgi, Greece and Sklave, Bulgaria), which protects plants and trees from freezing. Eco-Plant accelerates severally plant growth, increasing enormously productivity, quality and resistance of various plants, e.g. vegetables, cereals (wheat, barley etc.), trees (fruit trees, lemons, forest trees etc.) and crops (corn, sunflower, rapeseed, cotton), to diseases and unusual climatic and soil conditions. It enhances severally plant production and increases the oil content of oil crops, the sugar content of fruits and grapes, nutrient value of food crops and the specific weight of cereals (more than ~15%), even at unusual conditions (very low or high temperatures, drought, high salinity etc.), minimizing enormously the use of fertilizers and agrochemicals. Furthermore, it enhances the resistance of plants against microorganisms, viruses, insects, worms and various plant diseases. Surely, it can contribute enormously in solving semantic global problems in coming years regarding soil pollution, health as well as agricultural and bioenergy production. Aside other carbohydrate, Eco-Plant has also been produced from crude glycerol from Greek Biodiesel industry. A novel photosynthetic biomass producing system has also been identified and grown at Pythia Institute of Biotechnology, producing large sheets of cellulosic material like thick paper from atmospheric CO_2 (second-generation cellulosic biomass) using Eco-Plant.

Notable biofuels and biochemical are usually microbial fermentation metabolites. Microorganisms have been broadly used to produce environmentally approachable fuels and commodity biochemical, e.g. *n*-butanol, via fermentation pathways, usually involving many redox transformations, typically requiring NADH and NAD^+ as cofactors for the reactions (Saini *et al.*, 2016). Saini *et al.* (2016) reported about *n*-butanol formation by adjusting intracellular redox state in *Escherichia coli* engineering three metabolic knots within its central metabolic network.

Ethanol biofuel: Ethanol (ethyl alcohol, C_2H_5OH, mp -114ºC, 78.4ºC,) is a water soluble solvent with a density of 789 g/l at 20ºC, which is synthetically produced by catalytic hydration of petroleum derived ethylene (Gnansounou and Dauriat, 2005).

$$\underset{Ethylene}{C_2H_4} + \underset{Steam}{H_2O} \rightarrow \underset{Ethanol}{CH_3CH_2OH}$$

Bioethanol production, accounting for 90-95% of globally produced ethanol, exclusively utilizes microbial and fermentation engineering (Sarris and Papanikolaou, 2016). Growing attention has gained in past years the perspective conversion of waste and residual biomass towards ethanol, considered as mostly clean fluid biofuel, alternatively to petrol fuel (Papanikolaou *et al.*, 2016; Sarris and Papanikolaou, 2016). Bioethanol is the predominant liquid biofuel worldwide, produced in North America from maize and in South America from sugarcane by microbial fermentations, as opposed to petrochemical alcohol (Walker, 2011). Bioethanol, otherwise called ethyl and grain alcohol, or in chemistry EtOH or C_2H_5OH, is an attractive sustainable energy source and worldwide the most produced and one of most commonly and widely used biofuels in transportation sector, substituting gasoline, to reduce greenhouse gases. It is commonly produced from a widespread variety of crops or materials such as sugar cane and corn (first generation), molasses, starch, sweet sorghum cane extract, lignocellulose, and other wastes, by well-established microbial processes and different extraction methods, mostly manufactured in Brazil and US accounting for over 80% of total global industrial production derived sugarcane or maize (*Saccharum* sp.), while sugar beet, wheat or potatoes (*Beta vulgaris* L.) are primarily usual feedstock in Europe and potential for biofuels in India and China grows significantly (Havlík *et al.*, 2011; Walker, 2011; Tesfaw and Assefa, 2014). Not like fossil oil, bio feedstock vary in synthesis, hence requiring distinct transformation procedures to harvest diverse biofuels (Devarapalli and Atiyeh, 2015). Agricultural carbohydrate raw materials for bioethanol production include a huge diversity of carbohydrates, like mono-, di- and polysaccharides, comprising sugary (sugarcane and sugar beet juice), starchy (wheat or maize), or celluloses, which are classified into three categories: simple sugars, starch andlignocellulose (Gnansounou and Dauriat, 2005; Balat and Balat, 2009; Walker, 2011).

Ethanologenic biomass crops comprise multipurpose crops devoted to food market and dedicated ethanol crops (Gnansounou and Dauriat, 2005). Bioethanol production from readily fermentable by *Saccharomyces cerevisiae* sucrose containing feedstock is easier, more effective and cost-effective in comparison with starches, which necessitate previous hydrolysis before fermentation (Walker, 2011). Sugar cane juice from sugar refining processes, usually contains ~15% sucrose, while residual molasses ~50%, comprising saccharides, including glucose, fructose and sucrose), fatty acids, minerals, organic acids, vitamins, etc. Not directly fermentable by *S. cerevisiae* starch requires prior to fermentation pretreatments and hydrolysis, comprising cereal heating, liquefaction of starch and starch hydrolysis (Walker, 2011). Brazil was a pioneer in replacing already in 1973 gasoline with sugarcane based alcohol fuel, as clean (with respect to CO_2 balances) petrol additive/substitute energy source (biofuel), and introduced in 1975 the so called National Alcohol Fuel Program (ProAlcool). It is utilized as road transport fuel either directly or commonly mixed at 10% with 90% gasoline (E10, gasohol). Middle of 1980s, around 95 % of Brazilian vehicles have been transformed to ethanol burning, showing biofuel infrastructure to be ecologically and cost efficient (Gnansounou and Dauriat, 2005; Demirbas, 2007; Granda *et al.*, 2007; Balat and Balat, 2009; Da Silva *et al.*, 2009; Havlík *et al.*, 2011). Bioethanol has been traditionally produced by the fermentative metabolic conversion of sucrose or simple sugars derived from biomass hydrolysis towards alcohol, which is located in *S. cerevisiae* and other yeast species within the cytoplasm. It may also be economically synthesized alike to sugarcane from cellulosic wood, straw and even household wastes, or also using crops with greater efficiencies and lesser ecological influence in a transformation procedure using little fossils, making it even more maintainable and ecologically gentle (Demirbas, 2007; Granda *et al.*, 2007; Sarris and Papanikolaou, 2016). Expenses of bioethanol manufacturing is determined by numerous varying aspects, comprising transformation pathways, facility extent and place, feedstock and byproducts. Connection however of food to ethanol market generates price instability of ethanol.

Sucrose composed of glucose and fructose is the mostly used disaccharide in ethanol manufacturing (Gnansounou and Dauriat, 2005).

Starch is converted to ethanol through a hydrolysis reaction catalyzed by gluco-amylase enzyme generating dextrose or D-glucose which is a glucose isomer, yielding anhydrous bioethanol after fermentation, distillation and dehydration (Gnansounou and Dauriat, 2005). 1 ton of hexoses produces theoretically 511 kg of ethanol, only about 92% of which is however efficiently reached in praxis (Gnansounou and Dauriat, 2005).

$$(C_6H_{10}O_5)_n + n\,H_2O \xrightarrow{gluco-amylase} n\,C_6H_{12}O_6$$

First generation ethanol production: Ethanol is synthesized both, chemically (synthetically) from petrochemicals like petrol or through microbial fermentation from a large number of agricultural carbohydrates and products (Czyrnek-Delêtre *et al.*; Badger, 2002; Brooks, 2008). Bioethanol is a promising biofuel of great alternative prospective to fossil fuels (Adnan *et al.*, 2014). Renewable alcohol is generally more expensive than synthetic ethanol which is derived from ethylene and of methanol which is derived from natural gas. Ethanol has been the first biofuel which has been manufactured from food feedstock such as sugarcane and maize (Devarapalli and Atiyeh, 2015). Bioethanol, by far world's most widely used non-fossil alternative transportation engine biofuel, is a promising gasoline additive/substitute with many great prospective, advantageously displaying greenhouse benefits. It is nearly entirely manufactured from food grains, primary originated from sugar-rich sugarcane (60%) and other starchy grains (40%), predominantly referring to grains corn (*Zea mays*, 60-70% starch) or wheat (*Triticum* spp.), depending on varying local conditions (Czyrnek-Delêtre *et al.*; Gnansounou and Dauriat, 2005; Demirbas, 2009; Adnan *et al.*, 2014), with Brazil being one of leading bioethanol countries (Lee and Lavoie, 2013), the worldwide biggest exporter and second biggest manufacturer after US (Crago *et al.*, 2010). Brazil has been the global largest ethanol manufacturer up to 2005 (Crago *et al.*, 2010). Large bioethanol quantities are produced worldwide from sugar and cereals like corn or grain (Jørgensen *et al.*, 2007). US corn ethanol using over 30% of corn produced in US and Brazilian sugarcane ethanol are world's leading sources of biofuel, from which sugarcane ethanol has a lower cost that then increases with the transportation, and lower GHG emissions (Crago *et al.*, 2010). Starch- or grain-based (e.g. maize) ethanol is currently the major biofuel in United States (Simmons *et al.*, 2008), while sugarcane is the primary biofuel resource in Brazil and other regions of the world (Simmons *et al.*, 2008). Relative ethanol cost in US and Brazil is highly sensitive to prevailing exchange rate and feedstock prices (Crago *et al.*, 2010). 1975 was the real birth certificate of "Brazilian Alcohol Program", aiming to reduce gasoline consumption and decrease oil imports, while agricultural sugarcane production increased in various Brazil's areas by 51% between 1977 and 2009. A number of countries followed the successful Ethanol Program of Brazil in recent years aiming to reduce CO_2 emissions from gasoline, mainly United States, which produces ethanol from corn. World ethanol production from sugar beet, maize and sugarcane raised from under 20 billion liters in 2000 to more than 40 billion liters in 2005, and approximately 46 billion liters in 2006 (Jørgensen *et al.*, 2007), representing about 3 % of worldwide use of gasoline, while its manufacture had been foreseen to nearly double until 2010. Bioethanol production in EU accounted 620 million liters in 2004, with Spain being the EUs leading bioethanol producer. Present global bioethanol request is growing due to low petrol fuel availability and growing quantity of ethanol/gasoline flex-fuel automobiles, therefore any small process improvement could save billions of dollars (Abreu-Cavalheiro and Monteiro, 2013). Brazil has cultivated sugarcane from sixteenth century and in recent times became the biggest sugar producer with about 25% of global manufacture.

Ethanol, also known as ethyl alcohol, is an alternative liquid biofuel that has been generally produced on large scale by the classical conversion route from various carbon sources utilizing *S. cerevisiae* (generally regarded as safe), especially in Brazil, the US and France and more modestly in Sweden and Spain. Carbohydrates, biomass feedstocks and residues, such sugar crops like sugarcane (C4 plant), barley, rice, corn starch, C6 sugars, mostly glucose (saccharides), sugar, sugar cane juice and molasses, maize, sunflower, sugar beets (*Saccharinae* plants), potatoes, sweet sorghum (C4 photosynthesis), plant oils, sugar beets, cereals, cassava, wheat and other grains, or even cornstalks, various fruit, vegetable, and other organic waste, are applied. *S. cerevisiae*, also well-known as Bakers' yeast usually applied in baking manufacture, is most often applied in ethanol fermentation, even though bacteria, fungi, and other yeasts might also be utilized. Lignocellulosic biomass and cellulosic feedstocks are also utilized for next generation ethanol production using classical or GMO yeast strains, providing the potential to reduce particulate emissions (Badger, 2002; Faaij, 2006; Energy4me, 2006-2014; Prasad *et al.*, 2007; Paterson *et al.*, 2009; Posada and Cardona, 2010; Suhaimi *et al.*, 2012; Lee and Lavoie, 2013; Tesfaw and Assefa, 2014). Simultaneously cultivating *S. cerevisiae* with other yeasts or microbes, cell immobilization on a carrier material, cell reuse, cell recycling and cell retention by centrifugation or filtration, or by cell sedimentation and exploiting cellular flocculation have also targeted to optimize ethanol production, shorten fermentation time, enhance ethanol productivity, facilitate product separation and reduce process cost (Tesfaw and Assefa, 2014; Westman and Franzén, 2015) (Westman and Franzén, 2015). Microbial ethanol production from biomass follows three steps: (1) formation of fermentable sugars, typically 6-carbon sugars and most commonly glucose, (2) sugar fermentation to ethanol and other byproducts by various microorganisms, and (3) separation as well as purification of ethanol, commonly via distillation (Badger, 2002). Theoretically, from 100 grams of glucose about 51.4 g ethanol and 48.8 g of CO_2 are formed. However, the actual yield is practically less than 100%, because of microorganisms' maintenance metabolism spending some of the glucose for growth and maintenance (Badger, 2002). Certain bioethanol fermentations reliably reach very high ethanol titers of ~20 % (v/v). Bioethanol process also generates diverse byproducts, comprising CO_2, fusel oils, grain remains, bagasse, stillage as well as yeast biomass (Walker, 2011). Most of currently produced bioethanol is derived from conventional food and animal feed crops, such as starch from maize grain, as well as single saccharides from sugarcane and beets (Himmel *et al.*, 2007; Abramson *et al.*, 2010). So, 1st generation biofuels are finally unmaintainable on the ground of insecure supply of nutrients and concerns regarding utilization of terrestrial area (Walker, 2011).

First generation of global sustainable renewable biofuels, primarily utilizing first generation biomass, such as food crops, sugar, starch, oil seeds or fats and oils used for food and feed, has been the most cost-effective route for manufacturing renewable liquid fuels. There are however concerns that they may be unsustainable in face of expanding demand for food, feed and fiber and cannot achieve future objectives for manufacturing of biofuel, reduction of climatic alterations and financial development (Mata *et al.*, 2010). Amongst present biofuels derived from food grains, biodiesel generated from soy beans displays semantic benefits above ethanol derived from maize crop. Net Energy Balance (NEB) for maize crop bioethanol is small since much energetic input is necessary for the cultivation of maize corn and its conversion to bioethanol, supplying only ≈25 % additional energy than has been spent for its manufacture, whereby nearly altogether is attributed to DDGS byproduct serving as feed of animals, instead of ethanol itself.

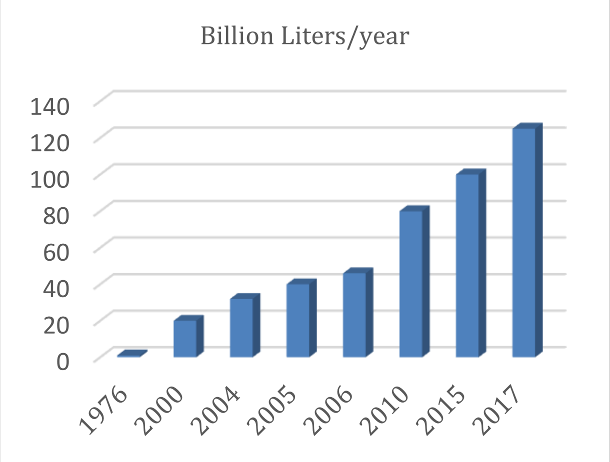


Fig. 3. Global ethanol production (Data received from (Gnansounou and Dauriat, 2005; Jørgensen *et al.*, 2007; Walker, 2011; Tesfaw and Assefa, 2014).

Comparably, biodiesel derived from soybean oil delivers ≈93% further vigorous energy from what is necessary for its generation (Hill *et al.*, 2006). Sugar producing crops have the highest effectiveness regarding land use as referring to replace fossil energy, whilst sugarcane from tropics semantically exceeds sugar beet and starch crops of temperate region (Niven, 2005; Von Blottnitz and Curran, 2007). Because of high percentage of sucrose of sugar cane syrup, sucrose extraction is a relatively simple process that requires no microbial or enzymatic treatment (Elshahed, 2010). Sugar cane doesn't thrive in colder climates, therefore United States uses corn starch as starting material for ethanol production, rather than sugar cane, which will always be a much more expensive alternative to oil (Elshahed, 2010). On the other side, it is doubtful if energy efficiency balance for corn ethanol in United States is not at all positive, or only marginally (Niven, 2005). Moreover, more than entire continent of North America is needed to provide ethanol to substitute oil needs of United State (Elshahed, 2010). Bioethanol manufacture is medially to highly beneficial from viewpoint of climate stability and fossil fuel preservation (Von Blottnitz and Curran, 2007). Flórez-Orrego *et al.* (2015) determined a ratio between renewable to nonrenewable exergy (cR/cNR) of 2.69 for biodiesel, 4.39 for electricity, 15.96 for ethanol, and a negligible ratio for fossil fuels.

Bioethanol is an attractive, renewable and sustainable energy source produced biotechnologically from sugar juice, starchy crops, and lignocellulosic materials, whereas microbial culture and maintenance is an essential step for effective fermentation (Zabed *et al.*, 2014). Brazil has been producing ethanol since the 1930s, basically operating a spontaneous fermentation (Andrietta *et al.*, 2011). Now todays, Brazilian ethanol processes utilize sugarcane and *S. cerevisiae*, operating at high temperature and cell concentration in exposed tanks with huge volume, applying cell recycling for efficient vital industrial production, genetic improvement of industrial yeast strains towards stress-resistant strains as well as substrate improvement (Abreu-Cavalheiro and Monteiro, 2013).

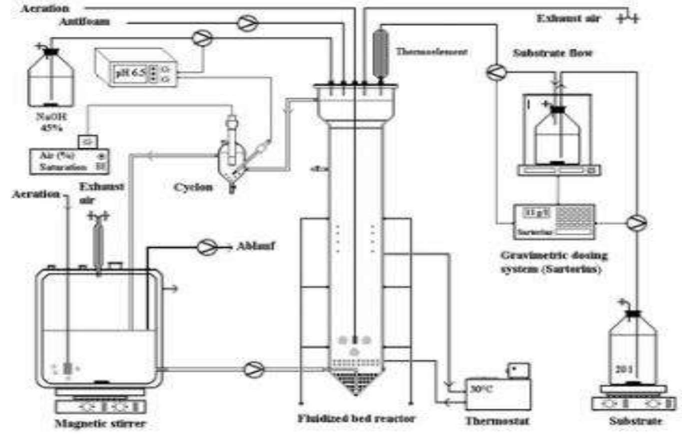


Fig. 4. Fluidized bed fermenter with immobilized cells.

S. cerevisiae is a facultative anaerobic yeast with enhanced glycolytic activity, as well as Pasteur-positive, meaning ethanol production under anaerobic conditions, as also Crabtree-positive microorganism, meaning ethanol production under aerobic circumstances in the presence of high glucose concentration (catabolite repression). Energy crops such as sugarcane (*Saccharum officinarum*, C4 plant,) grown in tropical and subtropical countries, sugar beet (*Beta vulgaris*) and its industrial byproduct beet molasses, highly efficient photosynthetic sweet sorghum (*Sorghum bicolor* L., C4 plant), and some fruits are most attractive, cost-effective and feasible carbon sources for ethanol production, employing batch, fed-batch, and continuous processes (Zabed *et al.*, 2014). Sugar cane has 12–17% sugar concentration consisting of 90 % sucrose and 10 % fructose and glucose (Zabed *et al.*, 2014). Batch, fed-batch, or continuous fermentation operations are used in bioethanol production, utilizing free or immobilized microbial cells of selected industrial yeasts (Gen amplifications) on different carriers, depending on microbial kinetics and nature of feedstock. Various abiotic operational parameters influence growth and production, including the pH, the temperature, agitation speed, fermentation time, sugar content and inoculation amount, wherein accumulated strains in fermenter have the ability to withstand great biotic and abiotic oscillations (Andrietta *et al.*, 2011; Zabed *et al.*, 2014). Continuous processes mainly operating in plug flow reactor or continuous stirred tank reactor offer several advantages over conventional batch processes. They are cost-effective, use cheaper bioreactors, require lesser maintainability and operational time, and provide easier controlling of fermentation, and greater production efficiency, operating in stationary steady state at 100% capacity (Andrietta *et al.*, 2011; Abreu-Cavalheiro and Monteiro, 2013; Zabed *et al.*, 2014). Batch processes with recycled yeasts are lesser vulnerable to microbial infections and in decreasing production rate compared with continuous fermentations (Abreu-Cavalheiro and Monteiro, 2013). Continuous process is usually conceived with 4 or 5 fermenters connected in series, wherein substrate and treated yeast cells are continuously and simultaneously added in first fermenter (Andrietta *et al.*, 2011). Accumulated ethanol during yeast fermentation is main toxic compound, slowing down yeast growth and ethanol production, resulting therefore in low final ethanol titers of about 8 to 9% (v/v). Semantic quantity of energy for ethanol recovery and large fermentation tanks are required, while also generating large amounts of vinasse (Silva *et al.*, 2015; Sonego *et al.*, 2016). Industrial fed-batch ethanol fermentation starts with 25-30% inoculum, resulting compared to conventional batch mode in a higher ethanol concentration due to reduction of sugar inhibitory effect on *S. cerevisiae* growth and ethanol production during first stages of fermentation process (Sonego *et al.*, 2016). Extractive fed-batch fermentation with 2.5 vvm CO_2 stripping at 34.0°C has been reported to overcome inhibitory effect of ethanol, wherein ethanol is removed during the fermentation process, resulting in 33% higher ethanol concentration of 110.3 g/L and 9.2 g/(L*h) productivity from 240 g/L substrate (Sonego *et al.*, 2016). Using a 5 liter bubble column fermenter, temperature and entrainment factor were positively affected by the starting temperature and specific flow rate of carbon dioxide, and negatively by the ratio between the height and the diameter (Silva *et al.*, 2015). Taylor *et al.* (1995) described a continuous fermenter/stripper process with heat recovery as a lower-cost alternative to conventional fermentation and distillation, reducing heating and cooling costs. Adapted to galactose thermotolerant yeast strain *Pichiakudriavzevii kudriavzevii* isolated from sugarcane juice produced 30% more ethanol than non-adapted cells, reaching 71.9 g/L ethanol at 4.0 g L/(1*h) and 40°C (Dhaliwal *et al.*, 2011).

The 2nd generation biofuels: Raw materials dominantly determine the price of commodity products and predominant expenditure in bioethanol production (Walker, 2011). Large-scale bioprocess or chemical transformations might be very costly and worthy uneconomical for the commercial supply of biofuels (Savaliya *et al.*, 2015). First generation production systems, competing with farming for arable territories utilized for the production of food, have substantial financial and ecological limits (Méndez-Vilas, 2010). Convertible into fluid biofuels renewable bio resources are available worldwide, including residual agricultural biomass and wastes, currently being the only logical, economic and environmentally accepted substitute for green development. Greatest easily accessible biomass exists in agrarian and civic remains (Martin, 2014). Technological advances, certain land-use changes and the development of a diversity of innovative, renewable, resistant bioenergy feedstock can make biofuels sustainable and cost effective. This may overcome the high cost of biomass feedstock and their conversion to sugars, to meet growing energy demands and provide economic and environmental benefits and sustainability, without to need more net land and to impact food production (Himmel *et al.*, 2007; Lewis *et al.*, 2015; Anastassiadis, 2016). Primary phase in biofuel production is to find an alternative, inexpensive and plentiful feedstock. Woodlands contain around 80 % of global biomass like lignocellulosic biomass as well as materials, which are the most abundant renewable biological resources, as well as attractive and relatively inexpensive raw material and feedstock for ethanol production, since they are outside the human food chain (Badger, 2002; Pereira *et al.*, 2012). 2nd generation bioethanol means that it is generated using inedible resources, such as lignocellulose and lignocellulosic substrates, comprising the greatest plentiful carbon on Globe (Jørgensen *et al.*, 2007) and biggest resources of fermentable saccharides for biotransformation to bioethanol and further valued components (Harner *et al.*, 2015).

