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CARBON SOURCES FOR BIOMASS, FOOD, FOSSILS, BIOFUELS AND BIOTECHNOLOGY - REVIEW ARTICLE

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ABSTRACT

Carbon atom Carbon atom is most important and abundant constituent of existing and new generated biological mater and biomass and the basis of all forms of life on earth. It is involved in the composition and construction of organic micro- and macromolecules, cells and living organisms, storage molecules, fossils, fossil fuels, biofuels and energy resources of living and nonliving organic matter. Initially originated from atmospheric carbon dioxide, it is absorbed and incorporated into organic molecules by photosynthetic plants and microorganisms through photosynthetic processes to form glucose and other less or more complex organic molecules, enabling and sustaining life on Earth. A semantic part of CO_2 has been captured, trapped and immobilized in various forms of fossils, not participating in biogeochemical carbon cycles for millions of years, or is dissolved in oceans. Carbon sources is also one of most important parameters, strongly influencing microbial growth and the accumulation of cellular metabolites, fermentation technologies, process economics and feasibility of industrial production. Advanced developments in recombinant technologies, such as metabolic and genetic engineering, systems and synthetic biology, as well as in bioengineering, biotechnology, industrial microbiology and fermentation technology will expand the opportunities of literally unseen microbial world.

Key word: Carbon sources for biomass, food, fossils, biofuels and biotechnology.

INTRODUCTION

Historical developments of biomass, fossil fuels and biofuels: Since the early human history and existence, humans cultured and used various food crops, energy rich plants and forest cellulosic material for fire, light, heating, cooking and other daily activities. They prepared foods, drinks and tools as well as constructed huts and houses, transport cars and ships, hence permanently affecting and alternating expansion and consistence of landscapes and forests. Historically, management and control of land, agricultural and agro-industrial production and production strategies, preparation, distribution and utilization of carbon sources, food and energy resources followed up geopolitical, socioeconomic and ecological developments, competitions, conflicts and in worst case confrontations. As a result, availability, distribution, production costs and prices for carbon sources, biomass resources and their products remained rather unstable, challenging continuous scientific research and development in our times. In the ever-increasing human population, predictively expanding to about 10 billion by 2050, various carbon resources are used for the preparation of animal feed, food, renewable energy and as substrates in microbial fermentation processes for the production of a vast variety of metabolites.

The vastly growing global population, the steadily rising living standards and expected anticipation inevitably enforce a conflict between satisfying people′s demands for goods and services on one site and sustainable development requirements as well as considerable treatment of nature and earth′s resources on the other (Przybylski *et al.*, 2012; Ahlgren and Di Lucia, 2014). Entering the 21st century an urgent need has risen for clean and sustainable bio-production and alternatives to an economy predominantly depending on fossil resources, (Brandberg *et al.*, 2005; Przybylski *et al.*, 2012). Growing food grains for the production of biofuels squanders land, water and energy resources being vital for the production of food, while our agricultural systems are concurrently degrading land, water, biodiversity and climate on a global scale. This has already caused food shortages for the world′s poor, exacerbating the malnourishment problem worldwide, while nearly 60% of world′s population is currently malnourished (Pimentel *et al.*, 2008; Pimentel *et al.*, 2009), or approximately a billion people are chronically malnourished (Foley *et al.*, 2011) and malnutrition is the leading world′s cause of death (Pimentel *et al.*, 2007). Protein and energy malnutrition, as well as micronutrient deficiencies is a major health burden in developing countries, and globally the most important risk factor for illness, infections and death, particularly affecting hundreds of millions of pregnant women and young children, mainly owing to poverty (Müller and Krawinkel, 2005). To meet the world′s future food security and sustainability needs in coming decades, food production must grow substantially, agriculture′s environmental footprint must shrink dramatically, and the world has to expand energy supplies in a safe, secure, affordable and environmentally responsible way. Economic output will more than double, whereas prosperity and middle class will be expanding, largely in India and China, across a world that will reach nearly 9 billion people. Global energy demand will increase by about 35 percent in 2030 compared to 2005 and about 30 percent in 2040 compared to 2010, whereby electricity generation accounts for more than 40 percent of global energy consumption. Energy demand growth will slow as economies mature, efficiency accelerates and population growth moderates (exxonmobil.com) (Foley *et al.*, 2011). There has been increasingly awareness about human activities, impinging on Earth's ability to sustain future growth of food supply and population. There is serious concern about ecological and environmental consequences of further expanding food supply to feed the still rapidly growing human population, while global food demand is expected to double within the coming 50 years (Cohen and Fedoroff, 1999), and global demand for transportation fuels is expected to increase even more rapidly (DOE, 2006).

Humanity lives in an unprecedented historical time with respect to economic development based on available energy sources, whereas petroleum-based economy comes slowly to the end (Demain, 2009). World's politically and economically unstable main energy source hydrocarbon-based fossil fuels is clearly running out inevitably, while energy demand and prices of fuel petroleum are unstable, steadily fluctuating and increasing. Hence, an intense interest in alternative energy sources revealed worldwide. New advanced alternative renewable and maintainable energy resources and carbon neutral sources for biofuels, as also clean technologies must be found, developed, offered and applied, to satisfy the rising global energy demand and for environmental and economic sustainability (Aristidou and Penttilä, 2000; Lin and Tanaka, 2006; Charles *et al.*, 2007; Patil *et al.*, 2008; Pimentel *et al.*, 2008). We caused an energy crisis neglecting alternative energy sources for years, like bioethanol, biobutanol, biodiesel, biohydrocarbons, methane, methanol, electricity-generating microbial fuel cells, and production of hydrogen via photosynthetic microbes (Demain, 2009). Chemistry has always had a fundamental role in almost every aspect of modern society, whereas only a minor fraction of chemical industry products is currently made from renewable biobased raw materials (Hatti-Kaul *et al.*, 2007). Biotechnology is another competing sector using a great variety of carbon sources and agricultural byproducts for the production of a vast number of bio products and metabolites. Biotechnology can also produce a variety of alternative energy sources from different biomass feed stocks and enhance crop production supplying bio stimulators and superior soil and plant conditioners.