Economic dependency on fossil fuels and resulting effects on climate and environment have put tremendous focus on utilizing fermentable sugars from lignocellulose (Jørgensen *et al.*, 2007). Land use for fuel production introduced ethical problems in increasing food price (Aro, 2016). A controversial debate “food versus fuel” arose, due to conflict of existing food supply with the questionable, unviable first generation of limited yield biofuels, which are derived from edible agricultural substrates like corn, rapeseed, sugar beet and others, being traditionally destined for food and animal purposes, resulting in price inflation of goods, primarily food and feed for livestock, and in a negative impact on food security. This led to the development of advanced, effective second-generation biofuels (bioethanol, bio hydrogen, methane), relying on a diversity of non-food lignocellulosic biomass and bioenergy species (Lewis *et al.*, 2015; Sambusiti *et al.*, 2015; Aro, 2016). Second-generation alcohol fuel processes, especially utilizing alternative crude residual agricultural biomass and bio-wastes, are much more environmentally and ethically acceptable also providing their disposal, compared with exploiting edible starch and sugar resources (Walker, 2011; Sarris and Papanikolaou, 2016). Low processing cost cellulosic cell wall biofuels potentially offer sustainable and economically attractive petroleum-based alternatives (Tian *et al.*, 2016). Sustainable non-food lignocellulosic carbon is greatly capable of producing alternative, renewable precursors for hydrocarbons, such as microbial lipids, primarily palmitic and oleic acid, alike to conventional oilseed plants (Fei *et al.*, 2016). Ethanol from sugarcane is superior over other biofuels, like ethanol from grains or sugar beet, and biodiesel from soybeans or rapeseed (LEAL and Walter, 2010). Attention to the production of biomass derived bioethanol continues to grow, attempting to make road transport environmentally maintainable, whereas many attempts assessed environmental merit of biofuels (Von Blottnitz and Curran, 2007). Ethanol supplementation in gasoline may affect urban air quality, and substantively endanger water resources and biodiversity, whereas relatively land intensive agricultural biomass production pollutes water sources with fertilizers and phytochemicals, which are used to improve plant development, whereas overall sustainability is largely unknown (Niven, 2005). Involvement of lignocellulosic feedstock to replace fossil power with ethanol is similar to starch crops (Niven, 2005). 2nd generation of biofuels aims to increase their quantity, sustainably applying residues of inedible parts of existing crops, including leaves, stems, and husks, and further inedible plants, like switch grass (*Panicum virgatum*), grass, different assortments of prairie grasses, forbs, as well as woody plants, *Jatropha*, entire corn, miscanthus and cereals bearing not so much grain, as well as industrial wastes like woodchips, skins and pulp derived from pressing fruits (Hill *et al.*, 2006; Inderwildi and King, 2009). They should be cultivated on agrarian marginal areas using no or little pesticides, fertilizers, and energy and then converted into synfuels or cellulose based bioethanol (Hill *et al.*, 2006). Sorghum, which is world's fifth most significant cereal creating much of agricultural residues (leaves, stalks, panicles and peduncles) in tropical regions, is as an inedible feedstock potentially able to provide large-scale high yields of ethanol, following an efficient pretreatment of its lignocellulosic material (Nasidi *et al.*, 2016). One ton sweet sorghum delivers about 640 kg sweet liquor and 360 kg bagasse after extraction, whereas about 121 liter of ethanol are produced from one ton of sweet liquor, 77l liter per ton sweet sorghum, and 157 liter per ton of bagasse corresponding to 56 l/ton of sweet sorghum (Gnansounou and Dauriat, 2005). Rice (*Oryza sativa*), reaching an annual global production of about 721.4 MT, which is major staple food in most nations of Asia, generates enormous quantity of rice straw residues of about 973.89 MT in the fields with a content per kilogram of about 14 MJ energy and 10 % humidity, which may generate bioethanol (Singh *et al.*, 2016). Rice straw contains mainly 33 to 47% cellulose, 19 to 27% hemicelluloses, 5 to 24% lignin, silica and 18.3% ash, and the highest accessible cellulose among all of agrarian crop remains (Singh *et al.*, 2016). Rice straw is rich in fermentable saccharides, and except of structural lignocellulosic carbohydrates cellulose and xylan, also contains substantial quantities of non-structural carbohydrates (62 to 303 g/kg), soft carbohydrates like sucrose, starch, fructose, glucose, and β-1,3-1,4-glucan (Park *et al.*, 2011). Bioethanol has been reported to be produced from rice straw in India, sustainably providing clean energy to supply a continuously growing energy consumption. Rice straw is usually used as source of soil nutrients, whereas bioethanol production through four main operational steps, including a pretreatment, polysaccharide hydrolysis, sugar fermentation and bioethanol distillation, is more supportable than to burn rice straw (Singh *et al.*, 2016).

Humans used during the pass of millennia cellulose of firewood as an elementary energy source, whereas recent developments in bioenergy, ranging from pellets to advancing cellulose bioethanol, request high yielding feedstock (WIKI net). Numerous different feedstock must be sustainably cultured and cost efficiently processed in various areas, to maximize production and sustainability and provide the desirable capacity of 2nd generation biofuels at a minimal cost, whereas dedicated energy crops with improved features would be an important advancing step (Simmons *et al.*, 2008). High yielding food crops such as rice, an important cereal crop of Asia with abundant residuals (polysaccharide-rich straw), are received by advanced genetic engineering techniques, to precisely manipulate the regulating mechanisms of grain production, cellulose and lignin content, and stress tolerance, and to enhance biomass saccharification. They could effectively generate biomass feedstock for animal feed and bioenergy production, reducing conflicts over land use (Phitsuwan and Ratanakhanokchai, 2014). Genetic engineering and synthetic biology have modified plant composition, to reduce conversion process cost or to develop and produce perennial feedstock with desired traits, reaching very quickly high energy capacities by spending lesser fertilizers and water (Simmons *et al.*, 2008). Alternative biomass resources, such as lignocellulosic biomass, from either forestry or agriculture, are prerequisite to lower water and energy consumption without competing with food supplies (Lavoie *et al.*, 2011). Nonfood feedstock, including high developing plantation trees like *Populus* and *Eucalyptus* which are broadly representative for the Northern or Southern Hemisphere, which produce large amounts of woody biomass

(>50 m^3 ha^{-1} yr^{-1}), offer advantages for energetic, environmental and economic criteria and renewable sources of lignocellulose feedstock (Hill *et al.*, 2006; Mizrachi *et al.*, 2012). Urban solid wastes are further large resource, followed by agricultural remains and devoted energy crops. Amongst biomass sources, comprising short cycling woody and herbaceous crops, mainly tall grasses are the biggest and mostly favorable feedstock resources in coming years. They achieve many harvests from a sole planting, semantically reducing average expenses yearly for establishment and management of energy crops, specifically compared with conventional crops (Hoogwijk *et al.*, 2003; Lin and Tanaka, 2006). Corn based bioethanol technology is well-known, though the exploration of inedible feedstock is strengthened due to argument over consumption of edible feedstock (Devarapalli and Atiyeh, 2015). Evidencing shortcoming of fossil fuels, direct processing of lignocellulosic biomass into ethanol has gained attraction as financially practicable route towards manufacturing of fuels and other high value chemicals (Akinosho *et al.*, 2007). 2nd generation biofuels do not depend on grain crops, but use a diverse set of sustainably grown and cost-effectively processed feedstock (Anastassiadis, 2016). Second generation ethanol production utilizes cheaper, more competitive, and non-food feedstock like lignocelluloses or municipal solid waste, by a more complex upstream process, which is transformed into bioethanol by hydrolysis and subsequent fermentation. Long-term, lignocellulosic bioethanol will expectably succeed because of its independence from food industry, while its production cost will fall by forth on technological advances and developments (Gnansounou and Dauriat, 2005; Anastassiadis, 2016). Recalcitrant and challenging to process lignocellulosic biomass will be the global bulk feedstock resource in future's bio economy (Kumar and Murthy, 2011). Carbohydrate hydrolysis is accomplished by interaction with cellulases or xylanases, or by treatment with inorganic acids (Martin, 2014). Lignocellulosic bioethanol industry relies on cellulases and hemicellulases, almost exclusively stemming from filamentous fungus *Trichoderma reesei*, generating various monomeric sugars such as d-glucose, d-mannose, d-galactose, l-arabinose and d-xylose (Xu *et al.*, 2015). Enzymatic cellulose degradation is a procedure yielding fermentable glucose, consequently being very significant for the biotechnology (Guerriero *et al.*, 2016). Microbial cellulosic ethanol production basically comprises four critical steps: (1) Biomass pretreatment of lignocelluloses by thermal, chemical, biochemical, microbial approaches or their combinations (e.g. dilute acid or alkali, hot water and steam explosion) to decrease the recalcitrance, (2) Chemical or biological hydrolysis by cellulase enzymes of sugar polymer (cellulose, hemicellulose etc.) to fermentable carbohydrates containing either monomeric or polymeric C6 and C5 saccharides, (3) Microbiological conversion of sugar monomers into ethanol by fermentation and (4) Downstream processes comprising the purification and concentration of ethanol (Akinosho *et al.*, 2007; Kumar and Murthy, 2011). Barrier with biofuels is the lack of economic conversion technologies (Lin and Huber, 2009). Some of these steps need to be further improved to break through the barriers and commercialize the process. Harnessing structural sugars of complex plant fibers and commencement of lignocellulosic biomass and cellulose processing requires primary to overcome plant's biomass recalcitrance. Cell wall bound sugars are easier released and converted into renewable fuels, by improving (i) rather low kinetic rates of cellulosic conversion towards saccharides, (ii) lower sugar yields compared with other plant polysaccharides, and (iii) lignin removal, which is a rather inflexible polymer consisting of phenylpropanoid subunits (Himmel *et al.*, 2007). Due to complexity of cellulose and cellulase, the mechanism of enzymatic hydrolysis of cellulose is not yet sufficiently understood, whereas inexpensive maximization of sugar titers has not been adequately achieved (Yang *et al.*, 2011). Cellulose, plant cell wall's furthermost plentiful component, is a linear homo-polysaccharide with a polymerization degree of up to 10,000 and higher, and average molecular mass of ~100,000 Da, entirely composed of D-glucose units linked together by β-1,4-glucosidic as well as intra- and intermolecular hydrogen bonds, while the long β-glucose chains create crystalline non soluble microfibril bundles (36 chains), packed strongly together with van der Waals interactions and hydrogen bonds via the -OH groups (Gnansounou and Dauriat, 2005; Jørgensen *et al.*, 2007; Walker, 2011; Guerriero *et al.*, 2016). Cellulose includes also amorphous (disordered) regions (Guerriero *et al.*, 2016). Hemicellulose is an extensively branched heteropolysaccharide with an average molecular mass of 30,000 Da, containing pentose (arabinose and xylose) and hexose saccharides (glucose, mannose and galactose). Lignin is a very tough, three-dimensional recalcitrant material of plant's cell wall, containing di- and mono-methoxylated, and non-methoxylated phenylpropanoid units, while other minor constituents of lignocellulosic biomass include ash (inorganic minerals), pectins (highly branched polysaccharides of galacturonic acid and its methyl esters), acids and extractives (extracellular and non-cell wall material) (Walker, 2011). Enzyme hydrolysis is affected by the physical characteristics of cellulose as well as the function of enzyme action (Yang *et al.*, 2011). Cellulose accessibility is a main factor in efficient bio-conversion of lignocellulosic biomass to fermentable sugars (Ni *et al.*, 2006). Imaging (auto-fluorescence) revealed that enzyme binds mainly to areas with structure damage and removed lignin, occurring throughout the pretreatment process, confirming the significance of lignin removal during pretreatment (Luterbacher *et al.*, 2015). Crystallinity and specific surface area of cellulose influence speed and degree of hydrolysis. Economics of enzyme action of cellulose cleavage, suffering under relatively low hydrolysis rates and high cost of enzymes, can be significantly improved by pretreatment. Disruption of lignocellulosic carbohydrates into fermentable sugars necessitates the action of numerous diverse cellulase and hemicellulase enzymes (Jørgensen *et al.*, 2007). Cellulose hydrolysis is a stepwise cleavage of the linear chains of glucose units, whereas hemicellulases hydrolyze knotted molecular chains comprising diverse saccharides and functional groups (Jørgensen *et al.*, 2007). Genomic information collected across the biosphere, including potential energy crops and cellulolytic microorganisms, is vital for improving cellulosic biofuel production (Rubin, 2008). Highly specific bacterial (high specific activity) and fungal (*Trichoderma reesei*) cellulase enzymes, a mixture of enzymes operating in mild environments at pH 4.8 and high temperatures of 45 to 50ºC, usually hydrolyze cellulose to form reducing sugars, including glucose, while sugar fermentation is carried out with different yeasts and bacteria (Sun and Cheng, 2002). Numerous organisms, including fungi, protozoa, bacteria, animals and plants, synthesize different cellulases, which cleave the β-1,4 linkages of cellulose molecules. Many bacteria possess cellulase systems, usually comprising numerous diverse enzymes with specific kinetics and activities which are similar to cellulolytic fungi, from which some are glycoproteins (Gilkes *et al.*, 1991). Long *et al.* (2016) isolated hemicellulolytic and cellulolytic fungus *Eupenicillium parvum* from soil, optimally grown at 37°C, while its hemicellulolytic and cellulolytic enzymes displayed maximum catalytic ability at acid pH between 4,5 and 5,0 and temperatures between 55°C and 70°C, as well as high efficiency at releasing ferulic acid (4-hydroxy-3-methoxycinnamic acid) from wheat bran. Porosity (permeability) of waste materials referring to the accessibility of surface region, fiber crystallinity of cellulose, as well as sharing amounts of hemicellulose and lignin are parameters that have been detected to influence hydrolytic cleavage of cellulose (McMillan, 1994). The removal of hemicelluloses is highly desirable for their bioconversion into ethanol (Jianguo *et al.*, 2015), whilst lignin removal can dramatically increase hydrolysis rate, because it blocks entrance of cellulase enzymes to cellulose complex (McMillan, 1994; Sun and Cheng, 2002). Cellulose hydrolysis follows three steps, namely adsorption of cellulase enzymes onto cellulose surface, cellulose biodegradation to fermentable sugars, and desorption of cellulase, while cellulase activity decreases during hydrolysis (Sun and Cheng, 2002). The cellulase enzymes might be reextracted mainly from fluid supernatant and solid remains, thus reducing the production cost (Sun and Cheng, 2002). Significant improvements in pretreatment modifying lignocellulosic structure, and hydrolysis by different cellulases and hemicellulases, as well as yield and cost reduction enable large-scale fermentation of lignocellulosic substrates, the largest known renewable carbohydrate source (Jørgensen *et al.*, 2007). Ethanol production cost and thus selling price, as well as utilization of energy are strongly dependent on process conditions, whereas semantic production cost decreases can be achieved by raising the effectiveness of fermentative pentose utilization and lowering feedstock and enzyme costs (Kumar and Murthy, 2011). Recycling of challenging in large amounts required high cost cellulolytic enzymes from insoluble fraction can maximize productivity by 30-50% and yields of renewable fuels derived from lignocellulosic biomass (e.g. sugarcane bagasse, corn stover), decrease necessary enzyme amount by 30%, and reduce operating costs. Increasing lignin concentrations did not negatively influence hydrolysis efficiency (Weiss *et al.*, 2013; Visser *et al.*, 2015). The combination of peroxide and acid prior treatment is an efficient and eco-friendly procedure for enzymatic hydrolysis of napier grass, dramatically improving substrate hydrolyzability, producing 287.81 mg of glucose and 245.81 mg of xylose per g of starting dry material (Bohórquez *et al.*, 2014). The addition of surfactants to hydrolysis blend, such as ethylene oxide polymers like poly (ethylene glycol), adsorbing lignin surfaces and thus reducing unproductive enzyme binding has been reported to increase enzymatic hydrolysis of lignocellulose and softwood lignocellulose from 42% without addition to 78% in 16 h. Moreover, the requirement of large amounts of enzymes and the conversion time are both reduced operating at 50ºC (Börjesson *et al.*, 2007). Cellulose substrate is primarily treated either by chemical or enzymatical means, to break down the polymeric components to access C5 and C6 saccharides for microbial ethanol production (Devarapalli and Atiyeh, 2015). Diverse pretreated lignocellulosic biomass feedstocks, like grass clipping, catalpa sawdust, corn straw, and pine sawdust, by homogenization under elevated pressure (10 MPa) enhances enzymatic digestibility, yielding more reducing fermentable sugar under mild natural conditions by decreasing particle size, destructing structure, and changing crystallinity (Jin *et al.*, 2015). Hydrophobic kraft lignin enhances slightly enzymatic hydrolysis, while hydrophilic sulfonated lignin improves effectively the enzymatic cleavage of green liquor and pretreated resources by acidic bisulfite, but has slight influence on sulfite formaldehyde pretreatment (Wang *et al.*, 2015). Cellulase is currently commercially produced either by submerse or solid fermentation mostly using *Trichoderma* or *Aspergillus* and derivative strains, nevertheless almost all of commercial factories prefer the submerse fed-batch processes, reaching titers (by weight) higher than 100 g/l of low-cost rough cellulases at an estimating cost between 10 and 40 $/kg dry protein (Zhang and Zhang, 2013). Recombinant cellulolytic engineering introduces heterologous cellulase genes into well-established ethanol producing microorganisms like *Saccharomyces cerevisiae*, or combines cellulolytic and ethanologenic genetic engineering in model organisms, like *E. coli* or regarded as safe industrial microorganisms, e.g., *Bacillus subtilis* (Zhang and Zhang, 2013). Genetically engineered strains of *T. reesei* by pathway engineering coding pyruvate decarboxylase (ScPDC1) and alcohol dehydrogenase (ScADH1) from ethanologen *S. cerevisiae* display significantly higher ethanol yield and dramatically decreased byproduct formation of acetic acid, in comparison to wild strain. Different methodologies have been applied to reduce the inhibitory effects of various inhibitory compounds on ethanol fermentation, which are produced during the hydrolysis of lignocellulosic biomass, besides the five- and six-carbon sugars (Tesfaw and Assefa, 2014). Cellulase enzymes are also applied in various industry sectors, including textile, detergents, pulp and paper, additives for improving animal feed digestibility, as well as food (Zhang and Zhang, 2013). Reconstitution of *Neurospora crassa*'s high-affine cellodextrin transport system in *Saccharomyces cerevisiae* has been reported to efficiently promote growth on cellodextrins (Galazka *et al.*, 2010). Fan *et al.* (2016) designed and engineered a heterologous cellulose-utilization system with a cellodextrin degradation path as well as bi-functional mini-cellulosomes from cellulosomal *C. thermocellum* and cellulolytic fungus into non-cellulolytic *S. cerevisiae*, able to cleave and utilize cellulose, without inhibition or repression of glucose on cellulases and mixed saccharide uptake, to produce in a single step cellulosic ethanol at a high specific productivity. The thermophile, anaerobe *Thermoanaerobacterium saccharolyticum* digesting hemicellulose and utilizing main biomass saccharides, has been engineered and reported to reach high ethanol yields at high temperatures of 51 to 55 °C from priory treated hardwood, reaching 70 g/L in batch fermentation using a combination of maltodextrin and cellobiose (Herring *et al.*, 2016). Biomass conversion into cleanest liquid fuel ethanol has gained growing attention as an alternative to fossil fuels, however complete and efficient sugar utilization is an essential prerequisite for cost effective ethanol production. Significant developments have been accomplished in ethanol processes, comprising fermentation converting xylose to ethanol, cellulase enzymes hydrolyzing lignocellulosic materials, microorganism immobilization in large fermenters, simultaneously applying saccharification and ethanol fermentation (Lin and Tanaka, 2006; Tesfaw and Assefa, 2014), as well as strain improvement by genetic engineering and recombinant DNA technology (Aristidou and Penttilä, 2000).

Ethyl alcohol (ethanol) is a product of primary microbial metabolism, synthesized by the fermentative conversion of saccharides, or fermentable sugars derived by polysaccharide depolymerization. *S. cerevisiae* ferments hexoses, reaching about 10 to 12 % ethanol (v/v) after 5 days, which slows down growth and ceases fermentation, whereas *Pichia stipitis* or *Candida* species utilize pentose. Special yeasts can reach titers of 20 % alcohol (v/v), but it takes months or years. *Kluyveromyces fragilis* or *Candida species* can convert lactose or a pentose, respectively (Demain, 2009). Temperature, pH, oxygen (pO_2), medium composition, initial sugar concentrations, organic acids, dissolved solids, and yeast immobilization essentially influence yeast growth and ethanol production. Cell immobilization enables biocatalyst recycling, enhances ethanol productivity, reduces contamination risk, facilitates cell separation, elongates cell activity, minimizes production costs, reduces fermentation time, and protects cells from inhibitors. Industrial cellulosic ethanol production is still challenging due to high processing cost (Tesfaw and Assefa, 2014). High investment costs and slow fermentation rates are challenging the bio economy, which can be overcome by using high cell density cultures emphasizing on local cell density in smaller bioreactors facilitating sustainable first and second generation yeast bioethanol production (Westman and Franzén, 2015). Pejin *et al.* (2015) reported about increased triticale's amylase activity increasing glucose and maltose content, and yeast's enzymatic activity by adding calcium and magnesium ions during bioethanol fermentation during SSF processing of triticale.

Complete conversion efficient conversion of all major C5 and C6 biomass sugars in potential hydrolysates is critical and essential for the economics and efficient production of biofuel and other desired products (Harner *et al.*, 2015; Mohagheghi *et al.*, 2015). Renewable feedstock for many chemicals, hemicellulosic xylan (1,4-β-D-heteroxylans) is second highest plentiful lignocellulosic polymer of terrestrial plant cell wall, reaching depending upon plant species 35% (Dekker, 1989). Low ethanol tolerant native pentose-fermenting yeasts such as *Pachysolen tannophilus*, *Scheffersomyces* (*Pichia*) *stipitis*, *Spathaspora passalidarum* and *Scheffersomyces* (*Candida*) *shehatae* convert lignocellulosic glucose and mannose and less efficiently xylose (C5) into ethanol.Microbial strain improvement by classical and molecular means has been practiced to improve ethanol formation from xylose and lignocellulosic materials (Harner *et al.*, 2015). Traditional ethanol producing microorganisms like *S. cerevis*iae and *Zymomonas mobilis* cannot metabolize pentose sugars, being therefore of limited use for lignocellulose substrates with high pentose content, unless necessary pathway genes are inserted and expressed (Inui *et al.*, 2005). *Z. mobilis* is a unique fermentative Gram negative bacterium offering numerous advantages over other ethanol forming microbes for large-scale bio-ethanol production. Being a prokaryote, it is more amenable to genetic engineering, i.e. conventional mutagenesis, applying UV light and mutagens like 1-methyl-3-nitro-1-nitrosoguanidine (NTG), ethyl methane sulfonate and caffeine, or transposon mutagenesis, specific gene knock-out, adaptive laboratory evolution, and metabolic pathway engineering. Thus, biomass resource range is extended, pentose and hexose sugars from lignocellulosic hydrolysates are simultaneously fermented and acetate resistance is increased, thus attracting great attention in ethanol technology (Panesar *et al.*, 2006; Linger *et al.*, 2010). Recombinant *Z. mobilis* strain 8b has been improved via a continuous adaptation on hydrolysate of corn stover which was pretreated by dilute acid (Mohagheghi *et al.*, 2015). Simultaneous saccharification/fermentation processes (SSF) have been described utilizing *Z. mobilis* for the conversion of liquefied starch or cellulose to ethanol (Spangler and Emert, 1986). Simultaneous saccharification and co-fermentation has been reported for efficient ethanol production from dilute-acid pretreated biomass by metabolically engineered *Z. mobilis* or for other microorganisms. It co-fermented glucose and xylose, preferring glucose over xylose due to higher affinity of sugar transporter for glucose (Himmel *et al.*, 2007), to reach 94%

ethanol yield of theoretical maximum in a continuous co-fermentation by recombinant *Zymomonas* 39676:pZB4L (Lawford *et al.*, 2000). Fermentation was influenced by pH and acetic acid concentration. In NREL simultaneous saccharification and co-fermentation process, microorganisms and enzymes are added simultaneously to slurry, simultaneously converting cellulose and fermenting saccharides to ethanol (Badger, 2002). The central metabolism of *Corynebacterium glutamicum* has been genetically modified to form ethanol, bearing and expressing the genes of *Z. mobilis* coding pyruvate decarboxylase (pdc) and alcohol dehydrogenase (adhB) (Inui *et al.*, 2005). Oxygen deprived growth-arrested cells of recombinant ethanologenic *Corynebacterium glutamicum* R. reached high volumetric productivity and significant yield of ethanol from glucose and lignocellulosic hydrolysates in absence of cellular growth (Inui *et al.*, 2005; Sakai *et al.*, 2007). Moreover, the addition of pyruvate in trace amounts and acetaldehyde as well as the disruption of lactate dehydrogenase gene (ldhA) and inactivation of phosphoenolpyruvate carboxylase (ppc) significantly increased ethanol production, dramatically decreasing succinate formation without any lactate production, whereby intracellular concentrations of NADH in *C. glutamicum* are associated to oxygen deprived metabolic flows (Inui *et al.*, 2005).