Photosynthesis, Biomass and Carbon resources: Carbon atom (C) is the most important and abundant constituent of biological mater and biomass (50% of dry biomass). It is involved in the composition and construction of organic micro- and macromolecules, cells and living organisms, storage molecules, fossils, fossil fuels, biofuels and energy resources of living and nonliving organic matter. Photosynthesis is the process during which organisms use energy from the sun to combine water with carbon dioxide (CO_2) to create biomass. Photosynthetic organisms like plants and some microorganisms such as algae, microalgae and photosynthetic bacteria absorb and incorporate CO_2, initially originated from atmosphere, via photosynthetic processes (Fig. 1).

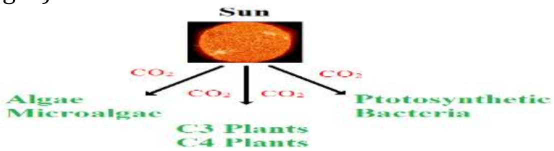

Fig. 1: Biomass formation from sun energy.

They capture CO_2 applying solar energy to synthesize a variety of useable compounds, such as glucose and other organic molecules, combining water with carbon dioxide to create biomass, enabling and sustaining life on Earth. Various fossils, such as liquid, gaseous and solid fossils, coral sediments and islands have captured, trapped and immobilized a part of existing CO_2, not participating in biogeochemical carbon cycle for millions of years, while oceans dissolve another one. Biomass formation in various forms is a very complicated process that has been developed through the long lasting process of evolution. International Energy Agency (IEA) defines biomass as any plant matter, serving directly as a fuel or delivering fuels, electricity or heat. Biomass is any organic matter, synthesized by green plants (including algae, trees and crops) utilizing sunlight through photosynthesis, whereby energy of sun light is stored in organic chemical bonds (McKendry, 2002). More than 3800 zettajoules (one zettajoule = 1×1021 joules) of solar energy are annually absorbed by Earth's atmosphere and surface, from which only 0.05% is captured through photosynthesis (Sayre, 2010). CO_2 is also adsorbed by some enzymatic anaplerotic sequences of cellular metabolism, e.g. pyruvate carboxylase, phosphoenol carboxylase.

Oxygen (O_2) content of the Archaean atmosphere was generally less than ca 10^{-5} of present level (Holland, 2006). Oxidation of atmosphere and portions of hydrosphere occurred during the Great Oxidation Event in Paleoproterozoic at ca. 2400 Ma (Huston David L, 2015). Oxygenic photosynthesis, involving photolysis of water into electrons, protons and free oxygen, transformed largely or entirely the anoxic atmosphere and oceans, with the possible exception of oxygen oases in shallow oceans, into an oxidizing one. Photosynthesis evolved in the anoxygenic ancestors of Cyanobacteria at least 3.4 or even 3.8 billion years ago. Integration, engulfment and enslavement of a cyanobacterium (single origin, monophyletic) into the cellular machinery of a heterotrophic eukaryote led to the development of a new organelle through endosymbiosis, the plastid of Archaeplastida comprising green plants, red algae and the glaucophytes (Holland, 2006; De Clerck *et al.*, 2012). Evolvement of Cyanobacteria using photosystem-II exceeded atmospheric O_2 concentrations to ca 2 ppm at about 2.45 Ga (Holland, 2006). Minimum oxygen pressure (PO_2) for the 3.0–2.2 Ga atmosphere was about 1.5% of present atmospheric PO_2 level that has not changed significantly since 1.9 Ga (Ohmoto, 1996). Principally, the evolution of large vascular land plants created probably the high atmospheric O_2 levels (Berner, 2004). Fossil spore tetrads indicate that the ubiquitously found in modern soils higher plants first appeared on the continents during the Mid-Ordovician (Kenrick and Crane, 1997; Rye and Holland, 1998). Origin of land plants from a green algal ancestor and early evolution of land plants in mid-Palaeozoic era between about 480 and 360 million years ago was a very important key event in life history and in the evolution of life on earth (Kenrick and Crane, 1997; De Clerck *et al.*, 2012). Chronologically uncertain when, gradual oxygenation of Proterozoic oceans and atmosphere enabled more complex aerobic organisms, cellular compartmentalization separating metabolic activities, and ultimately eukaryotic cells, as well as new biochemical pathways to evolve, changing our planet's face in all aspects once forever (De Clerck *et al.*, 2012). Paleosols, meaning a soil forming on a landscape of the past at the atmosphere-lithosphere interface, provide semi quantitative data regarding atmospheric oxygen levels (Valentine and Dalrymple, 1976; Rye and Holland, 1998). Earth has experienced major episodic and irreversible changes over its long history. Meanwhile, changes in composition of atmosphere and hydrosphere influenced secondarily secular zinc distribution as well (Huston David L, 2015).

Evolution of photosynthesis completely altered planet's biogeochemistry, permitting to more complex multicellular organisms to evolve (Finazzi *et al.*, 2010). Evolutionary, succession of marine photoautotrophs began with the origin of photosynthesis in Archean Eon, about 3.8 billion years ago (Katz *et al.*, 2004). Marine microbes, representing only less than 1% of photosynthetic biomass found on land, synthesize approximately equally biomass (Finazzi *et al.*, 2010). Red algae or Rhodophyta are a distinct lineage of eukaryotic algae containing about 5000–6000 species of mostly multicellular marine algae, which absorb light energy by phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin), instead of chlorophyll accessory pigments (De Clerck *et al.*, 2012). Photosynthetic plants and other photosynthetic organisms (algae, microalgae, and photosynthetic bacteria) are principally natural solar energy collectors, which transform solar energy through the fundamental biological process of photosynthesis into chemical energy and thermochemical storage systems, necessary for life. They generate sugar building blocks $(CH_2O)_n$ and oxygen combining water and CO_2, and store the energy in diverse range of molecules, such as oils, carbohydrates (starch, lignin, cellulose) and proteins. They can be later accessed via thermochemical or enzymatic conversion, or converted into biofuels, hence distinguishing biomass from other renewable energy sources, as being primarily a form of solar energy (Huber *et al.*, 2006; Rubin, 2008; Schenk *et al.*, 2008; Demain, 2009; Demirbas, 2009; Edwards *et al.*, 2010). Calvin cycle is integral part of photosynthetic process, responsible for fixing CO_2 in a diverse range of organisms from primitive algae through to higher plants (Schenk *et al.*, 2008; Chaudhary *et al.*, 2014). C3 plants fixing CO_2 through photorespiration and stomatal opening, as well as C4 plants from malate without requiring open stomata are the two main photosynthetic plant categories (Agbor *et al.*, 2011). Ancestral C3 photosynthesis evolved more than 2800 million years ago in a CO_2 rich atmosphere, which has been depleted about 30 Ma million years ago, thus reducing carbon uptake in many terrestrial plants (Sage, 2004). C4 photosynthesis arose suddenly in grassland to ecological dominance 3 to 8 million years ago by a series of coordinated anatomical, biochemical and physiological modifications, which concentrate CO_2 around the C3 photosynthetic carboxylating machinery and promote high rates of photorespiration, transforming ecosystems across large regions of Earth (Sage, 2004; Edwards *et al.*, 2010). C4 plants have a higher water use efficiency and biomass production in hotter and drier climates; whereas C3 plants have an advantage in CO_2 fixation and growth at moderate temperatures and available soil water. Plants typically capture 0.1 to 1.0% of solar energy in a proportion to plant growth rate. Plant breeding, biotechnology, and genetic engineering can accelerate growth rates and enhance production efficiency, simultaneously reducing energy requirements of plants (Huber *et al.*, 2006). EcoPlant, a novel superior soil conditioner of microbial origin, has been developed and is produced by fermentation by Dr. Savas G. Anastassiadis at Pythia Institute of Biotechnology. It protects plants and trees significantly from freezing and it has shown many beneficial properties for plant growth and production (Greece, Bulgaria etc.). It enhances severally photosynthesis activity, energy production and resistance of plants to cold, heat, drought and diseases, enhances plant and root growth, increases semantically growth and the production of crops, vegetables, fruit trees etc., elongates the life of trees, vegetables and other plants and elongates the conservation of fruits and other plant products. Moreover, it accelerates and enhances the absorption of nutrients, macro- and micro-minerals and can semantically reduce the use of fertilizers and pesticides, as well as the final production cost. EcoPlant can contribute semantically to reduce environmental pollution, increase food and feed production helping to overcome the global hunger problem, and overproduce feedstocks for bioenergy production, highlighting the importance of industrial microbiology and biotechnology in solving global future problems.