Industrial cellulosic ethanol production is still a challenge due to high processing cost and significant scientific and technological issues (Walker, 2011; Tesfaw and Assefa, 2014). A combination of diverse enzymes is essential for the hydrolysis of cellulose and hemicellulose into monomeric saccharides (Devarapalli and Atiyeh, 2015). Consolidated bioprocessing (CBP) for biofuel production requiring an industrially relevant CBP microorganism (e.g. *C. thermocellum* DSM 1313) developed by genetic engineering, combining various phases into a single stage like hydrolytic enzyme production, rapid solubilization and hydrolysis, as well as fermentation, would improve process efficiency, eliminate the addition of exogenous hydrolytic enzymes, and reduce inhibition of sugar on cellulases (Tian *et al.*, 2016). Tian *et al.* (2016) obtained by strain evolution selected recombinant strains of *C. thermocellum* with improved growth rate, balanced expression of metabolic enzymes, reduced glucose accumulation, improved cellobiose consumption, and improved ethanol production, in both ethanol yield and titer. In contrast with hexoses, the fermentative bioconversion of pentoses in hydrolysates into ethanol and some high-value chemicals remains inefficient (Harner *et al.*, 2015). Effective simultaneous consumption of glucose and xylose is significant for cost-effective biofuel manufacture from lignocellulosic feedstocks (Wang *et al.*, 2016), specific transporters of xylose suffer however under low total activity mostly due to glucose inhibition (Wang *et al.*, 2016). To enable glucose–xylose co-utilization, an unaffected by glucose extremely active xylose-specific transporter is greatly desired in cost-effective manufacture of lignocellulosic biofuels (Wang *et al.*, 2016) F. Genuine glucose–xylose co-fermentation has been reported, using an engineered sugar transporter (Wang *et al.*, 2016). Wang *et al.* (2016) obtained mutant AN25-R4.18 from xylose-specific transporter AN25 from *Neurospora crassa* by directed evolution strategies with 43-fold capacity of xylose transport, while still keeping its high glucose specificity, enabling rather effective glucose-xylose co-consumption in high titers of mixed saccharides and high cell-density fermentation. Immobilized cells of yeast *Scheffersomyces stipites* in calcium alginate beads has been reported to produce bioethanol from hardwood spent sulfite liquors (HSSLs), a coproduct of pulp and paper production with a high pentose sugar content (xylose), under appropriate microaerophilic and pH control (pH 5.5) conditions. Moreover, Abubackar *et al.* (2015) reported about the formation of ethanol and acetic acid from carbon monoxide (CO) by *C. autoethanogenum* in gas-fed fermenters operating in continuous and batch mode. Because of substantial worries connected with longevity and sustainability, energy security, and ecological impact of fossil fuels, the anaerobe, rod shaped, Gram positive, thermophile and biofilm forming *C. thermocellum*, which was firstly isolated in 1926 by Viljoen *et al.*, has gained attention for the economical manufacture of bioethanol and additional high value chemicals like hydrogen, butanol, lactic acid, formic acid, and acetic acid, directly from lignocellulosic biomass in consolidated bioprocessing (Viljoen *et al.*, 1926. Tian *et al.* (2016) engineered successfully a *C. thermocellum* strain with improved ethanol yield and titer by adaptive evolution, deleting central metabolic pathways of byproduct formation and selecting by a two-step selection method, reaching 22.4 ethanol from 60 g/L cellulose at 39% yield, corresponding to 75 % of maximum theoretical yield. Commercial bioethanol processes can operate in batch, repeated batch fill-and-draw, fed-batch, continuous, semi-continuous or potentially immobilized and recycling systems (Walker, 2011). Future process improvements, requiring more comprehensively cell's and metabolism's knowledge, will join important process stages, thus decreasing overall process complexity and cost (Himmel *et al.*, 2007). Microbial cells will expectably conduct efficiently multiple conversion reactions and remain robust to process conditions. New generation hydrolytic enzymes will act near theoretical limits and utilize modified energy crops, serving as improved substrates, or will carry genes encoding for self-deconstruction, which are stimulated before harvest or at growth cycle termination (Himmel *et al.*, 2007). The development of high effectiveness and viable biofuel generating systems in industrial microorganisms requires the engineering of more robust and superior strains for commercial manufacture to bypass stress tolerance (Fu *et al.*, 2016). Fu *et al.* (2016) reviewed regulators related to biofuels tolerance and regulatory mechanisms that have been principally constructed based on proteomic and transcriptomic analyses to improve biofuel stresses tolerance.

Metabolite and lipid production by oleaginous and non-oleaginous microorganisms: Replacement of chemical production by ecologically friendly energy-efficient technologies to produce of valuable metabolites is a principal strategy of developing biotechnological industry all over the world (Morgunov *et al.*, 2013). Biofuel production using engineered microbes has gained interest due to current CO_2 emission trends (Zhang *et al.*, 2016), while bio-oil based chemistry is one of most promising alternatives to petroleum for the production of fuels and chemicals. Industrial biotechnology provides environmentally friendly bio-based products replacing petroleum-based industries (Fei *et al.*, 2016). Microorganisms overproduce under certain conditions a variety of metabolites utilizing various carbon sources (Anastassiadis, 2016). Restrictions of 1st generation biofuels have caused attention in genetically engineered microorganisms to convert abundant and inexpensive feedstock into biofuels (Zhang *et al.*, 2016). Fermentation carbon sources are usually obtained from agricultural commodities, used as foodstuffs (Fei *et al.*, 2016). All forms of life, including bacteria, yeast, plants and animals synthesize triacylglycerol (TAG) under nutritional stress and excess and depletion (Coleman and Lee, 2004). Microbial biodiesel fuels (fatty acid methyl esters) originated from oleaginous microorganisms, like microalgae, bacteria and yeasts, are potential renewable alternatives for petrol diesel (Coleman and Lee, 2004). Microbiological oil is an alternative substitute to biodiesel obtained from edible crops (Sitepu *et al.*, 2014). Biological lipids share striking similarity with petroleum hydrocarbons (Zhang *et al.*, 2016). Microbial fatty acids are overproduced by numerous strategies and converted into biofuels such as alkanes, fatty alcohols, and fatty acid methyl or ethyl esters (Sherkhanov *et al.*, 2016). Synthetic biology tools and methods simplify microbial modification and improvement, drastically affecting productivities and titers as well as growth rates of modified microorganisms (Zhang *et al.*, 2016). Microbial biofuels are more ecologically benign and financially feasible, helping to dispose various industrial wastes (Dobrowolski *et al.*, 2016). Practical interest has received heterotrophic microbial lipid and single-cell oil production (enriched in essential polyunsaturated fatty acids) by oleaginous microorganisms (more than 20% up to >70%, de novo synthesis ad ex novo accumulation), fatty-acid bioconversion and substrate valorization for the synthesis and manufacturing of fuels, specialty chemicals, and bulk chemicals from inexpensive biomass sources (Beopoulos *et al.*, 2009; Donot *et al.*, 2014; Qiao *et al.*, 2015; Friedlander *et al.*, 2016). Bulk chemicals refer to production capacity of more than 10,000 tons yearly. Lipogenic organisms provide an ideal platform for biodiesel and oleochemical industry (Liu *et al.*, 2015). Oleaginous microbes convert a diverse range of feedstock into single cell oils, which are transesterified to biodiesel as an important alternative renewable biofuel (Dobrowolski *et al.*, 2016), presenting several advantages over plant-based oils, suffer however under high production costs (Ledesma-Amaro *et al.*, 2015). Oleaginous yeasts convert sugars accumulating over 20% of their cell mass as intracellular lipids with similar fatty acid profiles to plant oils containing primarily C16 and C18 fatty acids, which are suitable to produce alternative second-generation fuels including biodiesel (Sitepu *et al.*, 2014; Slininger *et al.*, 2016). Among certain oleaginous yeasts, *Cryptococcus humicola* UCDFST 10-1004 accumulated 15.5 g/L lipids from 36 g/L authentic cellular biomass, suitable for the production of biodiesel and other valuable oleochemicals (Sitepu *et al.*, 2014). *Y. lipolytica* uses an ex novo process to synthesize intracellular lipids by incorporating exogenous fatty acids and lipids (Zhu and Jackson, 2015), which is in contrast to de novo lipogenesis a growth-associated process entirely independent of nitrogen exhaustion (Donot *et al.*, 2014).

Lipid yields of lignocellulosic biomass vary depending on oleaginous yeast species, feedstock types, and cultivation conditions (Sitepu *et al.*, 2014). De novo synthesis of intracellular lipids belongs to anabolic metabolism generating fatty acids from acetyl-CoA of intermediate cellular metabolism by means of an inverted β-oxidation (Donot *et al.*, 2014). Under certain abiotic (pH, temperature), excess carbon and nutrient limiting conditions affecting biomass production (especially nitrogen), many oleaginous microorganisms significantly differing in lipid content and fatty acid profile, especially the well-characterized yeast *Yarrowia lipolytica* developing very sophisticated and effective mechanisms for regenerating and assimilating hydrophobic compounds, produce grown on various carbon sources including glycerol in various fermentation configurations such as batch, repeated batch, fed-batch, repeated fed-batch or continuous manner, above 60 % of their dry biomass in single cell oils and microbial lipids (fatty acids), especially unsaturated triglycerides, infrequently existing in plant or animal world, which are of great attention in numerous industrial areas, e.g., food, chemical, and energy manufacturing (Beopoulos *et al.*, 2009; Donot *et al.*, 2014; Friedlander *et al.*, 2016). *Y. lipolytica*, regarded as safe and robust yeast with a long history of commercial use, is an excellent cell host for metabolic engineering due to its physiological, metabolic and genomic characteristics, and a platform cell factory for economical manufacture of chemical compounds and fuels generated from fatty acids, lipids and acetyl-CoA (Zhu and Jackson, 2015). *Y. Lipolytica* is usually considered as a harmless non-pathogenic and versatile yeast, displaying unique physiological and biochemical properties, which are considerably significant in food-related applications, such as production of food-additives like aromatic substances, organic acids, polyalcohols as well as surfactants and emulsifiers (Ledesma-Amaro *et al.*, 2015). Wild type *Y. lipolytica* strains utilize glucose, fructose, glycerol and hydrophobic substrates as single carbon source, while using sugars from in vivo lignocellulosic processes would significantly reduce the sugar cost (Zhu and Jackson, 2015). Crude glycerol from biodiesel derived from plants has been applied by numerous authors as single carbon for fungi and yeasts synthesizing oils (Donot *et al.*, 2014; Dobrowolski *et al.*, 2016). Dobrowolski *et al.* (2016) reported about the direct production of single cell oil with a defined fatty acid composition from various industry low-cost unpurified crude glycerol wastes by wild type *Yarrowia lipolytica* A101 (green chemistry), reaching 4.72 g L^{-1} with a biomass yield of 0.21 g g^{-1}, composed majorly of oleic acid (C18:1) with 44 to 55 % of fatty acids, palmitic (C16:0) and linoleic (C18:2) acids (10–20%) and less stearic acid (<10%). High mono-unsaturated fatty acid amount, such as oleic acid, is favoured in biofuel business due to better cold flow characteristics and decreasing tendency to crystalization. A high enhancement of linoleic acid production by *Y. lipolytica* occurring during scale-up has observed reported by various authors (Tai and Stephanopoulos, 2013; Dobrowolski *et al.*, 2016). Dimorphic, non-pathogenic obligate aerobic ascomycetous yeast *Yarrowia lipolytica*, the most widely studied and engineered oleaginous yeast by means of different molecular and bioinformatics tools and systems metabolic engineering strategies, can produce usual and unusual fatty acids in white biotechnology and it accumulates high amounts of lipids from glucose as sole carbon sources (Ledesma-Amaro *et al.*, 2015). Genome sequencing and strain engineering applying genetic manipulations and metabolic modifications by suitable genetic tools and rational metabolic engineering of *Y. lipolytica* can potentially increase lipogenesis titers, lipid yield by upper regulation of lipid biosynthesis and down regulation or removal of competing pathways in high lipid producing biocatalysts, to cost-effectively produce large-scale microbial lipids (bio-oil) and functional fatty acids for biofuels, biochemical and biotechnological use (Beopoulos *et al.*, 2009; Liu *et al.*, 2015; Zhu and Jackson, 2015; Friedlander *et al.*, 2016). Tai and Stephanopoulos (2013) first developed an expression platform utilizing an intron containing translation elongation factor-1α (TEF) promoter, which is able to increase gene expression by 17-fold above intron less TEF promoter. Overexpression of acetyl-CoA carboxylase (ACC1), the first step of fatty acid formation, increased lipid content by 2-fold, whilst overexpression of diacylglycerol acyltransferase (DGA1), the final step of triglyceride (TAG) formation pathway, gained a 4-fold increase (Tai and Stephanopoulos, 2013). Technological, it is much easier to utilize a wild type microorganism to generate biodiesel or PUFA's rich diet additive by lonely altering carbon substrate, than genetically engineered microorganisms, which have poorer constancy and growth stability. Otherwise, wild type strains can be excellent lipid producers (Dobrowolski *et al.*, 2016). (Slininger *et al.*, 2016) identified oleaginous yeast strains capable to convert acetic acid and almost all accessible saccharides of low cost, and renewable hydrolyzed lignocellulosic plant biomass to lipids, to accumulate 50-65% lipid (w/w) in cell biomass and to reach 25 to 30 g/L lipid at pH 6 and 7, increasing the market capacity for lignocellulosic biofuels beyond bioethanol. Fed-batch cultivation is commonly used in various industrial applications, achieving high cell densities along with high lipid yield and productivity on lignocellulosic materials (Fei *et al.*, 2016).

Biodiesel (methyl esters of fatty acids) is presently completely produced from plant oils, while microbial fatty acid biosynthesis is firmly controlled at manifold levels. *E. coli* genome has been multiply engineered, to produce minimum 50 % of fatty acids as free acids. Considerable knowledge has been gained meanwile about enzyme catalysis and regulation in triacylglycerol biosynthesis (Coleman and Lee, 2004). Carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase is the first step in fatty acid metabolism, followed by the iterative condensation of acetyl-CoA starter and several malonyl-CoA extender molecules, to synthesize depending of organism a linear acyl chain consisting usually of 12 to 22 carbons, which are freed in form of acyl thioesters, linked to either coenzyme A or to acyl carrier protein (Zhang *et al.*, 2016). ATP-citrate lyase (ACL), Acyl-CoA carboxylase (ACC) and Malic enzyme (ME) principally generating NADPH are the key enzymes for lipid synthesis, which utilize acetyl-CoA, malonyl-CoA and glycerol, as well as energy (ATP, NADPH) to (Beopoulos *et al.*, 2009). AMP deaminase activated by medium nitrogen exhaustion in oleaginous microorganisms is involved in the regulation of lipid synthesis. Consequently, mitochondrial AMP inhibiting isocitrate dehydrogenase decreases, interrupting TCA at isocitrate level, simultaneously increasing cellular ammonium concentration. Aconitase enzyme mediates citrate accumulation in mitochondria that exits via citrate/malate cycle, providing huge quantities of acetyl-CoA for cytosolic fatty acid production and oxaloacetate by ATP-citrate lyase (ACL). Malonyl-CoA necessary for fatty acid formation is formed from acetyl-CoA, in a reaction catalysed by acetyl-CoA carboxylase (Beopoulos *et al.*, 2009). Malic enzyme (ME; $NADP^+$-dependent; EC 1.1.40) converts L-malate to pyruvate and CO_2 providing NADPH for lipid synthesis in oleaginous microorganisms, its role is however unclear and isn't the main source of NADPH for lipid accumulation in oleaginous *Y. lipolytica*. Conclusively, auxiliary resources of NADPH are essential for lipogenesis (Zhang *et al.*, 2013; Ratledge, 2014). ^{13}C-Metabolic flux analysis identified oxidative pentose phosphate pathway as the major source of lipogenic cofactor NADPH correlating with the lipogenesis, instead of flux through malic enzyme, in *Y. lipolytica*. Genomic sequencing of evolved *Y. lipolytica* strains revealed the importance of gamma-aminobutyric acid assimilation in lipogenesis, at succinate semialdehyde dehydrogenase level (Liu *et al.*, 2015). The theoretical maximum of sugar to lipid conversion is 0.276 g/g (Ratledge, 2014), whilst reaching theoretical yield is a big challenge for most products because of still our limited knowledge of the physiology and biochemistry of wild type and engineered strains (Zhu and Jackson, 2015). High yield and rate microbial conversion of carbohydrates to lipids is essential for cost-effective industrial production of renewable biodiesel, however conversion yields and rates are usually low, primarily due to allosteric inhibition of lipid biosynthetic pathway by saturated fatty acids at delta-9 stearoyl-CoA desaturase (SCD) as a rate limiting step (Qiao *et al.*, 2015).

Creative innovations in fermentation engineering and downstream processing would realize widely used industrial biological applications of *Yarrowia* or other microorganisms, for example advanced continuous high yield, rate and titer fermentation processes utilizing raw and waste materials as substrate, thereby decreasing capital investment in fermentation and downstream process equipment (Ledesma-Amaro *et al.*, 2015; Zhu and Jackson, 2015). Ledesma-Amaro *et al.* (2015) expressed heterologous α-amylase and glucoamylase in *Y. lipolytica* to utilize starchy raw materials towards fatty acids, which was enhanced even more by expressing a second set of these enzymes and in addition to metabolic engineering by culture condition optimization. Other metabolites are often conjointly produced by *Y. lipolytica* beside the production of SCO like citric acid, sodium citrate, a sublement for environmentally harmless phosphate-free cleaners replacing the environmentallys hurmfull sodium triphosphate, isocitric acid for sport medicin and food industry, and enzymes like lipases, invertases, amylases, and b-galactosidases (Donot *et al.*, 2014; Kamzolova *et al.*, 2015). Recombinant *Y. lipolytica* has been created through heterologous expression of itaconic acid synthesis enzyme, able to serve as a platform for itaconic acid production, a naturally formed organic acid with various utilizations to replacement petroleum based products. Metabolic pathway engineering, enzyme localization, and media optimization improved itaconic acid formation in bioreactors 140-fold over initial titer.

World's energy demand has seen a rapid rise in recent decades, owing to population growth and global industrial development (Souza *et al.*, 2014). Rising worries over energy security and climatic alterations led in to development of more maintainable and ecologically friendly biofuels as substitutes (Fu *et al.*, 2016). Rapid development in biofuel industries, including biodiesel currently produced in large-scale operations, provides geopolitical, environmental and economic benefits, offering one of little choices for mobility requirements (Rywinska *et al.*, 2010). Transesterification of plant oils to biodiesel is connected to semantic by production of glycerol rich water, so-called "crude" or "raw" glycerol (Rywinska *et al.*, 2010; Przystałowska *et al.*, 2015). Synthesis of 10 kg of biodiesel from esterified rapeseed oil generates 1 kg of glycerol (Meesters *et al.*, 1996). Purification of crude glycerol is uneconomical, consequently production of valuable substances from raw glycerol by various means, such as 1,3-propanediol, poly(3-hydroxybutyrate), mannitol, erythritol, and citric acid, is critical for a durable sustainability of biodiesel businesses (Ferreira *et al.*, 2016). Majority of worldwide microbial production of citric acid, particularly in developed countries, utilizes *Aspergillus niger* in a carbohydrate based submerged batch fermentation, mostly with molasses as carbon source (Arzumanov *et al.*, 2000; Crolla and Kennedy, 2001; Darvishi *et al.*, 2009). Citric acid is a product of energy metabolism, rising in appreciable titers only under conditions of substantive metabolic imbalances. The mechanism of citric acid overproduction is yet not entirely understood (Papagianni, 2007). Nonconventional yeast *Y. lipolytica* utilizes highly effectively hydrophobic compounds, like *n*-alkanes, raw agro-industrial fat, fatty acids, fats, and oils (e.g. rapeseed oil), to produce and secrete under conditions of nitrogen limitation a broad range of organic acids including tricarboxylic acid (TCA) cycle intermediates, like citric, isocitric (ICA), 2-ketoglutaric and pyruvic acid, single-cell protein, single-cell oil, various lipases (extra- and intracellular, membrane-bound activities), and so forth (Kamzolova *et al.*, 2003; Darvishi *et al.*, 2009; Kamzolova *et al.*, 2011). Restriction of inorganic nutrients (particularly nitrogen) and surplus of carbon source is a precondition for CA accumulation in *Y. lipolytica* (Kamzolova *et al.*, 2003; Liu *et al.*, 2015). *Y. (Candida) lipolytica* produces CA under intracellular nitrogen limitation, beginning CA excretion a few hours after depletion of nitrogen grown on glucose (Anastassiadis *et al.*, 2002; Anastassiadis *et al.*, 2008) or ethanol (Arzumanov *et al.*, 2000) and a wide range of other carbon sources (Stottmeister *et al.*, 1981; Kamzolova *et al.*, 2005; Ferreira *et al.*, 2016). CA biosynthesis is an indirectly growth-associated metabolism (Elmer and Gaden, 1959), necessitating to separate growth and synthesis either in time or in space (Arzumanov *et al.*, 2000). During CA/ICA overproduction by glucose-grown *Saccharomycopsis lipolytica*, the specific activities of citrate synthase, aconitate hydratase, NAD+-linked and NADP+-linked isocitrate dehydrogenase decline semantically after nitrogen depletion, whereas pyruvate carboxylase activity stays rather constant corresponding to production rate changes (Franke-Rinker *et al.*, 1982). CA/ICA ratio of *Y. lipolytica* depends on particular yeast strain and fermenter parameters, such as pH, oxygen availability and medium composition (Ferreira *et al.*, 2016). Kamzolova *et al.* (2013) reported about ICA production from rapeseed oil by *Y. lipolytica*. *Y. lipolytica* advantageously displays a broader substrate variety, a lower sensitivity to low dissolved oxygen saturations and heavy metals, and higher product yields, compared to *Aspergillus* (Darvishi *et al.*, 2009; Ferreira *et al.*, 2016). Utilization of glycerol as sole carbon source to synthesize compounds of commercial interest such as organic acids, including citric acid, has been very attractive in recent times (Da Silva *et al.*, 2009). West (2013) reported concerning the formation of citric acid by five different *Candida* species grown on soy biodiesel-based crude glycerol, whereby *C. guilliermondii* ATCC 9058 was furthermost efficient species. Insoluble hydrocarbons serve as carbon sources for a great diversity of microorganisms, comprising bacteria, filamentous fungi and yeasts (Zinjarde and Pant, 2002). Hydrocarbons have the ability to provide high amounts of citric acid by yeasts like *C. lipolytica* (Stottmeister *et al.*, 1981; Crolla and Kennedy, 2001). Amongst investigated plant oils, olive oil showed to be the greatest medium for lipase and CA formation (Darvishi *et al.*, 2009). *Y. lipolytica* 187/1 grown on rapeseed oil has been reported to reach 135 g/L CA and a specific production rate of 127 mg/(g·h) under optimized conditions (Kamzolova *et al.*, 2005). Citric acid has also been reported to be produced from waste cooking oil as sole carbon source by *Y. lipolytica* SWJ-1b (Liu *et al.*, 2015), or from ethanol by a strain of *Y. lipolytica* in efficient continuous repeated batch (RB) mode eliminating time required for sterilization and inoculation, reaching 105.4 g/l CA within 3 days (Arzumanov *et al.*, 2000). Kamzolova *et al.* (2015) reported about the production of technical sodium citrate from crude glycerol by *Y. lipolytica*, substituting the ecologically harmful synthetic detergent sodium triphosphate. Continuous RB is a dynamic process operating in continuous mode, taking advantage of batch and continuous fermenter, wherein different values of abiotic fermenter parameters can be set during growth or production. CA concentrations between 99 and 110 g/l have been reached under optimized conditions in continuous repeated batch fermentations (RB) by *Y. lipolytica* from glucose within 45-51 h in stirred fermenter at 80% air saturation. A maximum differential volumetric productivity of up to 5.5 g L^{-1} h^{-1}, integral productivities between 1.9 and 2.22 g L^{-1} h^{-1} and a maximum CA/ICA ratio of 18.6 had been determined. A maximum integral biomass specific productivity (BSP) of 0.6 g g^{-1} h^{-1} and differential BSP of 0.13 g g^{-1} h^{-1} were also calculated. Moreover, the fastest growth and production was obtained with fructose among glucose, fructose and sucrose as single carbon source (unpublished). 250 g/l of citric acid have been produced from glucose by *Y. lipolytica* in continuous repeated fed batch process with continuous feeding into fermenter at MIT and Pythia Institute of Biotechnology (Anastassiadis *et al.*, 2008). Similarly, 504 g/l of gluconic acid have been produced from dextrose by isolated strains of *Aureobasidium pullulans* in fed batch process and 420-450 g/l in continuous chemostate cultures at more than 90-95% yields, extending the frontiers of capability of unseen microbial world, industrial microbiology and fermentation technology. 272–308 g/l have been produced under continuous cultivation of free-growing cells of *Aureobasidium pullulans* at 19.5-24 h residence times (RT) in the first bioreactor and 350–370 g/l in the second bioreactor at total RT of 30.8–37 h in a cascade process of two fermenters (Anastassiadis *et al.*, 2003; Anastassiadis and Rehm, 2006). As a multifunctional bulk carbonic acid, gluconic acid itself, the gluconolatone form and its salts (e.g., alkali metal salts, in sodium gluconate, especially) have found extensively multipurpose usages in chemical, pharmaceutical and in cement industry as a cement additive (Anastassiadis and Rehm, 2006; Anastassiadis *et al.*, 2008). Organic acids represent the third largest category in global market of large volume bulk chemicals obtained by biotechnological processes after antibiotics and amino acid (Anastassiadis *et al.*, 2008). Various yeasts of different genera and species has been reported to produce pyruvic and alpha-ketoglutaric acid (KGA) from glucose or ethanol under growth limitation by thiamine (Chernyavskaya *et al.*, 2000; Kamzolova and Morgunov, 2016), which's concentration has been reported to have a crucial effect on pyruvic acid formation by thiamine-auxotrophic yeast *Y. lipolytica* depending on carbon and energy source used (Morgunov *et al.*, 2004). α-ketoglutaric acid has been produced under thiamine limitation by thiamine-auxotrophic *Yarrowia lipolytica* VKM Y-2412 growth on ethanol, reaching under optimal conditions 88.7 g/L of a-ketoglutaric acid (Kamzolova *et al.*, 2012). a-ketoglutaric acid has been transformed by chemical treatment with hydrogen peroxide to significant quantities of succinic acid (71.7 g/L), which formed diethyl succinate by further direct esterification of succinic acid with excess absolute ethanol (Kamzolova *et al.*, 2012). *Blastobotrys adeninivorans* VKM Y-2677 produced 43.2 g l^{-1} of pyruvic acid with a product yield of 0.77 g/g as a result of thiamine-dependent pyruvate and α-ketoglutarate dehydrogenase inhibition (Kamzolova and Morgunov, 2016), which has thiamine as a cofactor. Thiamine-auxotrophic *Y. lipolytica* N 1 has been reported to produce alpha-ketoglutaric acid from ethanol reaching 49 g/l and a yield from ethanol consumed of 42%. Thiamine, nitrogen and oxygen concentration, and pH level are influencing (KGA) (Chernyavskaya *et al.*, 2000). *Y. lipolytica* VKM Y-2412 produced 106.5 g l^{-1} of KGA from rapeseed oil with a mass yield of 0.95 g g^{-1}, applying a three-stage pH controlling (Morgunov *et al.*, 2013).