Carbon cycle means present-day's biogeochemical cyclic transfer of carbon between life (biosphere), atmosphere, soil, rocks and Earth's oceans (Gallagher, 2011), whilst (Berner, 2004) describes another carbon cycle operating over millions of years, which involves carbon transfer between rocks and the combination of life, the atmosphere, and the oceans. CO_2 is an important ecumenical greenhouse gas with a short residence time of ~4 yrs., potentially regulating climate over a vast range of timescales from years to millions of years as either a climate driver or an important amplifier, and the primary driver for global warming the last 100 years

(Shackleton, 2000; Royer *et al.*, 2004). Periodic cosmic ray flux fluctuations may be of some climatic significance, but are likely of second-order importance on a multi-million year timescale (Royer and Berner, 2003). Cheap solar biomass has been planet's primary energy source for millennia (Nault, 2005) and the earliest source of energy and biofuel, providing heating, illumination and cooking for centuries (Abramson *et al.*, 2010). Our society fully depended on plant biomass energy prior to discovery of inexpensive crude oil and fossil fuels in 19th century that helped to industrialize the world and semantically improve living standards (Huber *et al.*, 2006). Biomass, mainly wood and woody materials, is the oldest form of reliable resource of energy used by humans since first manmade fire, up to current utilization of pelletized wood as a feed for thermal plants. Humans in developing countries combusted biomass mainly directly as an important energy source, along with hazelnut shell and forest products, such as wood, logging residues, trees, shrubs. They utilized waste materials, such as agricultural waste residues, energy crops and crop residues, starch crops, such as corn, wheat and barley, sugar crops, grasses, woody crops and wood wastes, vegetable oils, hydrocarbon plants, urban wastes, waste paper and wheat straw, tea waste and olive husk. They also used aquatic biomass like algae, waterweed and water hyacinth (Balat *et al.*, 2006; Huber *et al.*, 2006; Berni *et al.*, 2013; Lee *et al.*, 2014). Biomass, always being a major energy source for the humanity, supplies nowadays 10-14% of world's energy (McKendry, 2002). Biomass represents an abundant carbon-neutral renewable resource for bioenergy and biomaterials. However, increased production and enhanced use of biomass will have an impact on energy, environment, economy, as well as society and culture in coming years (Williams *et al.*, 2006). Energy demand has outpaced the potential biomass supply the last two centuries, requesting large land areas to meet only a significant portion of current primary energy demand using existing types of plants, even the fastest-growing of known energy crops like switch-grass (Nault, 2005). About one third of world's population depends still on traditional biomass as the main energy source, including wood, agricultural, livestock and forestry residues among others, which are more accessible and less expensive, whereas dry biomass is also easily obtainable and storable (Berni *et al.*, 2013). A variety of biomass feedstocks generate currently electricity and produce heat and liquid transportation fuels, whereas a number of ways can convert biomass into energy (McKendry, 2002). Research in biomass production, which is convertible to transportation biofuel, utilizing high yield crops that grow on arid land ideally requiring little nutrients, less water, fertilizers and energy input, is a very desirable issue, which easily fits into existing infrastructure, aiming to reduce air pollution (Huber *et al.*, 2006). Hence, biomass majorly derived from dedicated biomass plantations can become an important primary energy source in future's global energy system, significantly expanding worldwide(Berndes, 2008).