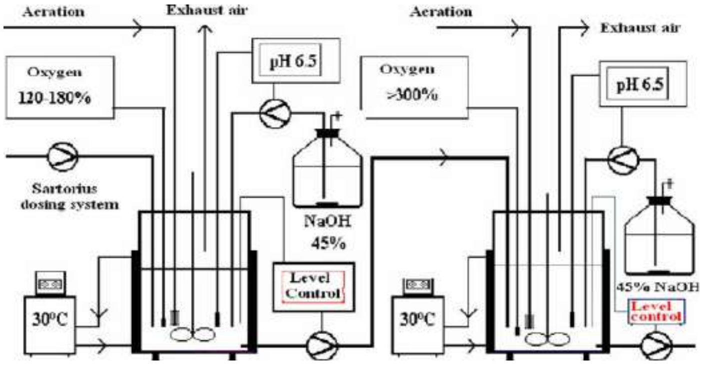


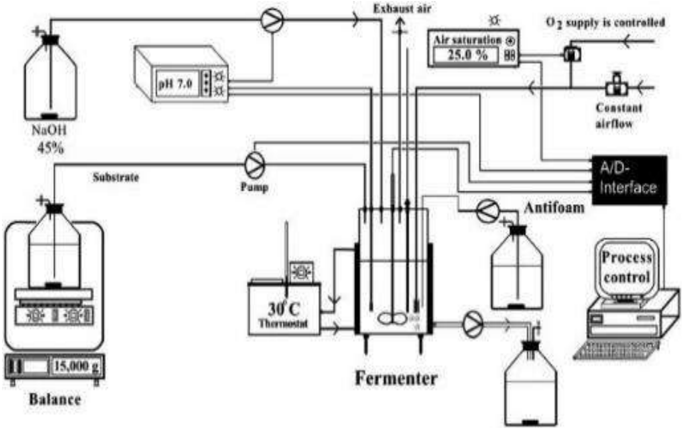
Fig. 5. Continuous gluconic acid production in a Cascade of 2 fermenters.

Liu *et al.* (2015) reported about enhanced citric acid formation by *Y. lipolytica* SWJ-1b from glucose as sole carbon source via the pyruvate carboxylation pathway, utilizing the main byproduct of corn steeping corn steep liquor (CSL), as an economic alternative source of organic nitrogen and vitamins to more expensive peptone or yeast extract, which deliver essential amino acids and growth factors for microbial growth and metabolism (Liu *et al.*, 2015). CSL has been broadly utilized in the manufacture of additional organic acids, such as succinic acid, fumaric acid and calcium malate (Liu *et al.*, 2015). Liu *et al.* (2014) reported about co-cultivation of free cells of *Y. lipolytica* SWJ-1b with immobilized mycelia of *T. reesei* in sodium alginate that produce higher enzyme activities than free cells, to produce CA from pretreated straw as sole carbon source or supplemented with glucose (83.4 g/l CA). Straw is an all over the world cheap and renewable cellulosic carbon with a complex composition (cellulose and hemicellulose intervened with lignin) (Liu *et al.*, 2014). Transformant *Y. lipolytica* PG86 which was obtained from marine yeast strain *Y. lipolytica* SWJ-1b, after the expression of a pyruvate carboxylase gene (PYC) that had been cloned from *Meyerozyma guilliermondii*, had much higher PYC activity than SWJ-1b, greatly enhancing citric acid (CA) production (Tan *et al.*, 2016). Similarly, Fu *et al.* (2016) cloned, expressed and characterized pyruvate carboxylase gene (PYC1) from marine fungus *Penicillium rubens* I607 in *Y. lipolytica* SWJ-1b. Transformant PR32 displayed much higher (133.8%) specific pyruvate carboxylase activity (0.53 U/mg) than *Y. lipolytica* SWJ-1b (0.07 U/mg), greatly enhancing (100%) citric acid formation (70.2 g/l) compared to host *Y. lipolytica* SWJ-1b (27.3 g/l). Glycerol catabolism comprises phosphorylation by glycerol kinase and subsequent oxidation by a flavin adenine dinucleotide (FAD)-dependent glycerol 3-phosphate dehydrogenase, which is located on the outer surface of mitochondrial inner membrane. Dihydroxyacetone phosphate formed enters the glycolytic pathway (Rywinska *et al.*, 2010). *Y. lipolytica* is a strictly aerobic microorganism, hence oxygen supply has a tremendous effect on yeast's metabolism and products formation, as well as bioprocess overall performance. Citric acid production by *Yarrowia lipolytica* W29 from crude glycerol increased with increasing kLa (oxygen mass transfer rate) from 7 h^{-1} to 55 h^{-1} by 7.8-fold, and at increasing dissolved oxygen (DO) up to 60% of saturation. No differences occurred in specific growth rate, biomass yield and glycerol consumption rate with increased medium DO concentrations, while CA/ICA ratio increased along with increased DO (Ferreira *et al.*, 2016). Oxygen mass transfer rate (OTR) and pH highly influence on CA production by *Y. lipolytica* W29 (ATCC 20460) and CBS 2073 (Ferreira *et al.*, 2016). Anastassiadis and Rehm (2006) determined maximum citric acid production by *Candida lipolytica* in continuous chemostat cultures with a DO of 20% (pseudo-Crabtree effect). Confluent overexpression of PYC from *Pichia guilliermondii* Pcla22 and endogenous ACL1 gene encoding ATP citrate lyase has been reported to enhance formation of intracellular lipid of oleaginous yeast *Y. lipolytica* ACA-DC 50109 (Wang *et al.*, 2015).

Inexpensive carbon sources such as industrial wastes are required to reduce substrate and process costs and to compete with petroleum-based production. Advances in metabolic engineering expanded carbon source range of industrial yeast *Yarrowia lipolytica*, to produce biofuels and biochemical (Ledesma-Amaro and Nicaud, 2016). Lazar *et al.* (2011) reported about the concomitant formation of citric acid and invertase by *Y. lipolytica* SUC + transformants grown on different carbon sources including sucrose, mixture of glucose and fructose, glucose or glycerol, reaching 57.15 g/L CA with glycerol.

Fermentation technology and process development: A great variety of microbial metabolites and bulk chemicals are produced worldwide in various types of fermenters, including carbonic acids, antibiotics, biofuels, lipids, amino acids, vitamins and fine chemicals among others, or foods and beverages starting since ancient times. Process design involves two central actions, process synthesis and process analysis, whereby process synthesis refers to selection and arrangement of a set of unit operations (process steps) able to produce the desired product at a satisfactory cost and quality. Process analysis is the evaluation and comparison of diverse process synthesis solutions. The development of a new multistep biotechnological process necessitates three main steps, including (Anastassiadis *et al.*, 2008):

1. Identification and characterization of a suitable biological system (microorganism, biocatalyst).
2. Increase of bioreactor productivity and efficiency by methodical substrate optimization and adaptation of fermentation technology to a developing process (process development and fermentation technology) (Fig. 5).



3. Downstream process (cell separation by centrifugation or ultrafiltration, separation, evaporation and drying).

Stirred fermenters, fluidized bed fermenters, or variations are used operating at different operation modes, comprising batch, fed batch, continuous repeated batch, and continuous repeated fed batch fermentations with intermittent or continuous feeding (linear or logarithmic feeding), as well as continuous single or multistage chemostat CSTR enterprise with and without biomass retention utilizing free or immobilized cells. Batch processes display some benefits compared with continuous fermenter operating, e.g., easiness in controlling microbial infection and enhanced product titers; however, they necessitate greater investments for largescale manufacture, due to reactor's lower volumetric productivity (Anastassiadis and Rehm, 2006). Batch cultivation has disadvantageously limited duration (Arzumanov *et al.*, 2000) and low volumetric productivity. RB cultivation is simple and efficient for industrial application, has the advantages offered by a continuous cultivation and is free from its disadvantages (Arzumanov *et al.*, 2000). Continuous largescale manufacture of high value biological products has received growing interest, to significantly reduce cost and facility's operation volume, while enhancing product quality and simplifying facility design (Zydney, 2016). Repeated Batch represents a cultivation process in which a part of fermentation medium is withdrawn from fermenter and fresh cultivation medium is added at fixed periods of time. Time required for cleaning, sterilization, preparation and inoculation of bioreactor (about 1 day) is deducted, resulting in saving time, increasing bioreactor's overall volumetric productivity and efficiency, as well as in reducing expenditure of fermentation. It decreases the expenses of sterilization and preparation of fermenter, inoculum preparation and inoculation, increasing economic efficiency biosynthesis. In contrast to batch process time for filling in, emptying and cleaning and/or sterilizing the fermenter can be evaded in continuous CSTR enterprise, strongly shortening necessary total production time required for inoculum preparation, inoculation, preparation of necessary equipment and fermentation. A continuous product quality is guaranteed, operating at persistent process conditions and medium composition over longer times, substantially facilitating down-streaming and reducing production costs. Continuous repeated fed batch with intermittent or continuous feeding (linear or logarithmic feeding) is very powerfull for metabolite production (Anastassiadis *et al.*, 2008). Contrarily, conventional fermentation processes require large resources, well organized infrastructure and waste treatment facilities. Oxygen availability, agitation rate, pH are key parameters strongly influencing microbial production. Moreover, various fermenter types and configurations are applied for metabolite production, employing different carbon sources.

Fig. 6. Flow scheme of continuous single stage fermenter.

Biochemical production by marine microorganisms: Marine biosphere harbors the largest wealthy flora and fauna and microbial diversity on Earth, most of which remains unexplored, offering great potential in detection of novel types of surface-active agents and a huge physiological resource of imperative useful commercial rank products. Bio surfactants, bio emulsifiers and exopolysaccharides are produced by marine microorganisms, such as *Acinetobacter*, *Pseudomonas*, *Arthrobacter*, *Myroides, Halomonas*, *Bacillus*, *Corynebacteria* and *Alteromonas* sp. (Satpute *et al.*, 2010; Sałek and Gutierrez, 2016). Most of marine microbial world is still unexplored due to enormity of marine biosphere (Satpute *et al.*, 2010). Marine yeasts, including also those found in dark, cold and anoxic environments at depths ranging from 4000 up to 11,000 m, and their genes are so versatile to produce various fine chemicals, functional biomolecules and bio-products with a diversity of numerous uses in different industries like biofuel, food, chemical, agricultural, cosmetic and pharmaceutical industry, such as enzymes, bioactive substances (siderophores, riboflavin, killer toxins, immunestimulators), single cell protein (SCP) and nanoparticles with antibacterial function (Chi *et al.*, 2016). The production of pullulan and polymalic acid by *Aureobasidium* pullulan strains, CA and high active and pH stable bio surfactants by *Y. lipolytica*, xylitol by *Williopsis saturnus* and *Debaryomyces hansenii*, long-chain *n*-alkanes by *A. pullulas* var. *melanogenum* P5, lipids for biodiesel production by *Rhodotorula mucilaginosa*, SCP by *Candida* and *Y. lipolytica*, as well as ethanol from seeweed hydrolysate by marine *Candida* sp. are some examples of the great potential of marine yeasts (Amaral *et al.*, 2006; Chi *et al.*, 2016). Marine yeasts can produce many industrial enzymes like terrestrial microorganisms, including amylase, less cellulase, lactase, exo-β-1,3-glucanase and superoxidase dismutase. Genetic engineering can extend the biotechnological opportunities of marine yeasts (Chi *et al.*, 2016). Novel bio emulsifier Yansan, a complex molecule consisting of lipid, carbohydrate and protein, possessing a great emulsification activity and wide pH stability of between pH 3.0 and pH 9.0, able to stabilize oil-in-water emulsions with numerous aromatic and aliphatic hydrocarbons, has been produced by the Brazilian wild marine strain *Y. lipolytica* IMUFRJ in glucose-based medium (Amaral *et al.*, 2006). Tropical marine *Y. lipolytica* NCIM 3589 has been reported to produce emulsifier in presence of crude oil or *n*-alkanes. *Y. lipolytica* produced a cell associated emulsifier in the earlier stages of cellular growth (Zinjarde and Pant, 2002). Emulsifiers play a significant functional role in hydrocarbon degrading organisms, while hydrocarbon and alkane uptake by *Y. lipolytica* is mediated by the attachment to large droplets (via hydrophobic cell surfaces) or is assisted by surfactants and emulsifiers (Zinjarde and Pant, 2002; Ledesma-Amaro *et al.*, 2015). An extracellular emulsifier has been produced under carbon excess and nitrogen limitation in stationary phase, in the presence of 2–3% NaCl. Emulsifier production of marine *Y. lipolytica* was in sea water 3 times greater (3 units/ml) compared to 1 unit/ml obtained in fresh water at sea water's initial pH of 8.0 (Zinjarde and Pant, 2002). Emulsifiers exist as an integral part of cell wall, to enhance crude oil or alkane (C10 –C18) binding strength and increase floc formation and therefore alkane uptake cells, while cells grown on other carbons like alcohol, sodium acetate, glucose, or glycerol lack this complex and exhibit lower hydrophobicity (Zinjarde and Pant, 2002).

Bacteria degrading hydrocarbons are usually related to the formation of surface-active agents, such as bio surfactants and bio emulsifiers, to increase hydrocarbon solubility in aqueous media and their bioavailability for biodegradation (Sałek and Gutierrez, 2016). Petroleum hydrocarbons are major pollutants of marine environment due to terrestrial and freshwater run-off (Phetrong *et al.*, 2008). Specialized bacteria even utilize hydrocarbons nearly exclusively as single carbon and energy source (Sałek and Gutierrez, 2016). Surface-active biopolymers have ecologically significant purposes in marine environments, including microbiological adhesion to solid surfaces, biofilm development, and hydrocarbon oil emulsification to improve biodegradability, or mediating fate and mobility of nutritional heavy and trace elements in biogeochemical cycles (Sałek and Gutierrez, 2016). A number of marine strains of bacteria, fungi, and yeast produce unique amphipathic surface-active bio surfactants during growth on hydrocarbons, with high profitable potential and dynamic applicability in pharmaceutical, medical, cosmetic, food, pesticide, oil, and biodegradation industry (Shekhar *et al.*, 2015). Bio surfactants and bio emulsifiers have created great interest in recent years because of several advantages over synthetic chemical counterparts, including their widespread structural and functional multiplicity, environmental acceptability, great biodegradability and low toxicity (Sałek and Gutierrez, 2016). Versatile characteristics of surface active agents are finding plentiful and versatile applicability in distinct industries (Satpute *et al.*, 2010). Surface active substances are usually categorized on behalf of their chemical charge or their molecular weight (Sałek and Gutierrez, 2016). Surface-active agents are amphiphilic chemicals mainly produced by organo-chemical synthesis, which are applied in nearly every contemporary industry (Sałek and Gutierrez, 2016). Bio surfactants are unique complex amphipathic compounds composed of hydrophobic region and hydrophilic portion (Phetrong *et al.*, 2008). Their hydrophilic top is commonly amino acid, peptide, mono-, di-, or polysaccharide and the hydrophobic end is commonly saturated or unsaturated, linear or branched, or hydroxylated fatty acid (Shekhar *et al.*, 2015). (Phetrong *et al.*, 2008) isolated a bio emulsifier-producing bacterium *Acinetobacter calcoaceticus* subsp. anitratus SM7 from oil-spilled seawater (Thailand), which produced grown in minimal *n*-heptadecane salt medium (pH 7.0) a useful for bioremediation of oil-polluted seawater extracellular high molecular-weight emulsifying agent.

Syngas fermentation: Syngas fermentation is another indirect transformation procedure which generates alcohols, organic acids and other products (Devarapalli and Atiyeh, 2015). Syngas or otherwise synthesis gas is mainly a mix of CO, CO_2, and H_2 that are produced by the gasification of inedible feedstock like agricultural residues, solid urban wastes, energy crops, coal, and petcoke, or come from industrial waste streams (Devarapalli and Atiyeh, 2015). A diversity of gasification technologies exists in a range of sizes (Roddy, 2013). CO, CO_2 or H_2 can be converted in anaerobe environments by acetogens like *C. ljungdahlii*, *C. carboxidivorans*, *A. bacchi* and *C. ragsdalei* to biofuels like alcohols and chemicals like organic acids (Devarapalli and Atiyeh, 2015).

The 3rd generation biofuels: Energy requirement for mankind mainly basing on fossil fuels has steadily increased, depleting conventional sources of energy and rising very speedily fossil fuel prices (Behera *et al.*, 2015; Singh *et al.*, 2016). Consequently, global energy crisis and increasing releases of greenhouse gases have driven the exploration for alternate and ecological renewable energy sources (Medipally *et al.*, 2015). Despite different concerns, first generation biofuels have essentially established and developed an infrastructure, policies, and knowhow (Scott *et al.*, 2010) of a new challenging scientific pioneer field. Energy and world food crises have ignited interest and increased attention to 3rd generation biofuels, such as biodiesel, bioethanol, biobutanol, biomethane, biohydrogen and biomass-to-liquid biofuel in recent times, which are derived from sustainable algal microalgal feedstocks using unsuitable land for agriculture (Schenk *et al.*, 2008; Méndez-Vilas, 2010; Kröger and Müller-Langer, 2012; Mahmoud *et al.*, 2015; Dong *et al.*, 2016). Photosynthetic microalgae and cyanobacteria are the fastest growing, most efficient, sustainable and versatile source of biomass, an alternative energy source for the manufacture of biodiesel, bioethanol, biogas and bio hydrogen in a combined bio refinery concept (Jones and Mayfield, 2012). Third-generation biofuels are advanced and viable biofuels, specifically derived from single-celled photosynthetic microbes, and microalgae growing in different environments under a wide range of temperatures in warm, tropical, and subtropical climates, pH and nutrient availability (González-Delgado and Kafarov, 2011), while application of tropical aquaculture microalgae is rapidly increasing (Dong *et al.*, 2016). Algal biofuels have been considered as the best feasible alternative bio resource of energy with high potential to substitute or supplement fossil fuels, due to their high production potential, avoiding disadvantages of 1st and 2nd generation biofuels (Knothe, 2012; Medipally *et al.*, 2015). They can potentially replace sustainably and cost effectively fossil fuels, mitigate atmospheric CO_2, and overcome the main drawbacks connected with oil crops and lignocellulosic biofuels (Medipally *et al.*, 2015). Microalgal culture is one of modern biotechnologies, while the first unialgal cultures were developed in 1890 by Beijerinck with *Chlorella vulgaris* (Borowitzka, 1999). Large scale commercial production of microalgae *Chlorella* as food additive began early 1960s in Japan, expanding later in other countries (Borowitzka, 1999; Medipally *et al.*, 2015). 3rd generation biofuels introduced a continuing surge in microalgal biotechnology in recent times, concentrating on applications in bioenergy, nutrition, and cosmetics (Kim *et al.*, 2016). Microalgae cultivated on non-arable land have reemerged as a popular feedstock with high areal productivity of biomass and lipids for the production of most promising alternative sources for next generation biofuels, food, feed, cosmetics, pharmaceutical products and renewable energy and other valuable products as well as wastewater purification and biogas production, although associated publicity has often surpassed limits of reality (Stephens *et al.*, 2010; González-Delgado and Kafarov, 2011; Lim et al., 2012; Duong *et al.*, 2015; Rumin *et al.*, 2015; Selvarajan *et al.*, 2015). Microalgal biodiesel production is a recently developing field, while microalgal biotechnology owns a high potential through research on algae biodiversity for high capacity species, microbial metabolism, cultivation systems, heterotrophic cultivation and genetic engineering approaches, to significantly increase lipid content and microalgae-based biodiesel production (Huang *et al.*, 2010; Trentacoste *et al.*, 2013; Maity *et al.*, 2014). Microalgal culture is one of modern biotechnologies, is however still uneconomic and unsustainable due to low PFCE of biodiesel production (Cheng *et al.*, 2015). Photosynthetic organisms like higher plants, algae, and cyanobacteria utilize sunlight and CO_2 to synthesize a diversity of biomolecules, predominantly carbohydrates and lipids (Jones and Mayfield, 2012). Microalgae convert solar energy into carbon reserves including triacylglycerols (TAG), which can be converted into biodiesel, bioethanol and biomethanol

(Maity *et al.*, 2014). Cheng *et al.* (2015) reported about the effects of CO_2 on the cell wall carbohydrate composition of microalgae. Fast growing and photosynthetically more effective than oil crops microalgae, tolerating great salt concentrations, are a dynamic biomass feedstock, with an excessive biodiversity and consequently variability in biochemical structure (Satyanarayana *et al.*, 2011).