Biomass resources for bioenergy and biofuel production: Biomass is an abundant versatile resource for energy production via different routes (García-Olivares, 2015). Sustainability is a key principle in natural resource management, interdependently involving operational efficiency, minimization of environmental impact and socioeconomic considerations (Brennan and Owende, 2010), aiming to achieve current production goals without compromising the future in terms of resource degradation or depletion (Matson *et al.*, 1997). Completely new approaches in R&D, production and economy are necessary for future rearrangement of a sustainable economy to biological raw materials (Naik *et al.*, 2010). The use of biomass to provide energy has been fundamental for the development of civilization (McKendry, 2002), and sustainable energy is a necessity for human's civilization future in ongoing 21st century (Ndimba *et al.*, 2013). Substantial effort has to be devoted to renewable biomass for future's sake and the conservation of natural resources and environment (Bilgen *et al.*, 2015), to produce sustainably energy, fuels, organic chemicals and polymers in an integrated biorefinery, simultaneously reducing enslavement on limited fossil fuels (Maity, 2015). Bioresources technology involves exploitation of natural substances and/or biotechnological approaches in production processes (Gübitz *et al.*, 1999). Biomass may be converted into energy via several routes, including fermentation and anaerobic digestion (Cesaro and Belgiorno, 2015). Modern fuel biomass requires availability, versatility, sustainability, global and local environmental benefits, and developmental and entrepreneurial opportunities (Bilgen *et al.*, 2015). Biobased energy sources preserve the environment by reintegrating carbon dioxide, released from their combustion, into photosynthetic cycle, thus avoiding net CO_2 buildup into atmosphere(Inui *et al.*, 2005). Otherwise, environmental damage will accelerate, inequity and prices of energy supplies in many countries will increase, global economic growth will be jeopardized, and vulnerability to interruptions in supply will confront all of us (Goldemberg, 2000). Demand for biofuels is currently on the rise worldwide, and the use of biomass is one of most promising alternatives, with Brazil being a pioneer in the use of clean (with respect to CO_2 balances) energy sources (biofuel), replacing gasoline with alcohol fuel since 1973 (Thapa *et al.*, 2014). Since late 1990s, Brazil's atypical global power and influence is rising in international affairs as a non-military and emerging environmental power, participating only in peacekeeping missions. Brazil is world's second biggest food exporter, home to world's biggest tropical rainforest, one of world's largest renewable freshwater reservoir, contains planet's most diverse stock of biodiversity, addressing also Brazil's advocacy of renewable energy, particularly biofuels as a pioneer in using bioethanol since the late 1970s in developing countries (Dauvergne and BL Farias, 2012). Global biomass feedstocks are classified into three general groups of agricultural raw materials (Huber *et al.*, 2006; Lin and Tanaka, 2006; Balat and Balat, 2009; Lin and Huber, 2009): a) amorphous sugars (e.g., starch, simple sugars like glucose, etc.), b) Lignocellulosic or woody biomass (the non-edible portion of biomass, e.g., bagasse, corn Stover, grasses, wood, etc.) and wood residues from wood product industry, including paper mills, sawmills and furniture manufacturing, which are currently the largest biomass source for energy production, and c) Triglycerides (e.g. vegetable oil). (Maity, 2015) classifies the biomass into four general types based on their origin: (1) energy crops, (2) agricultural residues and waste, (3) forestry waste and residues and (4) industrial and municipal wastes (Table 1).

1st generation biomass	**2nd-generation biomass**	**3rd –generation biomass**
Amorphous sugars, e.g. starch, simple sugars like glucose, sucrose Fatty acids Edible plant oils	Lignocellulosic or woody biomass (mixture of cellulose, hemicellulose, and lignin), wood, agricultural, livestock and forestry residues, bagasse, corn Stover, grasses, wood residues Microbial cellulose	Algae, microalgae, algal fatty acids Algal cellulose

Table 1: Biomass for biofuels.

Biomass feedstock choice ultimately depends on crops yields, regional and climatic conditions, food coproduction, economics and life cycle thermal efficiency. We can also separate edible and nonedible part of biomass, to convert nonedible fraction into a fuel, and reuse residual nutrients from biomass for new biomass growth (Huber *et al.*, 2006). Biomass feedstock has also to be harvested, collected, transported and possibly stored, before processing into a suitable form for the chosen conversion technology (McKendry, 2002). Biofuels, including gaseous forms like methane and hydrogen, as well as liquid forms of fuel such as sugar- and starch-based or lignocellulosic ethanol, biodiesel, biohydrogen, expectably butanol and bioelectricity are fuels derived from biological sources, utilizing physical, chemical and/or biological processes. These include grain, sugarcane or sugar beet, cellulose, a variety of vegetable virgin oils and animal fats, waste oils, and a greater diversity of primary raw materials, including an array of recycled materials. Liquid biofuels replacing diesel and gasoline have gained particular interest as a promising alternative, while biomass conversion to liquid fuel via pyrolysis and the production of alkanes from the hydrogenation of carbohydrates, lignin, or triglycerides is also receiving attention (Hansen *et al.*, 2009; Berni *et al.*, 2013). Energy supply from biomass has gained an important role among renewable energy sources (RES) in several world regions, while bioenergy will expectably supply large amounts of CO_2 neutral energy (Berndes, 2008). Despite the existence of other renewable and sustainable electricity and heat producing resources, such as solar, wind and hydroelectric, only plant biomass can be converted directly into liquid biofuels like ethanol and biodiesel and chemicals, hence other forms of renewable energy should be used for stationary power generation (Huber *et al.*, 2006). Biomass derived bioenergy is an interesting energy source contributing to sustainable development. Biofuels or bio-renewable fuel (refuel) is referred to solid, liquid or gaseous fuels predominantly generated from biological material and biomass, a concept that has been narrowed down to renewable sources of carbon (Demirbas, 2009; Lee *et al.*, 2014). Biofuels derived from biomass are already available alternative fuels to fossil fuels today. They have in addition to being renewable and biodegradable also the advantage of substantially reducing oil imports (energy independence) as well as the emission of CO_2 and unburned hydrocarbons, carbon monoxide, hydrocarbons, soot and particulate. They address substantial challenges related to energy security, socioeconomic development, economic prosperity, environmental sustainability and climate change mitigation (Agarwal, 2007; Berndes, 2008; da Silva *et al.*, 2012; Gude *et al.*, 2013). Crucial issues, some uncertainties, constraints and consequences emerged that could influence bioenergy's future, re-evaluating large-scale bioenergy potentials. They comprise greenhouse gas balance of bio-energy and climate change, economic and population growth, environmental implications of large-scale bio-energy use, land use, availability and degradation, population and food demand. They refer also to yield levels and development in energy crop production, water scarcity as well as biodiversity protection, indirectly impacting food production and biodiversity (biodiversity loss) (Gurgel *et al.*, 2012). Agricultural productivity in many of worlds' parts is often today no greater than that on the fields of Roman Empire (Viala, 2008) (Table 2). Sugar cane has the lowest water footprint of energy crops, while rapeseed has a very high water footprint (Table 3). Oil palm requires lower inputs of fertilizers and agrochemicals compared with soya and rapeseed.