Photosynthetic organisms evolving millions years ago, play a very significant role nowadays in the production of biomass and bio products. Life on Earth originated and evolved in anoxic environments long before semantic O_2 appearance in Earth's atmosphere, while anoxygenic ancestors of Cyanobacteria invented oxygenic photosynthesis around 2.4 billion-years-ago (even 3.4 or 3.8 billion years ago), producing surplus of O_2 as a by-product of phototrophic water oxidation along with electrons and protons. More advanced antioxidant systems evolving over time oxidized largely or entirely the anoxic atmosphere and oceans, allowing Cyanobacteria to acclimate to an aerobic lifestyle and develop to the most significant environmental engineers in Earth's history (Holland, 2006; De Clerck *et al.*, 2012; Anastassiadis, 2016; Fischer *et al.*, 2016). Microphytes or microalgae are among the oldest living microorganisms and can be prokaryotic (Cyanophyceae) or eukaryotic (Chlorophyta) (Maity *et al.*, 2014). They photosynthesize light and are present extensively in a diversity of natural and man-made locations like freshwater, wastewater, and marine milieu, as well as in extreme environments, e.g. elevated temperatures, and display numerous advantages, comprising greater photosynthetic effectiveness, greater oil-rich biomass formation, and higher growth rates, in comparison to further energy crops (Huang *et al.*, 2010; Maity *et al.*, 2014; Duong *et al.*, 2015). Numerous species of microalgae, such as *Botryococcus braunii*, *Nannochloropsis* sp., *Dunaliella primolecta*, *Chlorella* sp., and *Crypthecodinium cohnii*, synthesize enormous amounts of a large number of hydrocarbons and lipids, or also manufactures other significant commercial substances, such as carotenoids and polysaccharides (Borowitzka, 1999; Medipally *et al.*, 2015). Commercial microalgal culture is a well-established manufacturing (Borowitzka, 1999). Phototrophic microalgae usually grow in two different cultivation systems such as open ponds and enclosed photobioreactors (Huang *et al.*, 2010), operating in batch, semi-batch, and continuous systems, usually applying 4 cultivation types such as phototrophic, heterotrophic, mixotrophic, and photoheterotrophic (Wang *et al.*, 2014; Medipally *et al.*, 2015), among which, only phototrophic mode is practicable for large-scale operation (Borowitzka, 1999; Medipally *et al.*, 2015). Aside specialized small-scale culture systems of less than 1000 l, various cultivation types predominate for commercial algal culture, namely large open ponds which are suitable only for few algal species (shallow large ponds, tanks, spherical ponds and raceway ponds), circular ponds with a rotating mixing arm, raceway ponds and large bags, or tanks, cascade system, and heterotrophic fermenter systems (Borowitzka, 1999). Algae can grow in closed systems either photoautotrophically, mixotrophically or heterotrophically, while closed photoautotrophic culture systems are extensively used in aquaculture industry for a range of algal species (Borowitzka, 1999). Main problem of commercialization of microalgae and microalgal products is intensive capital requirement and high cost of effective large-scale microalgal cultivation systems and harvesting process, relating to light requirement, relatively slow growth rate, low cellular lipid content, and small cell size (Borowitzka, 1999; Medipally *et al.*, 2015). Improved harvesting and drying technologies, and metabolic pathway engineering can overcome such obstacles (Medipally *et al.*, 2015). Abiotic parameters like light, temperature, pH, salinity, O_2, CO_2, nutrient stress, and toxic chemicals, biotic (pathogens and competition by other algae), and operational parameters including shear caused by mixing, dilution rate, depth, harvest frequency, and addition of bicarbonate affect microalgal growth and biomass production rate (Medipally *et al.*, 2015). Photosynthetic microalgae require light, sugars, CO_2, and simple nutritional requirements like nitrogen, phosphorus, and potassium for growth and production of large amounts of lipids, proteins, and carbohydrates, which can be furtherly converted into different biofuels and other valuable commercial coproducts (Brennan and Owende, 2010; Nigam and Singh, 2011; Selvarajan *et al.*, 2015). Temperature, light, pH and salt concentration are environmental parameters influencing microalgae growth, whereas downregulation of light-harvesting complexes increases the resistance to photooxidative damage and enhances the photosynthesis effectiveness (Medipally *et al.*, 2015). Microalgae-bacteria interactions may have positive or negative interactions between each other (Medipally *et al.*, 2015). Microalgae can utilize inorganic (CO_2) as well as organic carbon sources (glucose, acetate, etc.) to produce lipids, whereas constituents and amounts of microalgal lipids differ from species to species (Huang *et al.*, 2010). Availability of dissolved free CO_2 has been reported to have the strongest impact on photosynthetic productivity. Photosynthetic productivity increased with the light intensity and/or CO_2 concentration and was maximal at biofilm surface in artificial phototrophic biofilms, while stimulating influence of elevated CO_2 in gas phase was heightened by higher light intensities (Li *et al.*, 2016). Heterotrophic large scale cultivation of lipid-rich microalgae with fast pyrolysis may result into high yield of bio-oils (Huang *et al.*, 2010). As world's energy request is continuously rising and fossil resources are exhausted, aquatic and marine macro- (i.e., seaweed) and micro-algae is gaining growing attention, offering a huge potential as an attractive, advantageous over terrestrial plant biomass, renewable source for the manufacture of biofuels and biochemical (Wei *et al.*, 2013; Sambusiti *et al.*, 2015; Selvarajan *et al.*, 2015; Ji *et al.*, 2016). It is however still far away from realizing undoubted energetic, climatic and environmental potential of algal biodiesel (Scott *et al.*, 2010). Current cost of microalgae diesel oil is still too high to compete with fossil diesel (Ogbonna and Ogbonna, 2015). An integrated multiproduct producing bio refinery process is crucial for the commercialization of microalgal biofuel production (Lim *et al.*, 2012; Dong *et al.*, 2016). Algae are a huge and extremely differing group of organisms present in nearly all ecosystems (Selvarajan *et al.*, 2015). Oleaginous microalgal fatty acid profiles vary depending on specific species and environmental conditions (Selvarajan *et al.*, 2015). Unicellular microalgae, especially consistently productive, high-lipid wild or genetically improved strains under varying environmental conditions, with redirecting metabolite fluxes towards increased lipid contents, are more efficient and sustainable for biofuel-production than vascular plants, e.g. soya and palm (Hildebrand *et al.*, 2012; Schuhmann *et al.*, 2012; Vanthoor-Koopmans *et al.*, 2013). Selected strains should characteristically possess high biomass productivity as well as adaptation to regional climatic conditions (Selvarajan *et al.*, 2015). Immobilized microalgal cultivation in biofilms, particularly porous substrate bioreactors (PSBR), have recently displayed semantic dynamics to improve cost-efficiency of complete process (Kim *et al.*, 2016). (Sinha *et al.*, 2016) isolated eleven freshwater microalgal strains from northeast India and identified through internal transcribed spacer 2 region sequence alignment, from which three strains of *Chlorella* sp. higher biomass and lipid productivity. (Selvarajan *et al.*, 2015)isolated species of Chlorophyceae from freshwater as well as soda lakes in Hungary and Romania, from which *Chlorella vulgaris* LC8 reached high lipid quantity of ~42.1% with an advantageous C16-C18 fatty acid profile (77.4%) and appropriate biodiesel characteristics. Duong *et al.* (2015) isolated various local green microalgae strains in Northern Territory of Australia with great protein and lipid amounts and then classified applying 18S rDNA sequencing, which can potentially provide an alternative source of protein-rich feed for livestock, particularly for cattle (Duong *et al.*, 2015). Ogbonna and Ogbonna (2015) isolated and screened native microalgae strains from arid locations of North East Nigeria, four of which had great potentials for biodiesel oil manufacturing. Large scale microalgal industry suffers under high-energy input and high cost of key operations such as harvesting, nutrient quantity and oil extraction (Wrede *et al.*, 2014). Simultaneous cultivation of self-pelletizing fungal (*A. fumigatus*) and microalgal cells (known as lichens) is highly effective, because of bio-flocculation and high efficient trapping of microalgal cells with no prerequisite to add chemicals and low energy inputs, as well as the concomitant and synergistic effects on biomass formation, lipid yield and composition, and wastewater bioremediation effectiveness (Wrede *et al.*, 2014). Fungal-algal biomass can generate biodiesel via the extraction of lipids followed by their transesterification (Wrede *et al.*, 2014). Municipal, agricultural or industrial wastewater (e.g., pulp and paper effluent) would be an ideal microalgal feedstock system for isolated microalgae strains, while commensal bacteria may influence fatty acid profiles of microalgal feedstock. Mixotrophic growth on glucose or acetate was faster than photoautotrophic growth for the most of isolated strains (Stemmler *et al.*, 2016). Photosynthetic conversion of energy by natural systems has been more and more studied in contemporary times. Photosynthetic microorganisms like cyanobacteria display depending on light electrogenic features in photo-bio electrochemical cells (PBEC) generating considerable photocurrents (Sekar *et al.*, 2016). Cyanobacterium *Synechococcus elongatus* PCC 7942 has been genetically engineered to express a nonnative redox protein named outer membrane cytochrome S (OmcS) to enhance extracellular electron transfer, causing a nearly nine-fold higher photocurrent generation on the anode of a PBEC compared with the wild strain (Sekar *et al.*, 2016).

Third generation Algal biodiesel production: Microalgae, owing potential characteristics and special features, are promising bio refinery resources forth on for obtaining energy, biofuels and multiple, high value bio products (González-Delgado and Kafarov, 2011; Anastassiadis, 2016). Among various bio resources, microalgae oil has the potential to replace conventional fossil diesel fuel (Sekar *et al.*, 2016). High oil productivity and suitable fatty acid profile is crucial for achieving commercial feasibility in a microalgae-based oil industry and for microalgae selection, whereas a process optimization is necessary for all strains to enhance lipid production (Lim *et al.*, 2012; Duong *et al.*, 2015). Microalgae biofuel production is limited by low biomass concentration, small cell size and low oil content, while genetic engineering and biotic interaction with bacterial biofilms is are effective strategies to enhance biomass and biofuel formation (Medipally *et al.*, 2015). Microalgae biodiesel process involves several steps, including four main sub-procedures, such as growth of algae in engineered pools (growth), harvest of biomass in settling pools (harvesting), extraction from cellular algal oils (extraction), and algal oil transformation into biodiesel (conversion) through a process called transesterification (Méndez-Vilas, 2010; Gallagher, 2011). Microalgal synthesis of triglycerides comprises next three steps: (a) acetyl coenzyme A synthesis (acetyl-coA) in cytoplasm; (b) elongation and desaturation of fatty acid chains; and (c) triglyceride biosynthesis (Huang *et al.*, 2010). Microalgal strains should have high lipid content with appropriate fatty acids for being ideal resource of sustainable biodiesel with good properties (Islam *et al.*, 2013). Growth phase has been reported to affect the quality of biodiesel stronger than nutrients fertilization, achieving a better synthesis during stationary phase, whereas addition of organic carbon into medium had an influence as well (Islam *et al.*, 2013). Ma *et al.* (2016) reported about the improvement of nutrients removal and enhancement of lipid production of *C. vulgaris* by the supplement of 10 g/l waste glycerol into wastewater to balance its C/N ratio.

Third generation Algal bioethanol production: 3rd generation bioethanol is related to alcoholic biofuel produced from non-terrestrial biomass resources like macroalgae, predominantly gigantic brown seaweeds (Walker, 2011). Ji *et al.* (2016) reported about the direct utilization of promising fast growing brown macroalgae lacking structural lignin which is a large group of characteristically olive-green to dark brown (fucoxanthin) marine seaweeds of about 1800 species (Wei *et al.*, 2013), to bioethanol by thermophile bacterium *Defluviitalea phaphyphila* Alg1 possessing an inserted genomic degradation system from brown algae, which simultaneously utilizes mannitol, glucose, and alginate at elevated temperature, reaching high yields of ethanol, 0.47 g/g from mannitol, 0.44 g/g from glucose, and 0.3 g/g from alginate (Ji *et al.*, 2016).

Algal and microbial hydrogen and methane production: Hydrogen is one of most promising alternate energy resources and a passage to maintainable energy's future containing a huge energy yield of 122 kJ/g, 2.75 times greater than hydrocarbon fuels. As a secondary form of energy not freely existing in nature, hydrogen has to be generated from biomass and biomass related fuels, to serve as a non-CO_2 emitting clean energy carrier like electricity, generate electricity in fuel cells, or serve as chemical platform for the food, petrochemical, and metallurgical industry as well as electronics (Balat and Kırtay, 2010). Hydrogen, created via a variety of sustainable resources of primary energy including biomass, wind, and sun energy as well as fuel cell technics, could be well incorporated in future's sustainable energy systems and directly used in internal combustion engines, for gradually replacing fossil fuels (Yolcular, 2009; Balat and Kırtay, 2010). Hydrogen production is also a very important factor in hydrogen economy. Global market for hydrogen exceeded already US$40 billion per year, mainly serving as a chemical feedstock for petrochemical, food, electronics and metallurgical processing industries (Kraus, 2007; Balat and Kırtay, 2010). Thermochemical (pyrolysis and gasification) and biological (bio photolysis, water–gas shift reaction and fermentation) processes can practically produce hydrogen (Ni *et al.*, 2006). Barbarias *et al.* (2016) reported about continuous fast pyrolysis (500°C) of pine wood sawdust has in a conical spouted bed reactor (CSBR) followed by in-line steam reforming of pyrolysis vapours in a fluidised bed reactor on a Ni commercial catalyst for H_2 production from biomass. Various technologies generate hydrogen from a wide variety of primary energy sources, mainly (to 95%) from nonrenewable fossil sources such as oil, natural gas, and coal, coproducing CO_2, while Cyanobacteria, anaerobic and fermentative bacteria can form biohydrogen as well (Balat and Kırtay, 2010). Biomass gasification is the oldest and most suitable efficient way towards sustainable hydrogen formation (Balat and Kırtay, 2010). Biological H_2 can been produced through dark- as well as photo-fermentation, or biological photolysis, amongst which dark fermentation is considered as mostly encouraging (Sekar *et al.*, 2016). Fermentative H_2 formation is suffering under low carbon to H_2 yield. Simultaneous ethanol and H_2 formation has been therefore projected to solve this problem, introducing heterologous pathways or hybrid process, which resulted in recombinant *Escherichia coli* BW25113 that effectively produced simultaneously ethanol (1.38 mol/mol) and H_2 (1.32 mol/mol) from glucose, in absence of acetate. Analogous attempts with diverse carbon resources or target products have also been published (Sekar *et al.*, 2016). Microalgae can deliver numerous diverse categories of renewable biofuels, including methane production by anaerobe conversion of algal biomass, biodiesel produced from microalgal oil and photo-biological bio hydrogen (Chisti, 2007). Much interest occurred in bio hydrogen production from de-polymerized algae carbohydrates in a bio refinery through dark fermentation, as an alternative to fossil fuels (Sambusiti *et al.*, 2015). Production of hydrogen (H_2) from microalgal biomass and methane (CH_4) from the residues in a combined two-stage fermentation process has been reported, comprising dark fermentation for H_2 and CH_4 fermentation. H_2 production increased seven-fold applying enzymatic pre-treatment, while CH_4 yield was the same as in two-stage process (Wieczorek *et al.*, 2014).

The *n*-Butanol production: *n*-Butanol (C4 alcohol) is a potential alternative fuel, more efficient than ethanol regarding energy density, vapor pressure, hygroscopicity and higher combustibility, flexibility to its storing and transport and miscibility with diesel (Walker, 2011; Saini *et al.*, 2016). *n*-Butanol has been produced by fermentation with various *Clostridium* species, e.g. *C. beijerinckii*, using glucose and sucrose or soluble fermentable carbohydrates of the very efficient plant Agave (Mielenz *et al.*, 2015). Intracellular redox state in microorganisms is known as an important factor influencing manufactory competence of fermentative goods (Saini *et al.*, 2016). Defining whether alternative biofuels would deliver advantages above displaced fossil fuels necessitates the comprehensive taking into account the direct and indirect participations and outputs for their full manufacturing and using life cycles (Gude *et al.*, 2013). As fossil fuel oil reserves will diminish, governments will eventually impose substantial carbon taxes on fossil fuels worldwide, expectantly making biomass carbon based liquid fuels universally competitive (Openshaw, 2000). Today's industry can generate bio-based goods with a value satisfying an extraordinary consumer request, which releases a wide variety of choices for substituting fossil sources (Berndes, 2008). Wood can also be used for alternative manufacturing of products containing or storing carbon, which are otherwise made from more energy-intensive materials (Schlamadinger and Marland, 1996). Numerous industrial materials, including dyes, solvents and synthetic fibers, have been manufactured from trees and cultivated crops at the beginning of 20th century, while many of them had been substituted by derivative products of petroleum by the late 1960s (Ragauskas *et al.*, 2006).

The use of limited arable land for food and bioenergy production remains a controversial issue (Phitsuwan and Ratanakhanokchai, 2014). Marine plants (macroalgae) only need seawater, sunlight and carbon dioxide to grow much faster than terrestrial plants, not encroaching on land required for food crops (Walker, 2011). First and second generation biofuels relying on storage compounds of agricultural plants, presented as suitable alternatives to depleting fossil fuels, appear unsustainable. They imply a relatively strong demand in arable areas and increased pressure on arable land, resulting in increasing food market prices, potential stress on food commodities and severe food shortage, along with only moderately positive energy and environmental advantages compared to fossil fuels.

Large Brazilian and South East Asian rainforest regions have been cleared to cultivate soy beans and oil palm to produce biodiesel. Alternatively, second and third generation biofuels basing on generic lignocellulosic biomass and still high cost algal biomass cultivation on non-arable land to produce large quantities of lipids are expected to overcome these limitations (Gabrielle, 2007; Schenk *et al.*, 2008; Naik *et al.*, 2010; Singh *et al.*, 2011; Schuhmann *et al.*, 2012; Anastassiadis, 2016; Singh *et al.*, 2016). Dong *et al.* (2016) demonstrated an integrated algal biorefinery process, termed CAP, capable of producing multiple products, including bioethanol. One of technological platforms enabling a number of pathways of biomass transformation is based on applications of pulsed electric fields (PEF). Pulsed electric field technology finds beneficial involvement in manifold procedures in biorefinery (Golberg *et al.*, 2016). Cell exposing to PEF induces additional transmembrane voltage (TMV, ΔVm) across its membrane.

Carbon dioxide, global warming and climate change: Two kinds of important global issues and concerns have risen in recent years, specifically environmental and energy crisis. Environmental issue is the global warming induced by intensive usage of fossil fuels, resulting in increasing atmospheric concentrations of greenhouse effect gases, smog, acid rain, ozone depletion, climate change world-over, changing global heat equilibrium, etc. (Pearson and Palmer, 2000). Climatic change referring to a change in average weather conditions is a semantic chronical deviation in weather patterns taking place over times, fluctuating between decades to millions of years (Wikipedia, 2014). Weather conditions describe short-term occasions, while climatic alteration is a much more elongated process also affecting the weather (Shah, 2013). Global warming is the century-scale rise in overall temperature of global climatic system, air and sea at Earth's surface (Wikipedia, 2014). A heating planet is really dependable with raising cold, accumulating rain and further extremes, as a generally warmer globe alternates weather conditions the world over and at all year's epochs (Shah, 2013).

CO_2 emissions from burning fossil fuels are of great worry due to rising degree and increasing atmospheric CO_2 concentrations, the simultaneous alterations in climate, and direct influence on energy demand and ecosystems, adversely impacting human society (Andres *et al.*, 2012). Human activities have dramatically raised the emission of a number of greenhouse gases, e.g. CO_2, during the past 150 years, changing earth's atmospheric equilibrium by increasing air CO_2 content from 280 ppm to 365 ppm (Galbe and Zacchi, 2002). During the past 200 years, human activities have significantly changed Earth's carbon cycle; however, the degree of alteration in atmosphere's carbon dioxide also bases on climatic and biogeochemical developments and their interconnections with the carbon cycle (Falkowski *et al.*, 2000). CO_2 releases originate essentially from burning of the three major fossil fuels: solid (coal), gaseous (natural gas), and liquid fuel (petroleum), as well as from natural gas flaring, a byproduct of petroleum and natural gas extraction and processing, used in cement manufacture (Andres *et al.*, 2012). Climate has really varied, indeed sometimes considerably, throughout Earth's long history, whereby today's phenomenon of global warming has been openly accepted to be caused mainly by human industrialization, modernization and other anthropogenic activities, accredited predominantly to rising atmosphere's carbon dioxide levels in Earth's atmosphere (Shah, 2013; Wikipedia, 2014). CO_2 release from burning fossil fuels and changes of land use has caused a significant perturbation in natural carbon cycling between land, atmosphere and oceans (Malhi, 2002). From 10 billion tons of carbon (GtC) released by human actions yearly as CO_2 into air, primarily by burning fossil fuels and cement production, only around 40% of anthropogenic emissions stay in atmosphere, while the rest is absorbed by the oceans and the terrestrial biota to about equivalent amounts, reducing the degree of atmospheric CO_2 increase (Jones and Cox, 2005; Knorr, 2009; Flagel, 2014).

Oceans occupy a central role in the global carbon cycle and climatic regulation, mainly by single-celled photosynthetic phytoplankton that converts roughly half of Earth's CO_2 to organic carbon, although accounting for only <1% of photosynthetic biomass (Chisholm *et al.*, 2001). About one fourth of anthropogenic CO_2 emissions are taken up by the ocean, preventing additional climate change (Flagel, 2014). This led to increased CO_2 concentrations and acidity levels in seawater at an unprecedented rate at least over the last 300 million years, which predictably will continue to accelerate at least until mid-century. However, oceanic and terrestrial ecosystems lost partly their capability to sequester a large quantity of anthropogenic CO_2 releases (Knorr, 2009). Worldwide temperatures have risen semantically since 1880, the start of the so call "modern record" by scientists, whereas temperature has moved up most remarkably since late 1970s (Shah, 2013). An overwhelming scientific consensus exists that climate is really changing rapidly caused by humans, earth is warming up, species and their environments decrease steadily and the probabilities of natural adaptation of ecosystems are shrinking (Shah, 2013). Ecological alteration on earth is so old like the planet itself and of astronomical origin, while geological powers have perhaps caused more fundamental ecological alterations than has been knowledgeable throughout recent century (Van Wyk, 2001). Historically, knowledge of atmospheric CO_2 evolution throughout Earth's history is important to reconstruct the links between climatic and radiative forcing of Earth's surface temperatures (Pearson and Palmer, 2000). It is extremely likely that "unequivocally, humans dominantly influenced the experienced rising of temperature since middle 20th century", counting to worldwide warming by 95% and triggering changes being exceptional over decades of years to millennia. Consequently, atmosphere and oceans have become warmer, quantities of snow and ice have decreased and glaciers have continued to shrink, sea level has risen, and amount of greenhouse gases has raised. Emissions from burning fossils, combined with alterations of land use, have lifted atmosphere's levels of CO_2, methane and nitrous oxide to unprecedented heights not seen for at least last 800,000 years or probably many millions of years, while CO_2 levels have risen by 40 % since preindustrial periods, due to emissions firstly from fossil fuel burning and secondly from net land use alterations. Oceans has incorporated around 30% of anthropogenic release of CO_2, resulting in acidification of oceans (Letcher, 2013). China's emissions alone are forecasted to raise twofold by 2030, while new coal burning electro-power stations starting to operate around every five days (Letcher, 2013). Many approaches are used to estimate CO_2 releases over space and time (Andres *et al.*, 2012). According to ESRL (2015), CO_2 concentrations surpassed the 400 ppm, reaching 400.83 ppm in March 2015 in comparison to 398.10 ppm measured in March 2014. Global carbon dioxide levels break 400ppm milestone.

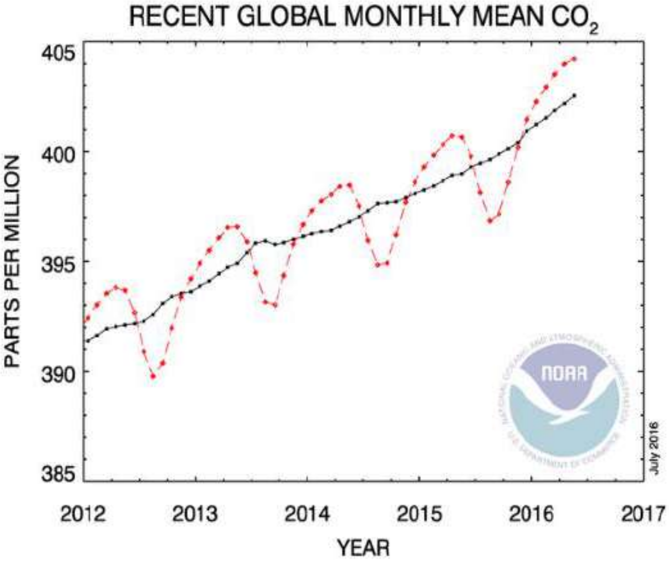


Fig. 7. Recent monthly globally averaged mean CO_2 over marine surface sites.

CO_2 reduction has become a central issue of environmental policy at least since the 1997 Kyoto conference on climate change (Zweigel and Gale, 2000). Photosynthetic fixation of CO_2 by plants and soils sequesters terrestrial CO_2, which's capacity and absorption rate can raise by reforestation and afforestation, as well as changing soil and land management practices (Bachu, 2008). Trees are effective in preventing atmospheric CO_2 accumulation either by removing atmosphere's C or by supplying a maintainable energy source substituting fossil C (Marland and Marland, 1992). Major challenge is to reduce CO_2 emissions to atmosphere hopefully to not more than double of preindustrial amount at about 550 ppm, for which an equivalent raising of 2-4.5°C is possible (Change, 2007; Bachu, 2008). CO_2 isn't lone climate regulator, nonetheless there are countless another radiative forcing ("net energy flow change at atmosphere's top"), affecting earth's energy imbalance, such as solar variations, volcanoes, clouds, methane and aerosols. Positive radiative forcing has a warming effect while negative radiative forcing obviously a cooling effect. Six are the key greenhouse gases, including carbon dioxide (CO_2), methane (CH_4) which is 20 times more potent than CO_2, nitrous oxide (N_2O) and additionally three commercial fluorinated gases, namely hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and Sulphur hexafluoride (SF_6), whereby water vapor is also seen as a greenhouse gas (Shah, 2013). It is widely believed that main cause of detrimental climate change and worldwide increase of temperature is the anthropogenic loading and raise in atmosphere's levels of greenhouse gases, such as CO_2, CH_4 and N_2O, that deplete stratospheric ozone (Change, 2007; Flagel, 2014). A comprehensive response of climatic system to elevating atmospheric CO_2 is uncertain, due to its inherent complexity and natural variability (Change, 2007), while a direct fundamental connection between rise in atmosphere's greenhouse gases and global warming that has been generally accepted has not yet been demonstrated (Bachu, 2008). Pearson and Palmer (2000) demonstrated that "atmosphere's CO_2 concentration usually viewed as the likely forcing mechanism on worldwide climate over geological time due to its great and predictable influence on temperature" is just plain false CO_2 (Science, 2000). After CO_2 spike, air's CO_2 level sinks enormously, decreasing to a minimal value near to of today, whereas the oxygen isotope fraction barely alters at all, defying and clearly contradicting the general hypothesis of "huge and foreseeable CO_2 influence" on temperature (Pearson and Palmer, 2000). CO_2 is a significant potent greenhouse gas driving climatic alteration the throughout Earth's history, whereby CO_2 concentrations have fluctuated only between 180 and 300 ppm over past 800,000 years till recent decades.

On the other side, deep oceans have been considered as potential storage places for CO_2 (Kheshgi *et al.*, 1994) and ocean has av incredible, much larger CO_2 storing ability than the entire CO_2 to be generated by burning of exploitable fossil fuels (Kaya, 1995). World's oceans enclose a total dissolved organic carbon content similar to atmosphere's CO_2 ((Sałek and Gutierrez, 2016). According to Kheshgi *et al.* (1994), ocean disposal isn't effective as an anti-CO_2 measure partly due to supplementary energy necessary to remove and dispose CO_2 removal, while some of this extra CO_2 reaches rapidly the atmosphere making marine disposal less favorable than direct atmospheric release, in contrary to Kaya (1995), who believes that oceanic CO_2 disposal is one of efficient measures to mitigate worldwide warming. Because of uncertainties, ocean storage is unlikely to be promoted as a mitigation option (Gale, 2004). Oceanic CO_2 disposal, being controversial regarding its efficiency in globally mitigating warming, is effective but necessitates more investigation regarding CO_2 behavior in oceans and ability of reducing global temperature, because it might act negatively on ocean ecosystems, while some of injected into ocean CO_2 could go out again eventually into atmosphere (Kaya, 1995). Sequestration of substantial CO_2 would necessitate enormously huge ocean zones likely and have harmful consequences to oceanic biotopes and biogeochemical cycles (Chisholm *et al.*, 2001; Buesseler and Boyd, 2003), while deep ocean circulation would bring back CO_2 into air after several centuries. Furthermore, flower-like ice formations are formed every winter on newly formed sea ice surface called frost flowers, holding extremely high concentrations of calcium carbonate, significantly impacting on the Arctic's CO_2 uptake potential. Oceans have a significant role in global carbon cycle and climatic regulation, where phytoplankton, single cell photosynthetic microorganisms forming <1 % of photosynthetic biomass, converts CO_2 to organic carbon in ocean's surface, is responsible for about half of Earth's carbon fixation. Most of phytoplankton's organic carbon is consumed by other organisms in surface waters, regenerating CO_2 by respiration (Chisholm *et al.*, 2001). Some of organic carbon precipitates to Deep Ocean, thus dropping CO_2 in surface layer and accumulating it in deep sea (Chisholm *et al.*, 2001). Speculatively, carbon flux rate to deep sea would increase if oceans would be fertilized, selling incremental carbon as credits in the emerging international carbon marketplace (Ney and Schnoor, 2000; Chisholm *et al.*, 2001).

Water management: Energy and water are to a large extent interdependent valuable resources underpinning human prosperity (IEA, 2012). Water is the blood of biosphere, connecting ecosystems across the landscape. 70% of water is withdrawn by agriculture, 20% by industry and 10% by municipalities. Surprisingly, approximately 3,000 liters of water transformed from liquid to vapor, meaning near 1 liter per calorie are required to produce sufficient food to fulfil a person's daily nutritional needs, while only around 2-5 liters of water are required for drinking (Viala, 2008). Water is an abundant source, but not always available for human usage in necessary amounts and quality as well as time and place. Only 2.5 % of Earth's water is freshwater, from which less than 1 % is accessible via surface sources and aquifers, whereas the rest is trapped in glaciers and ice caps or is deep underground (IEA, 2012). A large-scale expansion of energy crop cultivation would largely increase evapotranspiration appropriation for human consumption, potentially as large as current evapotranspiration from global cropland (Berndes, 2008). Irrigation can be implicitly included in water management factors. In fact, water scarcity has been shown to be an important limiting factor in growing bioenergy sector (Berndes, 2008; Van Vuuren *et al.*, 2009). Around 80% of agricultural evapotranspiration (turning water into vapor by crops) originates directly from rain, and around 20% from irrigation, while irrigated area doubled and water withdrawals tripled since 1950 (Viala, 2008). Earth has sufficient freshwater to produce enough food over the next half century on condition of improving water management beside non-miraculous modifications in policy and cultivation practices, while world leaders should act before the opportunities are lost (Moldon, 2007).

CONCLUSIONS

Humans used biomass and energy since the early human history and existence for their daily needs. Following the 20th century's era largely being shaped by fossil fuel energy and petrochemistry, and marked by world's crude oil depletion, atmospheric CO_2 accumulation and environmental pollution, global warming, earth's heat inbalances and climate changes causing an energy crisis, we entered in a new era of green energy, alternative energy resources, renewables and biofuels, along with the 21st century and forth on. At an ever-increasing human population, a search for various alternative, renewable energy sources have been continuing, to satisfy world's rising energy and nutritional demands, leading society's dependency away from petroleum to renewable biomass. Future outlook of biofuels is though beset by uncertainty, while Industrial Microbiology, Biotechnology and Genetic Engineering will play an important role in finding solutions to solve major problems that plague mankind. Developments in increasing productivity and resistance of existing and hybrid crops, for example advanced biological and ecological conditioners and bio stimulators of microbial origin like EcoPlant© (plant antifreeze, stimulator, protector etc.) will simplify those efforts. A combination of different of alternative energy sources, comprising solar, wind, wave power and cosmic energy, as well as diverse photosynthetic and advanced biofuels will be desirable. Recombinant microbial production promises to extensively produce alternatively to petroleum environment-friendly biochemicals and biofuels. Recombinant technologies, such as metabolic and genetic engineering, systems and synthetic biology as well as advanced developments in bioengineering, biotechnology, industrial microbiology and fermentation technology will expand the opportunities of literally unseen microbial world, offering answers and solutions to the problems that plague, perplex, and will perplex the unknown future of humanity, for which we should worry now.

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REFERENCES

Abramson, M., O. Shoseyov and Z. Shani, 2010. Plant cell wall reconstruction toward improved lignocellulosic production and processability. Plant Science, 178(2): 61-72.

Abreu-Cavalheiro, A. and G. Monteiro, 2013. Solving ethanol production problems with genetically modified yeast strains. Brazilian Journal of Microbiology, 44(3): 665-671.

Abubackar, H. N., M. C. Veiga and C. Kennes, 2015. Ethanol and acetic acid production from carbon monoxide in a clostridium strain in batch and continuous gas-fed bioreactors. International journal of environmental research and public health, 12(1): 1029-1043.

Adnan, N. A. A., S. N. Suhaimi, S. Abd-Aziz, M. A. Hassan and L.-Y. Phang, 2014. Optimization of bioethanol production from glycerol by escherichia coli ss1. Renewable Energy, 66: 625-633.

Agarwal, A. K., 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Progress in energy and combustion science, 33(3): 233-271.

Akinosho, H., K. Yee, D. Close and A. Ragauskas, 2007. The emergence of clostridium thermocellum as a high utility candidate for consolidated bioprocessing applications. Protein engineering and other bio-synthetic routes for bio-based materials: Current uses and potential applications: 92.

Almeida, J. R., L. C. Fávaro and B. F. Quirino, 2012. Biodiesel biorefinery: Opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnology for biofuels, 5(1): 1.

Amaral, P., J. Da Silva, M. Lehocky, A. Barros-Timmons, M. Coelho, I. Marrucho and J. Coutinho, 2006. Production and characterization of a bioemulsifier from yarrowia lipolytica. Process Biochemistry, 41(8): 1894-1898.

Amin, S., 2009. Review on biofuel oil and gas production processes from microalgae. Energy conversion and management, 50(7): 1834-1840.

Anand, P. and R. K. Saxena, 2012. A comparative study of solvent-assisted pretreatment of biodiesel derived crude glycerol on growth and 1, 3-propanediol production from citrobacter freundii. New Biotechnology, 29(2): 199-205.

Anastassiadis, S., A. Aivasidis and C. Wandrey, 2002. Citric acid production by candida strains under intracellular nitrogen limitation. Applied Microbiology and Biotechnology, 60(1-2): 81-87.

Anastassiadis, S., A. Aivasidis and C. Wandrey, 2003. Continuous gluconic acid production by isolated yeast-like mould strains of aureobasidium pullulans. Applied Microbiology and Biotechnology, 61(2): 110-117.

Anastassiadis, S., I. G. Morgunov, S. V. Kamzolova and T. V. Finogenova, 2008. Citric acid production patent review. Recent patents on biotechnology, 2(2): 107-123.

Anastassiadis, S. and H.-J. Rehm, 2006. Oxygen and temperature effect on continuous citric acid secretion in candida oleophila. Electronic Journal of Biotechnology, 9(4): 0-0.

Anastassiadis, S. G., 2016. Carbon sources for biomass, food, fossils, biofuels and biotechnology-review article. World Journal of Biotechnology, 1(1): 1-32.

Andres, R. J., T. A. Boden, F.-M. Bréon, P. Ciais, S. Davis, D. Erickson, J. S. Gregg, A. Jacobson, G. Marland and J. Miller, 2012. A synthesis of carbon dioxide emissions from fossil-fuel combustion. Biogeosciences, 9.

Andrietta, M. d. G. S., É. N. A. Stupiello and S. R. Andrietta, 2011. Bioethanol-what has brazil learned about yeasts inhabiting the ethanol production processes from sugar cane? : INTECH Open Access Publisher.

Aristidou, A. and M. Penttilä, 2000. Metabolic engineering applications to renewable resource utilization. Current Opinion in Biotechnology, 11(2): 187-198.

Aro, E.-M., 2016. From first generation biofuels to advanced solar biofuels. Ambio, 45(1): 24-31.

Arzumanov, T., N. Shishkanova and T. Finogenova, 2000. Biosynthesis of citric acid by yarrowia lipolytica repeat-batch culture on ethanol. Applied Microbiology and Biotechnology, 53(5): 525-529.

Bachu, S., 2008. Co 2 storage in geological media: Role, means, status and barriers to deployment. Progress in Energy and Combustion Science, 34(2): 254-273.

Badger, P., 2002. Ethanol from cellulose: A general review. Trends in new crops and new uses, 14: 17-21.

Balat, H. and E. Kırtay, 2010. Hydrogen from biomass–present scenario and future prospects. International Journal of Hydrogen Energy, 35(14): 7416-7426.

Balat, M., 2007. An overview of biofuels and policies in the european union. Energy Sources, Part B, 2(2): 167-181.

Balat, M. and H. Balat, 2009. Recent trends in global production and utilization of bio-ethanol fuel. Applied energy, 86(11): 2273-2282.

Barbarias, I., G. Lopez, J. Alvarez, M. Artetxe, A. Arregi, J. Bilbao and M. Olazar, 2016. A sequential process for hydrogen production based on continuous hdpe fast pyrolysis and in-line steam reforming. Chemical Engineering Journal, 296: 191-198.

Behera, S., R. Singh, R. Arora, N. K. Sharma, M. Shukla and S. Kumar, 2015. Scope of algae as third generation biofuels. Marine biomolecules: 81.

Beopoulos, A., J. Cescut, R. Haddouche, J.-L. Uribelarrea, C. Molina-Jouve and J.-M. Nicaud, 2009. Yarrowia lipolytica as a model for bio-oil production. Progress in lipid research, 48(6): 375-387.

Beringer, T., W. Lucht and S. Schaphoff, 2011. Bioenergy production potential of global biomass plantations under environmental and agricultural constraints. Gcb Bioenergy, 3(4): 299-312.

Berndes, G., 2008. Future biomass energy supply: The consumptive water use perspective. Water Resources Development, 24(2): 235-245.

Berni, M., I. Dorileo, J. Prado, T. Forster-Carneiro and M. Meireles, 2013. Advances in biofuel production. Biofuels Production: 11-58.

Bilgen, S., S. Keleş, İ. Sarıkaya and K. Kaygusuz, 2015. A perspective for potential and technology of bioenergy in turkey: Present case and future view. Renewable and Sustainable Energy Reviews, 48: 228-239.

Bohórquez, C., E. Amado-González and M. Martínez-Reina, 2014. Effect of pretreatment dilute acid-peroxide on napier grass (pennisetum purpureum schumach) to enhance reducing sugar yield by enzymatic hydrolysis.

Börjesson, J., R. Peterson and F. Tjerneld, 2007. Enhanced enzymatic conversion of softwood lignocellulose by poly (ethylene glycol) addition. Enzyme and Microbial Technology, 40(4): 754-762.

Borowitzka, M. A., 1999. Commercial production of microalgae: Ponds, tanks, tubes and fermenters. Journal of biotechnology, 70(1): 313-321.

Brennan, L. and P. Owende, 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and sustainable energy reviews, 14(2): 557-577.

Brooks, A., 2008. Ethanol production potential of local yeast strains isolated from ripe banana peels. African journal of Biotechnology, 7(20).

Brown, D., C. Polsky, P. V. Bolstad, S. D. Brody, D. Hulse, R. Kroh, T. Loveland and A. M. Thomson, 2014. Land use and land cover change. Pacific Northwest National Laboratory (PNNL), Richland, WA (US).

Budianto, A., S. Sumari and K. Udyani, 2006. Biofuel production from nyamplung oil using catalytic cracking process with zn-hzsm-5/γ alumina catalyst.

Buesseler, K. O. and P. W. Boyd, 2003. Will ocean fertilization work? Science, 300(5616): 67-68.

Cadenas, A. and S. Cabezudo, 1998. Biofuels as sustainable technologies: Perspectives for less developed countries. Technological Forecasting and Social Change, 58(1): 83-103.

Campbell, C. J. and J. H. Laherrère, 1998. The end of cheap oil. Scientific American, 278(3): 60-65.

Change, C., 2007. Intergovernmental panel on climate change. World Meteorological Organization.

Charles, M. B., R. Ryan, N. Ryan and R. Oloruntoba, 2007. Public policy and biofuels: The way forward? Energy Policy, 35(11): 5737-5746.

Cheng, Y. S., J. Labavitch and J. VanderGheynst, 2015. Elevated co2 concentration impacts cell wall polysaccharide composition of green microalgae of the genus chlorella. Letters in applied microbiology, 60(1): 1-7.

Chernyavskaya, O., N. Shishkanova, A. Il'chenko and T. Finogenova, 2000. Synthesis of α-ketoglutaric acid by yarrowia lipolytica yeast grown on ethanol. Applied microbiology and biotechnology, 53(2): 152-158.

Cherubini, F., G. P. Peters, T. Berntsen, A. H. STRØMMAN and E. Hertwich, 2011. Co2 emissions from biomass combustion for bioenergy: Atmospheric decay and contribution to global warming. GCB Bioenergy, 3(5): 413-426.

Chi, Z., G.-L. Liu, Y. Lu, H. Jiang and Z.-M. Chi, 2016. Bio-products produced by marine yeasts and their potential applications. Bioresource technology, 202: 244-252.

Chisholm, S. W., P. G. Falkowski and J. J. Cullen, 2001. Discrediting ocean fertilization. Science, 294(5541): 309-310.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnology advances, 25(3): 294-306.

Coleman, R. A. and D. P. Lee, 2004. Enzymes of triacylglycerol synthesis and their regulation. Progress in lipid research, 43(2): 134-176.

Conti, J. J., P. D. Holtberg, J. A. Beamon, A. Schaal, J. Ayoub and J. T. Turnure, 2011. Annual energy outlook 2011 with projections to 2035. United States of America Department of Energy Information. Office of Integrated and International Energy Analysis. Available at http://www. eia. gov/neic/speeches/newell_12162010. pdf.

Crago, C. L., M. Khanna, J. Barton, E. Giuliani and W. Amaral, 2010. Competitiveness of brazilian sugarcane ethanol compared to us corn ethanol. Energy Policy, 38(11): 7404-7415.

Crolla, A. and K. Kennedy, 2001. Optimization of citric acid production from candida lipolytica y-1095 using n-paraffin. Journal of Biotechnology, 89(1): 27-40.

Czyrnek-Delêtre, M. M., A. Chiodi, J. D. Murphy and B. P. Ó. Gallachóir, Impact of including land-use change emissions from biofuels on meeting ghg emissions reduction targets: The example of ireland. Clean Technologies and Environmental Policy: 1-14.

Da Silva, G. P., M. Mack and J. Contiero, 2009. Glycerol: A promising and abundant carbon source for industrial microbiology. Biotechnology advances, 27(1): 30-39.

Darvishi, F., I. Nahvi, H. Zarkesh-Esfahani and F. Momenbeik, 2009. Effect of plant oils upon lipase and citric acid production in yarrowia lipolytica yeast. BioMed Research International, 2009.

De Clerck, O., K. A. Bogaert and F. Leliaert, 2012. Diversity and evolution of algae: Primary endosymbiosis. Adv Bot Res, 64: 55-86.

De Souza, W. R., 2013. Microbial degradation of lignocellulosic biomass. Chandel A, Da Silva, S. Sustainable degradation of lignocellulosic biomass-techniques, applications and commercialization. Brazil: InTech.

Dekker, R. F., 1989. Biodegradation of the hetero-1, 4-linked xylans. In: ACS Symposium series-American Chemical Society (USA).

Demain, A. L., 2009. Biosolutions to the energy problem. Journal of industrial microbiology & biotechnology, 36(3): 319-332.

Demirbas, A., 2007. Progress and recent trends in biofuels. Progress in energy and combustion science, 33(1): 1-18.

Demirbas, A., 2009. Biofuels securing the planet’s future energy needs. Energy Conversion and Management, 50(9): 2239-2249.

Demirbas, M. and M. Balat, 2006. Recent advances on the production and utilization trends of bio-fuels: A global perspective. Energy Conversion and Management, 47(15): 2371-2381.

Devarapalli, M. and H. K. Atiyeh, 2015. A review of conversion processes for bioethanol production with a focus on syngas fermentation. Biofuel Research Journal, 2(3): 268-280.

Dhaliwal, S. S., H. S. Oberoi, S. K. Sandhu, D. Nanda, D. Kumar and S. K. Uppal, 2011. Enhanced ethanol production from sugarcane juice by galactose adaptation of a newly isolated thermotolerant strain of pichiakudriavzevii. Bioresource technology, 102(10): 5968-5975.

Di Lucia, L., S. Ahlgren and K. Ericsson, 2012. The dilemma of indirect land-use changes in eu biofuel policy–an empirical study of policy-making in the context of scientific uncertainty. Environmental science & policy, 16: 9-19.

Dobrowolski, A., P. Mituła, W. Rymowicz and A. M. Mirończuk, 2016. Efficient conversion of crude glycerol from various industrial wastes into single cell oil by yeast yarrowia lipolytica. Bioresource technology, 207: 237-243.

Dobson, R., V. Gray and K. Rumbold, 2012. Microbial utilization of crude glycerol for the production of value-added products. Journal of industrial microbiology & biotechnology, 39(2): 217-226.

Dong, T., E. P. Knoshaug, R. Davis, L. M. Laurens, S. Van Wychen, P. T. Pienkos and N. Nagle, 2016. Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Research.

Donot, F., A. Fontana, J. Baccou, C. Strub and S. Schorr-Galindo, 2014. Single cell oils (scos) from oleaginous yeasts and moulds: Production and genetics. Biomass and Bioenergy, 68: 135-150.