Biodiesel production: Biodiesel has been promoted as one of promising alternative renewable fuels for environmental and economic sustainability. It has been chemically defined as a mixture of lower simple monoalkyl fatty acid (chain length C14–C22) esters of short-chain alcohols, primarily methanol or ethanol, obtained by transesterification or alcoholysis. Oil triglycerides from various renewable feed stock lipid resources, such as seeds of nonedible plants like *Jatropha*, palm kernel, pongamia etc., edible vegetable oils (sunflower, rapeseed oil), animal oils or fats and wastes of cooking oils

Feedstock	Productivity (l/ha)	Energy content (MJ/l)	Fossil energy balance*	water footprint m^3/GJ
Sugar cane	6,000 liters	24	2.0 to 8.0	29
Oil palm and sugar beet	5,000-6,000	30.53 MJ/l	9.0	75 (oil palm)
Corn, cassava and sweet sorghum	1,500-4,000		1 to 4	
Rapeseed, wheat and soya	1,500		1 to 4	131 Rapeseed) 99 (soya)

Table 2: Biofuel productivity (Fossil energy balance - ratio between renewable energy output and fossil energy input).

consist of three long chain fatty acid molecules, which are esterified with a molecule of glycerol (Demirbas, 2009). Economic sustainability requires long-term profitability, minimal competition with food production and competitiveness with fossil fuels. Worldwide established first-generation biofuels, including biodiesel (bio-esters) produced by transesterification from edible sources such as rapeseed oil, palm oil, or other plant oils, ethanol from sugar cane, and biogas, appear unsustainable due to potential stress placed on food commodities, impacts on biodiversity, contamination from fertilizers and herbicides, especially in monocultures (Naik *et al*., 2010). Raw materials used for the production of first generation biofuels, such as corn and sugarcane used for bioethanol production and soybeans used for biodiesel, are typically of high added value, making biofuel production more expensive. A single biofuel satisfying all aspects completely does not exist. Amongst first generation biofuels, palm oil biodiesel is a highly sustainable feedstock far superior to corn, rapeseed and soya. First generation biodiesel production oilseed crop oils including peanut, soybean, rapeseed, linseed, sunflower, safflower, coconut and corn oils are potential renewable sources of biofuels, used as diesel fuel substitutes in many parts of the world (Banerji *et al*., 1985). Oil-producing plants contain triglycerides, carbohydrates, protein, fiber and ash (Huber et al. 2006). Biodiesel derived from non-toxic, renewable and biodegradable resources has become more attractive recently, gaining more and more importance as an attractive fuel, due to depleting fossil fuel resources and its environmental benefits (Berchmans and Hirata, 2008). Due to rapid decline in crude oil reserves, vegetable oils are promoted as alternative to diesel fuels in many countries, which depending upon climate and soil conditions are looking into different vegetable oils for diesel fuels triglyceride as basic constituent. Currently, vegetable oils are being used for biodiesel production by transesterification. Soya bean (soybean) oil and animal fats is being considered as substitutes for diesel fuels in US and primarily rapeseed (80%) and to a lesser extent sunflower oils in Europe. Palm oil serves in tropical countries, such as Southeast Asia, mainly Malaysia and Indonesia, coconut oil in Philippines, whereby the development of more productive plant species with high yield of oil seed is required and requested (Huber *et al*., 2006; Shang *et al*., 2010). Most of US biodiesel is made from soybeans, causing a resource limitation of arable land. Moreover, rising soybean prices and falling crude oil and diesel prices have hurt the competitiveness of biodiesel industry during 2009 (Gallagher, 2011). About 70% of biodiesel in Europe is produced from rapeseed oil, 17% from soybean oil, and to an even smaller extent from sunflower and palm oil (Havlík *et al*., 2011). Sunflower is the most suitable vegetable oil crop for biodiesel production in Greece, with high crop yield potential and high oil content, while cottonseed oil production is the least attractive option. Positive energy balances have been determined for the cultivation of rapeseed (maximum energy efficiency coefficients of 10.68), sunflower (6.16) and sweet sorghum (8.92) in Greece, while the highest energy was spent for tillage and irrigation. Palm oil biodiesel is a highly sustainable far superior feedstock, compared with corn, rapeseed and soya. Palm oil obtained from tropical plant Elaeis guineensis with the highest oil content of all crops (45-55% oil) contains 50% saturated fatty acids (palm kernel oil and palm oil). It is the second largest consumed vegetable oil in the world. Yet it is beneficial to health, whereas oxidized palm oil is potentially dangerous. Biodiesel production is widely questioned because of shortage and high cost of edible oils (Shang *et al*., 2010) and today's biodiesel is not completely sustainable. Biodiesel creation requires processing with dangerous chemicals such as methanol, most of which comes commonly from fossil fuel, and it is mainly produced in large-scale from edible crop oils, such as rapeseed oil in Europe, soybean oil in American, and palm oil in Southeast Asia (Shang *et al*., 2010). Production of biofuels from human nutrition sources and food chain materials such as edible oils can cause a food crisis (Srinivasan, 2009). Biodiesel has to compete economically with petroleum diesel fuels to be future prospective (Berchmans and Hirata, 2008). Vegetable oils will not entirely displace petroleum based diesel fuel but will be wider used due to technical, economic, and environmental considerations.

Second-generation biodiesel production: Using sufficiently available and sustainable supplies of less expensive feedstocks such as inedible oils, animal fats, waste food oil and byproducts of refining vegetables oils is one crucial way of reducing biodiesel production costs, delivering competitive biodiesel to commercials filling stations (Berchmans and Hirata, 2008). More than 350 oil-bearing crops have been identified so far, among which only sunflower, safflower, soybean, cottonseed, rapeseed and peanut oils are considered as potential alternative fuels for diesel engines. Increasing biodiesel demand and growing energy crops instead of food or animal feed crops arose concerns about globally rising food prices, creating social unrest in developing countries and expanding agriculture's environmental impact. Consequently, the interest in less expensive fatty acids containing feedstocks has grown, to reduce biodiesel production costs. Inedible oils like crude tobacco seed oil, castor, jojoba and Tung oil (Tung tree), karanja and *Jatropha* oil, yellow, as well as animal fats, waste food frying oil, grease, tallow and lard, and byproducts of refining vegetables oils are those feedstocks (Ghadge and Raheman, 2005; Berchmans and Hirata, 2008; Shang *et al.*, 2010). Non-edible unconventional oils obtained from plant species, such as *Jatropha curcas* (Ratanjyot), *Pongamia pinnata* (Karanja), *Calophyllum inophyllum* (Nagchampa), *Hevca brasiliensis* (Rubber), tobacco (*Nicotiana tabacum* L.) seed oil, sal, neem (*Azadirachta indica*), undi and mahua oil (*Madhuca indica*, 20% FFAs), as well as Tung tree etc. can be main resources for 2nd generation biodiesel production (Ghadge and Raheman, 2005; Agarwal, 2007; Shang *et al.*, 2010). Inedible vegetable oils, mostly produced by seed-bearing trees and shrubs, can provide an alternative biofuel not competing with food usages. Characteristically, special attention turns to tropical and subtropical *Jatropha curcas* L. growing in climates across the developing world (Openshaw, 2000). Oil seed bearing *Jatropha curcas* Linnaeus ("Jatropha") has been seen as an energy source and effective solution to combat greenhouse effect, help mitigate soil erosion and provide rural employment and higher incomes (Srinivasan, 2009). It has a high content of free fatty acids of ~15%, compared with 6.1% of crude palm oil and only 1.2% of coconut oil (Berchmans and Hirata, 2008). *Jatropha* oil contains about 14% free fatty acids, far beyond the limit of 1% FFA level that can be converted into biodiesel by transesterification using an alkaline catalyst. *Jatropha* biodiesel has comparable fuel properties with diesel and is compliant to latest biodiesel standards yielding above 99% (Tiwari *et al.*, 2007). Free fatty acids and moisture contents have significant effects on transesterification of glycerides with alcohol as catalyst. Oils of *Jatropha* species are rich in oleic and linoleic acids (72.2-84.0%); however, no correlation exists between energy values and fatty acid composition (Banerji *et al.*, 1985). *Jatropha* Linn. (Euphorbiaceae) is a genus comprising of 70 species, distributed in world′s tropical and sub-tropical parts. Among four species of *Jatropha* (*J. curcas*, *J. glandulifera*, *J. gossypifolia* and *J. multifida*), the energy value of *J. multifida* oil has been reported to be the highest (13647 kcal g^{-1}) (Banerji *et al.*, 1985). (Mofijur *et al.*, 2015) suggest the use of 20% Jatropha biodiesel in unmodified diesel engines to meet global energy demand and to reduce emissions into atmosphere, without creating food versus fuel conflict. *Jatropha curcas* L., often considered, as a magical biodiesel plant with multitude of environmental benefits on a global scale, did not match however the expectations in real performance under field condition according to Edrisi *et al.*, (2015). Yellow oleander, native to Peru and well growing in Kenya, is drought tolerant, requiring little care even grown in dry areas. Henceforth Kenya is now exploring the potential to produce biodiesel from its oily seeds (Ruth, 2008). The easy growing and maintainable candlenut plants contain high vegetable oil content of 60-65% (Budianto *et al.*, 2006). Crude tobacco (*Nicotiana tabacum* L.) seed oil, a by-product of tobacco leaf production and mahua oil (*Madhuca indica*) have been shown to be appropriate substitutes for biodiesel fuel with high free fatty acids (19% FFA) (Ghadge and Raheman, 2005). Greek tobacco seed, a byproduct of tobacco leaves, contains 38% oil with major constituent linoleic acid (18:2), oleic acid (18:1) and palmitic acid (16:0). Fatty acid composition varies with the origin of Tung oil and is a major factor, influencing the properties of biodiesel (Shang *et al.*, 2010). Tung oil usually contains more than 80 wt.% of α-elaeostearic acid, a long-chain (C18) fatty acid with three conjugated double bonds (at carbons 9 cis, 11 trans, 13 trans) (Shang *et al.*, 2010). Variation in unsaturation pattern in plants such as soybean, flax and corn can be controlled by genetic engineering techniques (Khot *et al.*, 2001).

Ethanol production: produced through fermentation of various sugars (Sun and Cheng, 2002). Ethanol has been adopted worldwide as a strategically important transportation fuel, providing to countries independence from nonrenewable fuels, for climate and economic reasons (Reboredo *et al.*, 2016). Ethanol production comprises three main steps, namely acquisition of fermentable sugars, conversion of sugars to ethanol via fermentation process and distillation and purification of produced ethanol (Özçimen and İnan, 2015). Ethanol is highly soluble in water, biodegradable and lightly toxic, while large fuel spills are far less environmentally threatening than spills of crude oil or gasoline. Large quantities of bioethanol are produced worldwide from sugar and cereals. Any sugar containing material can be converted into ethanol by fermentation after hydrolysis to simple sugars, including sugars (sugarcane, sugar beets, molasses, and fruits), starches (corn, wheat, oats, rice, cassava, potatoes, and root crops), and cellulose materials (wood, agricultural residues, waste sulfite liquor from pulp, and paper mills). Cellulose materials represent the most abundant global source of biomass (Lin and Tanaka, 2006; Sakuragi *et al.*, 2011) and lignocellulosic ethanol is considered as the expanding most potential next generation transportation fuel (Jin *et al.*, 2015). Starches are amorphous polymers consisting of glucose units bound by R-1,4 glycoside and a large amount of R-1,6 glycoside linkages (Huber *et al.*, 2006). Treatment in hot water releases the two principle components of starch: water-soluble amylose (10-20 wt %) containing only R-1,4 glycoside linkages and water-insoluble amylopectin (80-90 wt %) containing R-1,4 and R-1,6 glycoside linkages at a R-1,4 to R-1,6 linkage ratio of 20:1 (Huber *et al.*, 2006). Most of ethanol, used in gasohol or oxygenated fuels containing up to 10% ethanol by volume, is produced by fermentation of corn glucose in US or sucrose in Brazil since the 1970-1980s (Sun and Cheng, 2002; Lin and Tanaka, 2006). Cornstarch-based production of ethanol, referred to as first generation bioethanol, competes for the limited agricultural land needed for food and feed production

(Sun and Cheng, 2002).