Duong, V. T., F. Ahmed, S. R. Thomas-Hall, S. Quigley, E. Nowak and P. M. Schenk, 2015. High protein-and high lipid-producing microalgae from northern australia as potential feedstock for animal feed and biodiesel. Frontiers in bioengineering and biotechnology, 3: 53.

Edrisi, S. A., R. K. Dubey, V. Tripathi, M. Bakshi, P. Srivastava, S. Jamil, H. Singh, N. Singh and P. Abhilash, 2015. Jatropha curcas l.: A crucified plant waiting for resurgence. Renewable and Sustainable Energy Reviews, 41: 855-862.

Elshahed, M. S., 2010. Microbiological aspects of biofuel production: Current status and future directions. Journal of advanced research, 1(2): 103-111.

Energy4me, 2006-2014. Energy sources. Essential Energy Education. http://www.energy4me.org/energy-facts/energy-sources/.

ESRL, N., 2015. National oceanic & atmospheric administration, earth system research laboratory: Mauna loa co2 annual mean data by dr. Pieter tans, noaa/esrl (www. Esrl. Noaa. Gov/gmd/ccgg/trends/) and dr. Ralph keeling, scripps institution of oceanography (scrippsco2. Ucsd. Edu/).

Faaij, A. P., 2006. Bio-energy in europe: Changing technology choices. Energy policy, 34(3): 322-342.

Falkowski, P., R. Scholes, E. Boyle, J. Canadell, D. Canfield, J. Elser, N. Gruber, K. Hibbard, P. Högberg and S. Linder, 2000. The global carbon cycle: A test of our knowledge of earth as a system. science, 290(5490): 291-296.

Fan, L.-H., Z.-J. Zhang, S. Mei, Y.-Y. Lu, M. Li, Z.-Y. Wang, J.-G. Yang, S.-T. Yang and T.-W. Tan, 2016. Engineering yeast with bifunctional minicellulosome and cellodextrin pathway for co-utilization of cellulose-mixed sugars. Biotechnology for Biofuels, 9(1): 137.

Fei, Q., M. O'Brien, R. Nelson, X. Chen, A. Lowell and N. Dowe, 2016. Enhanced lipid production by rhodosporidium toruloides using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. Biotechnology for Biofuels, 9(1): 130.

Ferreira, P., M. Lopes, M. Mota and I. Belo, 2016. Oxygen mass transfer impact on citric acid production by yarrowia lipolytica from crude glycerol. Biochemical Engineering Journal, 110: 35-42.

Fischer, W. W., J. Hemp and J. S. Valentine, 2016. How did life survive earth's great oxygenation? Current opinion in chemical biology, 31: 166-178.

Flagel, J. S., 2014. Examining the effectiveness of ecotourism as a funding source for protected area management in guyana.

Flórez-Orrego, D., J. A. da Silva, H. Velásquez and S. de Oliveira, 2015. Renewable and non-renewable exergy costs and co 2 emissions in the production of fuels for brazilian transportation sector. Energy, 88: 18-36.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O'Connell, D. K. Ray and P. C. West, 2011. Solutions for a cultivated planet. Nature, 478(7369): 337-342.

Franke-Rinker, D., U. Behrens and E. Nöckel, 1982. [enzymatic study of citrate-isocitrate accumulation in yeast with glucose as the carbon source]. Zeitschrift fur allgemeine Mikrobiologie, 23(2): 75-80.

Friedlander, J., V. Tsakraklides, A. Kamineni, E. H. Greenhagen, A. L. Consiglio, K. MacEwen, D. V. Crabtree, J. Afshar, R. L. Nugent and M. A. Hamilton, 2016. Engineering of a high lipid producing yarrowia lipolytica strain. Biotechnology for biofuels, 9(1): 1.

Fu, G.-Y., Y. Lu, Z. Chi, G.-L. Liu, S.-F. Zhao, H. Jiang and Z.-M. Chi, 2016. Cloning and characterization of a pyruvate carboxylase gene from penicillium rubens and overexpression of the genein the yeast yarrowia lipolytica for enhanced citric acid production. Marine Biotechnology, 18(1): 1-14.

Fulton, L., 2004. International energy agency (iea) biofuels study–interim report: Results and key messages so far. Biomass and Agriculture Sustainability, Markets and Policies: 105-112.

Gabrielle, B., 2007. [significance and limitations of first generation biofuels]. Journal de la Societe de biologie, 202(3): 161-165.

Galazka, J. M., C. Tian, W. T. Beeson, B. Martinez, N. L. Glass and J. H. Cate, 2010. Cellodextrin transport in yeast for improved biofuel production. Science, 330(6000): 84-86.

Galbe, M. and G. Zacchi, 2002. A review of the production of ethanol from softwood. Applied microbiology and biotechnology, 59(6): 618-628.

Gallagher, B. J., 2011. The economics of producing biodiesel from algae. Renewable Energy, 36(1): 158-162.

Ghimire, D., J. Shrestha and K. Anup, 2015. Study on potentiality of biogas plants and their role for the conservation of environment. Nepal Journal of Science and Technology, 15(2): 51-56.

Gilkes, N., D. Kilburn, R. Miller and R. Warren, 1991. Bacterial cellulases. Bioresource technology, 36(1): 21-35.

Gnansounou, E. and A. Dauriat, 2005. Ethanol fuel from biomass: A review. Journal of Scientific and Industrial Research, 64(11): 809.

Golberg, A., M. Sack, J. Teissie, G. Pataro, U. Pliquett, G. Saulis, T. Stefan, D. Miklavcic, E. Vorobiev and W. Frey, 2016. Energy-efficient biomass processing with pulsed electric fields for bioeconomy and sustainable development. Biotechnology for biofuels, 9(1): 1.

Goldemberg, J., 2000. World energy assessment: Energy and the challenge of sustainability. United Nations Pubns.

González-Delgado, Á.-D. and V. Kafarov, 2011. Microalgae based biorefinery: Issues to consider. CT&F-Ciencia, Tecnología y Futuro, 4(4): 5-22.

Granda, C. B., L. Zhu and M. T. Holtzapple, 2007. Sustainable liquid biofuels and their environmental impact. Environmental Progress, 26(3): 233-250.

Greene, D. L., J. L. Hopson and J. Li, 2002. Running into and out of oil: Scenarios of global oil use and resource depletion to 2050. DEAC05-00OR22725 (US Department of Energy, Tennessee, Knoxville): 1-65.

Gude, V., G. Grant, P. Patil and S. Deng, 2013. Biodiesel production from low cost and renewable feedstock. Open Engineering, 3(4): 595-605.

Guerriero, G., J. F. Hausman, J. Strauss, H. Ertan and K. S. Siddiqui, 2016. Lignocellulosic biomass: Biosynthesis, degradation, and industrial utilization. Engineering in Life Sciences, 16(1): 1-16.

Harner, N. K., X. Wen, P. K. Bajwa, G. D. Austin, C.-Y. Ho, M. B. Habash, J. T. Trevors and H. Lee, 2015. Genetic improvement of native xylose-fermenting yeasts for ethanol production. Journal of industrial microbiology & biotechnology, 42(1): 1-20.

Havlík, P., U. A. Schneider, E. Schmid, H. Böttcher, S. Fritz, R. Skalský, K. Aoki, S. De Cara, G. Kindermann and F. Kraxner, 2011. Global land-use implications of first and second generation biofuel targets. Energy Policy, 39(10): 5690-5702.

Herring, C. D., W. R. Kenealy, A. J. Shaw, S. F. Covalla, D. G. Olson, J. Zhang, W. R. Sillers, V. Tsakraklides, J. S. Bardsley and S. R. Rogers, 2016. Strain and bioprocess improvement of a thermophilic anaerobe for the production of ethanol from wood. Biotechnology for Biofuels, 9(1): 125.

Hildebrand, M., A. K. Davis, S. R. Smith, J. C. Traller and R. Abbriano, 2012. The place of diatoms in the biofuels industry. Biofuels, 3(2): 221-240.

Hill, J., E. Nelson, D. Tilman, S. Polasky and D. Tiffany, 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the National Academy of sciences, 103(30): 11206-11210.

Himmel, M. E., S.-Y. Ding, D. K. Johnson, W. S. Adney, M. R. Nimlos, J. W. Brady and T. D. Foust, 2007. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. science, 315(5813): 804-807.

Holland, H. D., 2006. The oxygenation of the atmosphere and oceans. Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1470): 903-915.

Hoogwijk, M., A. Faaij, R. Van Den Broek, G. Berndes, D. Gielen and W. Turkenburg, 2003. Exploration of the ranges of the global potential of biomass for energy. Biomass and bioenergy, 25(2): 119-133.

Horn, S. J., G. Vaaje-Kolstad, B. Westereng and V. Eijsink, 2012. Novel enzymes for the degradation of cellulose. Biotechnology for biofuels, 5(1): 1.

Huaman, R. N. E. and T. X. Jun, 2014. Energy related co 2 emissions and the progress on ccs projects: A review. Renewable and Sustainable Energy Reviews, 31: 368-385.

Huang, G., F. Chen, D. Wei, X. Zhang and G. Chen, 2010. Biodiesel production by microalgal biotechnology. Applied energy, 87(1): 38-46.

Huber, G. W., S. Iborra and A. Corma, 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. Chemical reviews, 106(9): 4044-4098.

IEA, 2011. International energy agency. . Technology Roadmap, Biofuels for Transport". Paris, France.

IEA, 2012. International energy agency http://www.iea.org/publications/freepublications/publication/WEO2012_free.pdf.

IEA, 2014. International energy agency. World Energy Outlook. http://www.worldenergyoutlook.org/publications/weo-2014/.

Inderwildi, O. R. and D. A. King, 2009. Quo vadis biofuels? Energy Environ. Sci., 2(4): 343-346.

Inui, M., H. Kawaguchi, S. Murakami, A. A. Vertès and H. Yukawa, 2005. Metabolic engineering of corynebacterium glutamicum for fuel ethanol production under oxygen-deprivation conditions. Journal of molecular microbiology and biotechnology, 8(4): 243-254.

Islam, M. A., M. Magnusson, R. J. Brown, G. A. Ayoko, M. N. Nabi and K. Heimann, 2013. Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles. Energies, 6(11): 5676-5702.

Ji, S.-Q., B. Wang, M. Lu and F.-L. Li, 2016. Direct bioconversion of brown algae into ethanol by thermophilic bacterium defluviitalea phaphyphila. Biotechnology for biofuels, 9(1): 1.

Jin, S., G. Zhang, P. Zhang, S. Fan and F. Li, 2015. High-pressure homogenization pretreatment of four different lignocellulosic biomass for enhancing enzymatic digestibility. Bioresource technology, 181: 270-274.

Johansson, T. B., 1993. Renewable energy: Sources for fuels and electricity. Island press.

Jones, C. D. and P. M. Cox, 2005. On the significance of atmospheric co2 growth rate anomalies in 2002–2003. Geophysical Research Letters, 32(14).

Jones, C. S. and S. P. Mayfield, 2012. Algae biofuels: Versatility for the future of bioenergy. Current opinion in biotechnology, 23(3): 346-351.

Jørgensen, H., J. B. Kristensen and C. Felby, 2007. Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. Biofuels, Bioproducts and Biorefining, 1(2): 119-134.

Kamzolova, S. V., M. N. Chiglintseva, A. I. Yusupova, N. G. Vinokurova, V. Y. Lysanskaya and I. G. Morgunov, 2012. Biotechnological potential of yarrowia lipolytica grown under thiamine limitation. Food Technology and Biotechnology, 50(4): 412.

Kamzolova, S. V., E. G. Dedyukhina, V. A. Samoilenko, J. N. Lunina, I. F. Puntus, R. L. Allayarov, M. N. Chiglintseva, A. A. Mironov and I. G. Morgunov, 2013. Isocitric acid production from rapeseed oil by yarrowia lipolytica yeast. Applied microbiology and biotechnology, 97(20): 9133-9144.

Kamzolova, S. V., A. R. Fatykhova, E. G. Dedyukhina, S. G. Anastassiadis, N. P. Golovchenko and I. G. Morgunov, 2011. Citric acid production by yeast grown on glycerol-containing waste from biodiesel industry. Food Technology and Biotechnology, 49(1): 65.

Kamzolova, S. V. and I. G. Morgunov, 2016. Biosynthesis of pyruvic acid from glucose by blastobotrys adeninivorans. Applied Microbiology and Biotechnology: 1-9.

Kamzolova, S. V., I. G. Morgunov, A. Aurich, O. A. Perevoznikova, N. V. Shishkanova, U. Stottmeister and T. V. Finogenova, 2005. Lipase secretion and citric acid production in yarrowia lipolytica yeast grown on animal and vegetable fat. Food Technology and Biotechnology, 43(2): 113-122.

Kamzolova, S. V., N. V. Shishkanova, I. G. Morgunov and T. V. Finogenova, 2003. Oxygen requirements for growth and citric acid production of yarrowia lipolytica. FEMS Yeast Research, 3(2): 217-222.

Kamzolova, S. V., N. G. Vinokurova, J. N. Lunina, N. F. Zelenkova and I. G. Morgunov, 2015. Production of technical-grade sodium citrate from glycerol-containing biodiesel waste by yarrowia lipolytica. Bioresource technology, 193: 250-255.

Kaya, Y., 1995. The role of co2 removal and disposal. Energy Conversion and Management, 36(6): 375-380.

Khanna, S., A. Goyal and V. S. Moholkar, 2012. Microbial conversion of glycerol: Present status and future prospects. Critical reviews in biotechnology, 32(3): 235-262.

Kheshgi, H., B. Flannery, M. Hoffert and A. Lapenis, 1994. The effectiveness of marine co 2 disposal. Energy, 19(9): 967-974.

Kim, H. S., A. R. Guzman, H. R. Thapa, T. P. Devarenne and A. Han, 2016. Cover image, volume 113, number 8, august 2016. Biotechnology and Bioengineering, 113(8).

Knorr, W., 2009. Is the airborne fraction of anthropogenic co2 emissions increasing? Geophysical Research Letters, 36(21).

Knothe, G., 2012. Fuel properties of highly polyunsaturated fatty acid methyl esters. Prediction of fuel properties of algal biodiesel. Energy & Fuels, 26(8): 5265-5273.

Koonin, S. E., 2006. Getting serious about biofuels. Science, 311(5760): 435-435.

Körbitz, W., 1999. Biodiesel production in europe and north america, an encouraging prospect. Renewable Energy, 16(1): 1078-1083.

Kraus, T., 2007. Hydrogen fuel—an economically viable future for the transportation industry? Duke Journal of Economics, 19: 39.

Kröger, M. and F. Müller-Langer, 2012. Review on possible algal-biofuel production processes. Biofuels, 3(3): 333-349.

Kumar, D. and G. S. Murthy, 2011. Impact of pretreatment and downstream processing technologies on economics and energy in cellulosic ethanol production. Biotechnology for biofuels, 4(1): 1.

Lavoie, J.-M., M. Chornet, R. Beauchet and V. Berberi, 2011. Biorefining lignocellulosic biomass via the feedstock impregnation rapid and sequential steam treatment. INTECH Open Access Publisher.

Lawford, H. G., J. D. Rousseau, A. Mohagheghi and J. D. McMillan, 2000. Continuous fermentation studies with xylose-utilizing recombinant zymomonas mobilis. In: Twenty-First Symposium on Biotechnology for Fuels and Chemicals. Springer: pp: 295-310.

Lazar, Z., E. Walczak and M. Robak, 2011. Simultaneous production of citric acid and invertase by yarrowia lipolytica suc+ transformants. Bioresource technology, 102(13): 6982-6989.

LEAL, M. R. L. V. and A. d. S. Walter, 2010. Sustainability of the production of ethanol from sugarcane: The brazilian experience. In: Proc. Int. Soc. Sugar Cane Technol.

Leblond, D., 2006. Iea: Fossil energy to dominate market through 2030. Oil & Gas Journal, 104(43): 28-29.

Ledesma-Amaro, R., T. Dulermo and J. M. Nicaud, 2015. Engineering yarrowia lipolytica to produce biodiesel from raw starch. Biotechnology for biofuels, 8(1): 1.

Ledesma-Amaro, R. and J.-M. Nicaud, 2016. Yarrowia lipolytica as a biotechnological chassis to produce usual and unusual fatty acids. Progress in lipid research, 61: 40-50.

Lee, R. A. and J.-M. Lavoie, 2013. From first-to third-generation biofuels: Challenges of producing a commodity from a biomass of increasing complexity. Animal Frontiers, 3(2): 6-11.

Letcher, T. M., 2013. Future energy: Improved, sustainable and clean options for our planet. Elsevier.

Levitan, O., J. Dinamarca, G. Hochman and P. G. Falkowski, 2014. Diatoms: A fossil fuel of the future. Trends in biotechnology, 32(3): 117-124.

Lewis, S. M., S. Gross, A. Visel, M. Kelly and W. Morrow, 2015. Fuzzy gis-based multi-criteria evaluation for us agave production as a bioenergy feedstock. GCB Bioenergy, 7(1): 84-99.

Li, C., K. L. Lesnik and H. Liu, 2013. Microbial conversion of waste glycerol from biodiesel production into value-added products. Energies, 6(9): 4739-4768.

Li, K., S. Liu and X. Liu, 2014. An overview of algae bioethanol production. International Journal of Energy Research, 38(8): 965-977.

Li, M., T. Utigard and M. Barati, 2014. Removal of boron and phosphorus from silicon using cao-sio2-na2o-al2o3 flux. Metallurgical and Materials Transactions B, 45(1): 221-228.

Li, T., B. Piltz, B. Podola, A. Dron, D. de Beer and M. Melkonian, 2016. Microscale profiling of photosynthesis-related variables in a highly productive biofilm photobioreactor. Biotechnology and bioengineering, 113(5): 1046-1055.

Lim, D. K., S. Garg, M. Timmins, E. S. Zhang, S. R. Thomas-Hall, H. Schuhmann, Y. Li and P. M. Schenk, 2012. Isolation and evaluation of oil-producing microalgae from subtropical coastal and brackish waters. PLoS One, 7(7): e40751.

Lin, Y. and S. Tanaka, 2006. Ethanol fermentation from biomass resources: Current state and prospects. Applied microbiology and biotechnology, 69(6): 627-642.

Linger, J. G., W. S. Adney and A. Darzins, 2010. Heterologous expression and extracellular secretion of cellulolytic enzymes by zymomonas mobilis. Applied and Environmental Microbiology, 76(19): 6360-6369.

Liu, X., J. Lv, J. Xu, T. Zhang, Y. Deng and J. He, 2015. Citric acid production in yarrowia lipolytica swj-1b yeast when grown on waste cooking oil. Applied biochemistry and biotechnology, 175(5): 2347-2356.

Liu, X., J. Lv, T. Zhang and Y. Deng, 2014. Direct conversion of pretreated straw cellulose into citric acid by co-cultures of yarrowia lipolytica swj-1b and immobilized trichoderma reesei mycelium. Applied biochemistry and biotechnology, 173(2): 501-509.

Long, L., D. Ding, Z. Han, H. Zhao, Q. Lin and S. Ding, 2016. Thermotolerant hemicellulolytic and cellulolytic enzymes from eupenicillium parvum 4-14 displays high efficiency at releasing of ferulic acid from wheat bran. Journal of applied microbiology.

Lund, H., 2007. Renewable energy strategies for sustainable development. Energy, 32(6): 912-919.

Luterbacher, J. S., J. M. Moran-Mirabal, E. W. Burkholder and L. P. Walker, 2015. Modeling enzymatic hydrolysis of lignocellulosic substrates using fluorescent confocal microscopy ii: Pretreated biomass. Biotechnology and bioengineering, 112(1): 32-42.

Ma, X., H. Zheng, M. Addy, E. Anderson, Y. Liu, P. Chen and R. Ruan, 2016. Cultivation of chlorella vulgaris in wastewater with waste glycerol: Strategies for improving nutrients removal and enhancing lipid production. Bioresource technology, 207: 252-261.

Macrelli, S., M. Galbe and O. Wallberg, 2014. Effects of production and market factors on ethanol profitability for an integrated first and second generation ethanol plant using the whole sugarcane as feedstock. Biotechnology for biofuels, 7(1): 1.

Mahmoud, E. A., L. A. Farahat, Z. K. A. Aziz, N. A. Fatthallah and R. A. S. El Din, 2015. Evaluation of the potential for some isolated microalgae to produce biodiesel. Egyptian Journal of Petroleum, 24(1): 97-101.

Maity, J. P., J. Bundschuh, C.-Y. Chen and P. Bhattacharya, 2014. Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives–a mini review. Energy, 78: 104-113.

Malhi, Y., 2002. Carbon in the atmosphere and terrestrial biosphere in the 21st century. Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 360(1801): 2925-2945.

Marland, G. and S. Marland, 1992. Should we store carbon in trees? Water, Air, and Soil Pollution, 64(1-2): 181-195.

Martin, M. E., 2014. Comparing ethanol production of carboxydotrophic clostridium strains during syngas fermentation with a two-stage continuous culture. Cornell University.

Mata, T. M., A. A. Martins and N. S. Caetano, 2010. Microalgae for biodiesel production and other applications: A review. Renewable and sustainable energy reviews, 14(1): 217-232.

McKendry, P., 2002. Energy production from biomass (part 1): Overview of biomass. Bioresource technology, 83(1): 37-46.

McMillan, J. D., 1994. Pretreatment of lignocellulosic biomass. In: ACS symposium series (USA).

Medipally, S. R., F. M. Yusoff, S. Banerjee and M. Shariff, 2015. Microalgae as sustainable renewable energy feedstock for biofuel production. BioMed research international, 2015.

Meesters, P., G. Huijberts and G. Eggink, 1996. High-cell-density cultivation of the lipid accumulating yeast cryptococcus curvatus using glycerol as a carbon source. Applied microbiology and biotechnology, 45(5): 575-579.

Méndez-Vilas, A., 2010. Current research, technology and education topics in applied microbiology and microbial biotechnology.

Mielenz, J. R., M. Rodriguez, O. A. Thompson, X. Yang and H. Yin, 2015. Development of agave as a dedicated biomass source: Production of biofuels from whole plants. Biotechnology for biofuels, 8(1): 1.

Mittelbach, M., 2009. Biodiesel–quo vadis? European Journal of Lipid Science and Technology, 111(8): 745-746.

Mizrachi, E., S. D. Mansfield and A. A. Myburg, 2012. Cellulose factories: Advancing bioenergy production from forest trees. New Phytologist, 194(1): 54-62.

Mohagheghi, A., J. G. Linger, S. Yang, H. Smith, N. Dowe, M. Zhang and P. T. Pienkos, 2015. Improving a recombinant zymomonas mobilis strain 8b through continuous adaptation on dilute acid pretreated corn stover hydrolysate. Biotechnology for biofuels, 8(1): 1.

Morgunov, I. G., S. V. Kamzolova and J. N. Lunina, 2013. The citric acid production from raw glycerol by yarrowia lipolytica yeast and its regulation. Applied microbiology and biotechnology, 97(16): 7387-7397.

Morgunov, I. G., S. V. Kamzolova, O. A. Perevoznikova, N. V. Shishkanova and T. V. Finogenova, 2004. Pyruvic acid production by a thiamine auxotroph of yarrowia lipolytica. Process Biochemistry, 39(11): 1469-1474.

Murugesan, A., C. Umarani, T. Chinnusamy, M. Krishnan, R. Subramanian and N. Neduzchezhain, 2009. Production and analysis of bio-diesel from non-edible oils—a review. Renewable and Sustainable Energy Reviews, 13(4): 825-834.

Naik, S. N., V. V. Goud, P. K. Rout and A. K. Dalai, 2010. Production of first and second generation biofuels: A comprehensive review. Renewable and Sustainable Energy Reviews, 14(2): 578-597.

Nasidi, M., R. Agu, Y. Deeni and G. Walker, 2016. Utilization of whole sorghum crop residues for bioethanol production. Journal of the Institute of Brewing, 122(2): 268-277.

Nault, R. M., 2005. Report on the basic energy sciences workshop on solar energy utilization. Argonne National Laboratory USA.

Ney, R. and J. Schnoor, 2000. What course for carbon trading?: Although still without a legislative mandate, a marketplace for carbon trading is beginning to develop., envrion. Sci. Technol, 34.

Ni, M., D. Y. Leung, M. K. Leung and K. Sumathy, 2006. An overview of hydrogen production from biomass. Fuel processing technology, 87(5): 461-472.

Nigam, P. S. and A. Singh, 2011. Production of liquid biofuels from renewable resources. Progress in energy and combustion science, 37(1): 52-68.

Niven, R. K., 2005. Ethanol in gasoline: Environmental impacts and sustainability review article. Renewable and Sustainable Energy Reviews, 9(6): 535-555.

Ogawa, T., M. Tamoi, A. Kimura, A. Mine, H. Sakuyama, E. Yoshida, T. Maruta, K. Suzuki, T. Ishikawa and S. Shigeoka, 2015. Enhancement of photosynthetic capacity in euglena gracilis by expression of cyanobacterial fructose-1, 6-/sedoheptulose-1, 7-bisphosphatase leads to increases in biomass and wax ester production. Biotechnology for biofuels, 8(1): 1.

Ogbonna, I. O. and J. C. Ogbonna, 2015. Isolation of microalgae species from arid environments and evaluation of their potentials for biodiesel production. African Journal of Biotechnology, 14(18): 1598-1604.

Onion, G. and L. Bodo, 1983. Oxygenate fuels for diesel engines: A survey of world-wide activities. Biomass, 3(2): 77-133.

Openshaw, K., 2000. A review of jatropha curcas: An oil plant of unfulfilled promise. Biomass and bioenergy, 19(1): 1-15.

Panesar, P. S., S. S. Marwaha and J. F. Kennedy, 2006. Zymomonas mobilis: An alternative ethanol producer. Journal of chemical technology and biotechnology, 81(4): 623-635.

Papagianni, M., 2007. Advances in citric acid fermentation by aspergillus niger: Biochemical aspects, membrane transport and modeling. Biotechnology advances, 25(3): 244-263.

Papanikolaou, S., M. Rontou, A. Belka, M. Athenaki, C. Gardeli, A. Mallouchos, O. Kalantzi, A. A. Koutinas, I. K. Kookos and A. P. Zeng, 2016. Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. Engineering in Life Sciences.

Park, J.-y., E. Kanda, A. Fukushima, K. Motobayashi, K. Nagata, M. Kondo, Y. Ohshita, S. Morita and K. Tokuyasu, 2011. Contents of various sources of glucose and fructose in rice straw, a potential feedstock for ethanol production in japan. biomass and bioenergy, 35(8): 3733-3735.

Park, J.-Y., D.-K. Kim, Z.-M. Wang, P. Lu, S.-C. Park and J.-S. Lee, 2008. Production and characterization of biodiesel from tung oil. Applied Biochemistry and Biotechnology, 148(1-3): 109-117.

Paterson, A. H., J. E. Bowers, R. Bruggmann, I. Dubchak, J. Grimwood, H. Gundlach, G. Haberer, U. Hellsten, T. Mitros and A. Poliakov, 2009. The sorghum bicolor genome and the diversification of grasses. Nature, 457(7229): 551-556.

Pearson, P. N. and M. R. Palmer, 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. Nature, 406(6797): 695-699.

Pejin, J. D., L. V. Mojović, D. J. Pejin, S. D. Kocić-Tanackov, D. S. Savić, S. B. Nikolić and A. P. Djukić-Vuković, 2015. Bioethanol production from triticale by simultaneous saccharification and fermentation with magnesium or calcium ions addition. Fuel, 142: 58-64.

Pereira, E. G., J. N. da Silva, J. L. de Oliveira and C. S. Machado, 2012. Sustainable energy: A review of gasification technologies. Renewable and Sustainable Energy Reviews, 16(7): 4753-4762.

Phetrong, K., H. Aran and S. Maneerat, 2008. Production and characterization of bioemulsifier from a marine bacterium, acinetobacter calcoaceticus subsp. Anitratus sm7. Songklanakarin Journal of Science & Technology, 30(3).

Phitsuwan, P. and K. Ratanakhanokchai, 2014. Can we create “elite rice”—a multifunctional crop for food, feed, and bioenergy production? Sustainable Chemical Processes, 2(1): 1.

Pimentel, D., A. Marklein, M. A. Toth, M. N. Karpoff, G. S. Paul, R. McCormack, J. Kyriazis and T. Krueger, 2009. Food versus biofuels: Environmental and economic costs. Human ecology, 37(1): 1-12.

Pimentel, D. and M. H. Pimentel, 2007. Food, energy, and society. CRC press.

Popp, J., Z. Lakner, M. Harangi-Rákos and M. Fári, 2014. The effect of bioenergy expansion: Food, energy, and environment. Renewable and Sustainable Energy Reviews, 32: 559-578.

Posada, J. and C. Cardona, 2010. Design and analysis of fuel ethanol production from raw glycerol. Energy, 35(12): 5286-5293.

Prasad, S., A. Singh, N. Jain and H. Joshi, 2007. Ethanol production from sweet sorghum syrup for utilization as automotive fuel in india. Energy & Fuels, 21(4): 2415-2420.

Przystałowska, H., D. Lipiński and R. Słomski, 2015. Biotechnological conversion of glycerol from biofuels to 1, 3-propanediol using escherichia coli. Acta Biochimica Polonica, 62(1): 23-34.

Qiao, K., S. H. I. Abidi, H. Liu, H. Zhang, S. Chakraborty, N. Watson, P. K. Ajikumar and G. Stephanopoulos, 2015. Engineering lipid overproduction in the oleaginous yeast yarrowia lipolytica. Metabolic engineering, 29: 56-65.

Ragauskas, A. J., C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak and C. L. Liotta, 2006. The path forward for biofuels and biomaterials. science, 311(5760): 484-489.

Ratledge, C., 2014. The role of malic enzyme as the provider of nadph in oleaginous microorganisms: A reappraisal and unsolved problems. Biotechnology letters, 36(8): 1557-1568.

Ray, D. K., N. D. Mueller, P. C. West and J. A. Foley, 2013. Yield trends are insufficient to double global crop production by 2050. PloS one, 8(6): e66428.

Reijnders, L., 2009. Fuels for the future. Journal of Integrative Environmental Sciences, 6(4): 279-294.

Roddy, D. J., 2013. Biomass in a petrochemical world. Interface focus, 3(1): 20120038.

Rubin, E. M., 2008. Genomics of cellulosic biofuels. Nature, 454(7206): 841-845.

Rumin, J., H. Bonnefond, B. Saint-Jean, C. Rouxel, A. Sciandra, O. Bernard, J.-P. Cadoret and G. Bougaran, 2015. The use of fluorescent nile red and bodipy for lipid measurement in microalgae. Biotechnology for biofuels, 8(1): 1.

Ruth, L., 2008. Bio or bust? EMBO reports, 9(2): 130-133.

Rywinska, A., W. Rymowicz and M. Marcinkiewicz, 2010. Valorization of raw glycerol for citric acid production by yarrowia lipolytica yeast. Electronic Journal of Biotechnology, 13(4): 9-10.

Saini, M., S.-Y. Li, Z. W. Wang, C.-J. Chiang and Y.-P. Chao, 2016. Systematic engineering of the central metabolism in escherichia coli for effective production of n-butanol. Biotechnology for biofuels, 9(1): 1.

Sakai, S., Y. Tsuchida, S. Okino, O. Ichihashi, H. Kawaguchi, T. Watanabe, M. Inui and H. Yukawa, 2007. Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested corynebacterium glutamicum r. Applied and environmental microbiology, 73(7): 2349-2353.

Sakuragi, H., K. Kuroda and M. Ueda, 2011. Molecular breeding of advanced microorganisms for biofuel production. BioMed Research International, 2011.

Sałek, K. and T. Gutierrez, 2016. Surface-active biopolymers from marine bacteria for potential biotechnological applications.

Sambusiti, C., M. Bellucci, A. Zabaniotou, L. Beneduce and F. Monlau, 2015. Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review. Renewable and Sustainable Energy Reviews, 44: 20-36.

Sarris, D. and S. Papanikolaou, 2016. Biotechnological production of ethanol: Biochemistry, processes and technologies. Engineering in Life Sciences.

Satpute, S. K., I. M. Banat, P. K. Dhakephalkar, A. G. Banpurkar and B. A. Chopade, 2010. Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnology advances, 28(4): 436-450.

Satyanarayana, K., A. Mariano and J. Vargas, 2011. A review on microalgae, a versatile source for sustainable energy and materials. International Journal of energy research, 35(4): 291-311.

Savaliya, M. L., B. D. Dhorajiya and B. Z. Dholakiya, 2015. Recent advancement in production of liquid biofuels from renewable resources: A review. Research on Chemical Intermediates, 41(2): 475-509.

Schenk, P. M., S. R. Thomas-Hall, E. Stephens, U. C. Marx, J. H. Mussgnug, C. Posten, O. Kruse and B. Hankamer, 2008. Second generation biofuels: High-efficiency microalgae for biodiesel production. Bioenergy research, 1(1): 20-43.

Schlamadinger, B. and G. Marland, 1996. Full fuel cycle carbon balances of bioenergy and forestry options. Energy conversion and management, 37(6): 813-818.

Schuhmann, H., D. K. Lim and P. M. Schenk, 2012. Perspectives on metabolic engineering for increased lipid contents in microalgae. Biofuels, 3(1): 71-86.

Scott, S. A., M. P. Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith and A. G. Smith, 2010. Biodiesel from algae: Challenges and prospects. Current opinion in biotechnology, 21(3): 277-286.

Sekar, N., R. Jain, Y. Yan and R. P. Ramasamy, 2016. Enhanced photo-bioelectrochemical energy conversion by genetically engineered cyanobacteria. Biotechnology and bioengineering, 113(3): 675-679.

Selvarajan, R., T. Felföldi, T. Tauber, E. Sanniyasi, T. Sibanda and M. Tekere, 2015. Screening and evaluation of some green algal strains (chlorophyceae) isolated from freshwater and soda lakes for biofuel production. Energies, 8(7): 7502-7521.

Sen, B., A. Dabir, V. Lanjekar and D. Ranade, 2015. Isolation and partial characterization of a new strain of klebsiella pneumoniae capable of high 1, 3 propanediol production from glycerol. Global Journal of Environmental Science and Management, 1(2): 99-108.

Shafiee, S. and E. Topal, 2008. An econometrics view of worldwide fossil fuel consumption and the role of us. Energy Policy, 36(2): 775-786.

Shah, A., 2013. Poverty facts and stats, global issues-social, political, economic and environmental issues that affect us all.

Shay, E. G., 1993. Diesel fuel from vegetable oils: Status and opportunities. Biomass and bioenergy, 4(4): 227-242.

Sheehan, J., V. Camobreco, J. Duffield, H. Shapouri, M. Graboski and K. Tyson, 2000. An overview of biodiesel and petroleum diesel life cycles. National Renewable Energy Lab., Golden, CO (US).

Shekhar, S., A. Sundaramanickam and T. Balasubramanian, 2015. Biosurfactant producing microbes and their potential applications: A review. Critical Reviews in Environmental Science and Technology, 45(14): 1522-1554.

Sherkhanov, S., T. P. Korman, S. G. Clarke and J. U. Bowie, 2016. Production of fame biodiesel in e. Coli by direct methylation with an insect enzyme. Scientific reports, 6.

Silva, C., M. Esperança, A. Cruz, L. Moura and A. Badino, 2015. Stripping of ethanol with co 2 in bubble columns: Effects of operating conditions and modeling. Chemical Engineering Research and Design, 102: 150-160.

Simmons, B. A., D. Loque and H. W. Blanch, 2008. Next-generation biomass feedstocks for biofuel production. Genome biology, 9(12): 1.

Singh, A., P. S. Nigam and J. D. Murphy, 2011. Renewable fuels from algae: An answer to debatable land based fuels. Bioresource technology, 102(1): 10-16.

Singh, R., M. Srivastava and A. Shukla, 2016. Environmental sustainability of bioethanol production from rice straw in india: A review. Renewable and Sustainable Energy Reviews, 54: 202-216.

Sinha, S. K., A. Gupta and R. Bharalee, 2016. Production of biodiesel from freshwater microalgae and evaluation of fuel properties based on fatty acid methyl ester profile. Biofuels, 7(1): 69-78.

Sitepu, I. R., M. Jin, J. E. Fernandez, L. da Costa Sousa, V. Balan and K. L. Boundy-Mills, 2014. Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. Applied microbiology and biotechnology, 98(17): 7645-7657.

Slingerland, M. and M. Schut, 2014. Jatropha developments in mozambique: Analysis of structural conditions influencing niche-regime interactions. Sustainability, 6(11): 7541-7563.

Slininger, P. J., B. S. Dien, C. P. Kurtzman, B. R. Moser, E. L. Bakota, S. R. Thompson, P. J. O'Bryan, M. A. Cotta, V. Balan and M. Jin, 2016. Comparative lipid production by oleaginous yeasts in hydrolyzates of lignocellulosic biomass and process strategy for high titers. Biotechnology and bioengineering.

Sonego, J., D. Lemos, C. Pinto, A. Cruz and A. Badino, 2016. Extractive fed-batch ethanol fermentation with co2 stripping in a bubble column bioreactor: Experiment and modeling. Energy & Fuels, 30(1): 748-757.

Srinivasan, S., 2009. The food v. Fuel debate: A nuanced view of incentive structures. Renewable Energy, 34(4): 950-954.

Srivastav, D., A. P. Singh and A. Kumar, 2014. Fossil fuels running out: Third generation micro algal biofuels showing light of hope. Open Access Library Journal, 1(03): 1.

Srivastav, D., A. P. Singh and A. Kumar, 2014. Fossil fuels running out: Third generation micro algal biofuels showing light of hope. Open Access Library Journal, 1(3).

Srivastava, A. and R. Prasad, 2000. Triglycerides-based diesel fuels. Renewable and sustainable energy reviews, 4(2): 111-133.

Stemmler, K., R. Massimi and A. E. Kirkwood, 2016. Growth and fatty acid characterization of microalgae isolated from municipal waste-treatment systems and the potential role of algal-associated bacteria in feedstock production. PeerJ, 4: e1780.

Stephanopoulos, G., 2007. Challenges in engineering microbes for biofuels production. Science, 315(5813): 801-804.

Stephens, E., I. L. Ross, J. H. Mussgnug, L. D. Wagner, M. A. Borowitzka, C. Posten, O. Kruse and B. Hankamer, 2010. Future prospects of microalgal biofuel production systems. Trends in plant science, 15(10): 554-564.

Stottmeister, U., U. Behrens, E. Weissbrodt, G. Barth, D. Franke-Rinker and E. Schulze, 1981. [utilization of paraffins and other noncarbohydrate carbon sources for microbial citric acid synthesis]. Zeitschrift fur allgemeine Mikrobiologie, 22(6): 399-424.

Suhaimi, S. N., L.-Y. Phang, T. Maeda, S. Abd-Aziz, M. Wakisaka, Y. Shirai and M. A. Hassan, 2012. Bioconversion of glycerol for bioethanol production using isolated escherichia coli ss1. Brazilian Journal of Microbiology, 43(2): 506-516.

Sun, Y. and J. Cheng, 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresource technology, 83(1): 1-11.

Tai, M. and G. Stephanopoulos, 2013. Engineering the push and pull of lipid biosynthesis in oleaginous yeast yarrowia lipolytica for biofuel production. Metabolic engineering, 15: 1-9.

Talebian-Kiakalaieh, A., N. Amin, A. Zarei and H. Jaliliannosrati, 2013. Biodiesel production from high free fatty acid waste cooking oil by solid acid catalyst. In: Proceedings of the 6th International Conference on

Process Systems Engineering (PSE ASIA), Kuala Lumpur. pp: 2013-2094.

Tan, M.-J., X. Chen, Y.-K. Wang, G.-L. Liu and Z.-M. Chi, 2016. Enhanced citric acid production by a yeast yarrowia lipolytica over-expressing a pyruvate carboxylase gene. Bioprocess and biosystems engineering: 1-8.

Taylor, F., M. J. Kurantz, N. Goldberg and J. C. Craig, 1995. Continuous fermentation and stripping of ethanol. Biotechnology progress, 11(6): 693-698.

Taylor, J. A., S. V. Dhople and D. S. Callaway, 2016. Power systems without fuel. Renewable and Sustainable Energy Reviews, 57: 1322-1336.

Tesfaw, A. and F. Assefa, 2014. Current trends in bioethanol production by saccharomyces cerevisiae: Substrate, inhibitor reduction, growth variables, coculture, and immobilization. International Scholarly Research Notices, 2014.

Tian, L., B. Papanek, D. G. Olson, T. Rydzak, E. K. Holwerda, T. Zheng, J. Zhou, M. Maloney, N. Jiang and R. J. Giannone, 2016. Simultaneous achievement of high ethanol yield and titer in clostridium thermocellum. Biotechnology for biofuels, 9(1): 1.

Tilman, D., C. Balzer, J. Hill and B. L. Befort, 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences, 108(50): 20260-20264.

Trentacoste, E. M., R. P. Shrestha, S. R. Smith, C. Glé, A. C. Hartmann, M. Hildebrand and W. H. Gerwick, 2013. Metabolic engineering of lipid catabolism increases microalgal lipid accumulation without compromising growth. Proceedings of the National Academy of Sciences, 110(49): 19748-19753.

Türe, S., D. Uzun and I. E. Türe, 1997. The potential use of sweet sorghum as a non-polluting source of energy. Energy, 22(1): 17-19.

USDA, 2005. Biomass as feedstock for a bioenergy and bioproducts industry. The Technical Feasibility of a Billion-Ton Annual Supply [http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf]

Van Maris, A. J., D. A. Abbott, E. Bellissimi, J. van den Brink, M. Kuyper, M. A. Luttik, H. W. Wisselink, W. A. Scheffers, J. P. van Dijken and J. T. Pronk, 2006. Alcoholic fermentation of carbon sources in biomass hydrolysates by saccharomyces cerevisiae: Current status. Antonie Van Leeuwenhoek, 90(4): 391-418.

Van Vuuren, D. P., J. van Vliet and E. Stehfest, 2009. Future bio-energy potential under various natural constraints. Energy Policy, 37(11): 4220-4230.

Van Wyk, J. P., 2001. Biotechnology and the utilization of biowaste as a resource for bioproduct development. TRENDS in Biotechnology, 19(5): 172-177.

Vanthoor-Koopmans, M., R. H. Wijffels, M. J. Barbosa and M. H. Eppink, 2013. Biorefinery of microalgae for food and fuel. Bioresource technology, 135: 142-149.

Viala, E., 2008. Water for food, water for life a comprehensive assessment of water management in agriculture. Irrigation and Drainage Systems, 22(1): 127-129.

Visser, E. M., T. F. Leal, M. N. de Almeida and V. M. Guimarães, 2015. Increased enzymatic hydrolysis of sugarcane bagasse from enzyme recycling. Biotechnology for biofuels, 8(1): 1.

Von Blottnitz, H. and M. A. Curran, 2007. A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. Journal of cleaner production, 15(7): 607-619.

Walker, G. M., 2011. 125th anniversary review: Fuel alcohol: Current production and future challenges. Journal of the Institute of Brewing, 117(1): 3-22.

Wang, L., R. Quiceno, C. Price, R. Malpas and J. Woods, 2014. Economic and ghg emissions analyses for sugarcane ethanol in brazil: Looking forward. Renewable and Sustainable Energy Reviews, 40: 571-582.

Wang, M., C. Yu and H. Zhao, 2016. Directed evolution of xylose specific transporters to facilitate glucose-xylose co-utilization. Biotechnology and bioengineering, 113(3): 484-491.

Wang, W., Y. Zhu, J. Du, Y. Yang and Y. Jin, 2015. Influence of lignin addition on the enzymatic digestibility of pretreated lignocellulosic biomasses. Bioresource technology, 181: 7-12.

Watanabe, Y. and D. O. Hall, 1996. Photosynthetic co 2 conversion technologies using a photobioreactor incorporating microalgae-energy and material balances. Energy conversion and management, 37(6): 1321-1326.

Wei, N., J. Quarterman and Y.-S. Jin, 2013. Marine macroalgae: An untapped resource for producing fuels and chemicals. Trends in biotechnology, 31(2): 70-77.

Weiss, N., J. Börjesson, L. S. Pedersen and A. S. Meyer, 2013. Enzymatic lignocellulose hydrolysis: Improved cellulase productivity by insoluble solids recycling. Biotechnology for biofuels, 6(1): 1.

West, T. P., 2013. Citric acid production by candida species grown on a soy-based crude glycerol. Preparative Biochemistry and Biotechnology, 43(6): 601-611.

Westman, J. O. and C. J. Franzén, 2015. Current progress in high cell density yeast bioprocesses for bioethanol production. Biotechnology journal, 10(8): 1185-1195.

Wieczorek, N., M. A. Kucuker and K. Kuchta, 2014. Fermentative hydrogen and methane production from microalgal biomass (chlorella vulgaris) in a two-stage combined process. Applied Energy, 132: 108-117.

Wikipedia, 2014. Http://en.Wikipedia.Org/wiki/biofuel

Worldwatch, 2011. Biofuels make a comeback despite tough economy. http://www.worldwatch.org/biofuels-make-comeback-despite-tough-economy

Wrede, D., M. Taha, A. F. Miranda, K. Kadali, T. Stevenson, A. S. Ball and A. Mouradov, 2014. Co-cultivation of fungal and microalgal cells as an efficient system for harvesting microalgal cells, lipid production and wastewater treatment. PloS one, 9(11): e113497.

Xu, Q., M. E. Himmel and A. Singh, 2015. Production of ethanol from engineered trichoderma reesei. NREL (National Renewable Energy Laboratory (NREL), Golden, CO (United States)).

Yang, B., Z. Dai, S. Ding and C. Wyman, 2011. Enzymatic hydrolysis of cellulosic biomass. Biofuels, 2, 421-450.

Yang, F., M. A. Hanna and R. Sun, 2012. Value-added uses for crude glycerol--a byproduct of biodiesel production. Biotechnology for biofuels, 5(1): 1.

Yolcular, S., 2009. Hydrogen production for energy use in european union countries and turkey. Energy Sources, Part A, 31(15): 1329-1337.

Zabed, H., G. Faruq, J. N. Sahu, M. S. Azirun, R. Hashim and A. Nasrulhaq Boyce, 2014. Bioethanol production from fermentable sugar juice. The Scientific World Journal, 2014.

Zhang, H., L. Zhang, H. Chen, Y. Q. Chen, C. Ratledge, Y. Song and W. Chen, 2013. Regulatory properties of malic enzyme in the oleaginous yeast, yarrowia lipolytica, and its non-involvement in lipid accumulation. Biotechnology letters, 35(12): 2091-2098.

Zhang, J., N. Sonnenschein, T. P. Pihl, K. R. Pedersen, M. K. Jensen and J. D. Keasling, 2016. Engineering an nadph/nadp+ redox biosensor in yeast. ACS Synthetic Biology.

Zhang, X. Z. and Y. H. P. Zhang, 2013. Cellulases: Characteristics, sources, production, and applications. Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers, 1: 131-146.

Zhu, Q. and E. N. Jackson, 2015. Metabolic engineering of yarrowia lipolytica for industrial applications. Current opinion in biotechnology, 36: 65-72.

Zinjarde, S. S. and A. Pant, 2002. Emulsifier from a tropical marine yeast, yarrowia lipolytica ncim 3589. Journal of basic microbiology, 42(1): 67-73.

Zweigel, P. and J. Gale, 2000. Storing co2 underground shows promising results. Eos, Transactions American Geophysical Union, 81(45): 529-534.

Zydney, A. L., 2016. Continuous downstream processing for high value biological products: A review. Biotechnology and bioengineering, 113(3): 465-475.

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