Research and development is under way to develop and improve 2nd generation ethanol production from cellulosic biomass (Agarwal, 2007), after a first step of pretreatment and the hydrolysis of lignocellulosic biomass by various cheap and efficient hydrolysis processes, enabling low cost and efficient ethanol production from monomeric sugars, particularly in Sweden and US (Faaij, 2006; Dussan *et al.*, 2014). Lignocellulosic plant material represents an as-of-yet untapped source of fermentable sugars, because of many physio-chemical structural and compositional factors hindering enzymatic digestibility of cellulose (Mosier *et al.*, 2005). A cost-effective and efficient biomass pretreatment technology is necessary for lignocellulosic biofuel production (Jin *et al.*, 2015). Industrial scale lignocellulosic ethanol production seems almost a reality (Larsen *et al.*, 2012), preferably converting biomass from agricultural feedstock (wheat, rice and barley straws, corn stover and sugarcane bagasse) or energy crops, rather than woody biomasses, such as spruce, willow, poplar or aspen (arundo donax and miscanthus) (Cannella *et al.*, 2014). Well-known for tequila production in Mexico, Agave is a promising productive lignocellulosic feedstock for biofuels production, offering high productivity with low water and nutrient demand in hot and arid regions, which are too harsh for conventional energy feedstocks. It thrives on arid and semiarid lands in diverse locations which are unsuitable for conventional agriculture, as also other lignocellulosic feedstocks, such as poplar, switchgrass, miscanthus, and sugarcane, such as Brazil, southwestern region of United States, particularly Arizona, California, and Texas, Australia, Southern and Eastern Africa, and areas across the Mediterranean (Lewis *et al.*, 2015). Agave species with high water use efficiency and drought resistance can be sufficiently productive even with only modest rainfall in Mexico and western United States. Agave species are low lignin biofuels feedstocks, while ~54-57 % of total carbohydrates of *A. americana* and *Agave salmiana* and ~34 % in *A. tequilana* are soluble sugars (Lewis *et al.*, 2015; Mielenz *et al.*, 2015). Agave plants *A. deserti* and *A. tequilana* can potentially contribute to supply 4.8–9.6% of United States′ ethanol, and 2.5–4.9% electricity consumption (Lewis *et al.*, 2015). Agave is a very efficient plant for the production of polyfructose molecules and soluble fermentable carbohydrates, such as glucose and high levels of fructose and sucrose. Agave species grow well in semi-arid marginal agricultural lands throughout the globe, regarding water use because of its crassulacean acid metabolism, minimizing Agaves′ water loss by limiting transpiration during the heat of the day by closing the plant stomata (Mielenz *et al.*, 2015). Soluble carbohydrates can be converted into bioethanol by *Saccharomyces cerevisiae* or biobutanol by *Clostridium* species (Mielenz *et al.*, 2015). Previously considered as a waste, sugarcane bagasse is the most abundant co-product in Brazilian sugar and ethanol industry, creating 280 kg of bagasse from 1 ton of cane (Dussan *et al.*, 2014) and most abundant biomass in Brazil, expectably reaching during the 2010/2011 season 651.5 million tons (Gurgel *et al.*, 2012). Sugarcane bagasse (composed of fiber bundles and pith), owing a large fraction of polymerized sugars, is an interesting lignocellulose rich feedstock for the biobased economy. Efficiency of pretreated solid material for enzyme monomerization reached 81% for acid pretreatment, 77% for autohydrolysis and 57% for alkaline pretreatment after 72 h (Gurgel *et al.*, 2012; Batalha *et al.*, 2015).

Pretreatment of agro-residues, acting mechanistically and disorganizing hemicellulose, is an inevitable process to depolymerize hemicellulosic fraction into xylose, whereby acid load, temperature, residence time and solid-to-liquid ratio as well as reactor design are pivotal parameters in kinetics of dilute acid hydrolysis of agro-residues and minimization of side (or by)-products (Chandel *et al.*, 2012). Dilute sulfuric acid (acid hydrolysis with 2% of H_2SO_4 at 155°C for 10 min, 1:8 solid-liquid ratio) is most common and efficient separating process in pretreatment of agro-residues for obtaining cellulose, which is submitted to acid or enzymatic hydrolysis to release glucose after a delignification stage using sodium hydroxide as catalyst (Chandel *et al.*, 2012; Dussan *et al.*, 2014). It is a simple and fast method for obtaining hemicellulosic hydrolysates, mainly containing xylose (80% of sugar content), arabinose, glucose, galactose, mannose along with cell wall-derived inhibitors such as furans, phenolics, weak acids and others (Chandel *et al.*, 2012). Acceleration of biodetoxification improves process efficiency for cellulosic ethanol production from dilute acid pretreated lignocellulose feedstock by *A. resinae* ZN1 (YanQing and Jian, 2016). Qing *et al.*, (2015) reported about the successful use of oxalic acid as an alternative to mineral inorganic acid in acid pretreatment of corncob, achieving a very high fermentable xylose yield of 94.3 % at 140°C for 40 min with 0.5 wt% oxalic acid at a solid loading of 7.5%. Steam explosion is most widely employed physicochemical pretreatment for lignocellulosic biomass, while fed batch hydrolysis increased ethanol production by about 162% (de Albuquerque Wanderley *et al.*, 2013). Nano-particles-based membrane systems may improve hemicellulose hydrolysis shortening times and reducing by-product formation (Chandel *et al.*, 2012). Applying the simple, low-cost and environmental friendly autohydrolysis technology for generation of sugars from biomass, bioethanol production can have a financial return larger than 12% (Batalha *et al.*, 2015). Essential minimum requirement for more sustainable alternative biofuels compared to fossil fuels is to be produced from renewable raw material, having a lower negative environmental impact (Achten *et al.*, 2008). Lignocellulosic biofuels have great potential as environmentally friendly alternative in reducing greenhouse gas emissions by 60-90% when compared with fossil petroleum (Börjesson *et al.*, 2007). Agro-residues can be used to produce important bioproducts such as D-xylitol, an artificial sweetener, ethanol, organic acids, and industrial enzymes etc. for medical and nonmedical applications (Chandel *et al.*, 2012). Agronomic lignocellulosic woody residues, such as crop residues, grasses, sawdust, wood chips and solid animal waste, collectively termed "biomass", can be potentially converted into low-cost fuel ethanol (Sun and Cheng, 2002; Lin and Tanaka, 2006). Lignocellulose hydrolysis to fermentable monosaccharides is technically problematic due to many physico-chemical, structural and compositional factors hindering digestibility of cellulose (de Albuquerque Wanderley *et al.*, 2013). High sugar concentrations achieved after pretreatment are crucial factor for competitive commercial use of lignocellulosic materials (Chandel *et al.*, 2012).

Chemical composition of lignocellulosic biomass differs with the source of plant species (Chandel *et al.*, 2012). Cellulose (microfibrils with characteristic distributions and organization) of plant cell wall is generally cross-linked with hemicellulose and lignin (Chandel *et al.*, 2012; Dussan *et al.*, 2014). The number of glucose units of wood cellulose molecule (i.e. the degree of polymerization) varies from at least 9,000–10,000 up to as high as 15,000 (Rowell et al. 2005, (Chandel *et al.*, 2012). The susceptibility of cellulose to enzymatic hydrolysis has been attributed to various structural features of lignocellulose. Cellulose molecules consist similarly to starch molecules of long chains of glucose molecules, but with a different structural configuration, making them in addition to encapsulation by lignin more difficult to hydrolyze than starchy materials. Most abundant renewable biological resource lignocellulosic biomass is formed by biopolymers, such as the most abundant high-molecular-weight biomolecule on earth cellulose (35–50%), hemicellulose (20–35%) and lignin (10–25%). It is an attractive and relatively inexpensive raw material, which is also outside the human food chain (Elshahed, 2010; Sakuragi *et al.*, 2011; Pereira *et al.*, 2012; Morales-Martınez *et al.*, 2014). Cellulosic ethanol may become potentially cost competitive with corn grain ethanol through improved pretreatments, enzymes, and conversion factors (Hill *et al.*, 2006; Himmel *et al.*, 2007). Second-generation lignocellulosic bioethanol, produced from waste streams, constitutes the most feasible technical option of potential renewable energy source and attracting intensive research effort in recent years, and resulting in key technologies with improved yields and efficiencies (Novy *et al.*, 2015). Susceptibility of cellulosic substrates to cellulases depends on structural features of substrate. Pretreatment of lignocellulosic materials by physical, physicochemical, chemical and biological processes is usually used to alter structural and compositional impediments and to remove lignin and hemicellulose, reduce cellulose crystallinity and increase material porosity, in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose (Sun and Cheng, 2002; Mosier *et al.*, 2005). It meets following requirements: (1) facilitate enzymatic hydrolysis for sugar formation (2) avoid the degradation or loss of carbohydrate (3) avoid the formation of inhibitory byproducts for subsequent hydrolysis and fermentation and (4) be cost-effective (Sun and Cheng, 2002). Hydrolysis is usually catalyzed by highly specific bacterial (high specific activity) and fungal (*Trichoderma reesei*) cellulases, a mixture of enzymes operating at mild conditions of pH 4.8 and 45-50°C, which form reducing sugars, including glucose, while sugar fermentation is carried out by yeasts or bacteria (Sun and Cheng, 2002). Factors identified to affect hydrolysis of cellulose include porosity (accessible surface area) of waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content. Combination of peroxide and acid pretreatment is an effective and environmentally friendly method for enzyme hydrolysis of napier grass, dramatically improving substrate hydrolyzability, producing 287.81 mg glucose and 245.81 mg xylose per g of initial dry sample (Bohórquez *et al.*, 2014). The addition of surfactants such as ethylene oxide polymers like poly(ethylene glycol) to hydrolysis mixture, adsorbing lignin surfaces and thus reducing unproductive enzyme binding, has been reported to increase enzymatic hydrolysis of lignocellulose and softwood lingocellulose 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 50°C (Börjesson *et al.*, 2007). Gavilà *et al.*, (2015) reported about hydrolysis of chemically robust, highly crystalline cellulose into glucose containing liquid by synergistically combining dilute acid treatment and microwaves in a microwave reactor, to produce 98% optically pure d-lactic acid by *Lactobacillus delbrueckii*, a natural carboxylic acid with extended industrial applications, especially production of poly-lactic acid, a thermoplastic aliphatic polymer used as bioplastic. 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). Removal of hemicelluloses is highly desirable for their bioconversion into ethanol (Li *et al.*, 2015), whilst lignin removal can dramatically increase hydrolysis rate, because it blocks access of cellulases to cellulose (Sun and Cheng, 2002). Cellulases can be recovered mainly from liquid supernatant or solid residues, reducing cost (Sun and Cheng, 2002). Pretreating different lignocellulosic biomass sources (Grass clipping, corn straw, catalpa sawdust and pine sawdust) with high-pressure (10 MPa) homogenization 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 enzymatic digestibility of green liquor and acidic bisulfite pretreated materials, but has little effect on sulfite-formaldehyde pretreated samples (Wang *et al.*, 2015).

Ethyl alcohol (ethanol) is a primary metabolite produced by fermentation of sugar, or fermentable sugar derived by polysaccharide depolymerization. *Saccharomyces cerevisiae* ferments hexoses, reaching approximately 10-12% ethanol by volume within five days, which slows down growth and ceases fermentation, whereas *Pichia stipitis* or *Candida* species can utilize pentose. Special yeasts can reach alcohol concentrations of 20% by volume, however only after months or years of fermentation. *Kluyveromyces fragilis* or *Candida species* can be used to convert lactose or a pentose, respectively (Demain, 2000; Demain, 2009). Complete conversion of major C5 and C6 biomass sugars is critical for efficient biofuel production processes (Mohagheghi *et al.*, 2015). Traditional ethanol producing microorganisms such as *Saccharomyces cerevis*iae and *Zymomonas mobilis* do not 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; YanQing and Jian, 2016). *Zymomonas mobilis* is a unique Gram-negative fermentative bacterium offering a number of advantages over existing ethanol-producing microorganisms for large-scale bio-ethanol production. It is as a prokaryote more amenable to genetic manipulations, i.e. conventional mutagenesis, including UV light, 1-methyl-3-nitro-1-nitrosoguanidine (NTG), caffeine and ethyl methane sulfonate, transposon mutagenesis, adaptive laboratory evolution, specific gene knock-out, and metabolic pathway engineering. Biomass resource range is extended, pentose and hexose sugars from lignocellulosic hydrolysates are simultaneously fermented and acetate resistance is increased, attracting great attention in ethanol production (Panesar *et al.*, 2006; Linger *et al.*, 2010; YanQing and Jian, 2016). Recombinant *Zymomonas mobilis* strain 8b has been improved through continuous adaptation on dilute acid pretreated corn stover hydrolysate (Mohagheghi *et al.*, 2015). Simultaneous saccharification /fermentation processes have been described utilizing *Z. mobilis* for the conversion of liquefied starch (Lee *et al.*, 1983) or cellulose to ethanol (Spangler and Emert, 1986). Simultaneous saccharification and cofermentation has been reported for efficient ethanol production from dilute-acid pretreated biomass by metabolically engineered *Z. mobilis* or for other microorganisms. It cofermented 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 cofermentation 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, enzymes and microorganisms are added at the same time to the slurry, simultaneously converting cellulose and fermenting sugar to ethanol. The central metabolic pathway of *Corynebacterium glutamicum* has been engineered to produce ethanol, bearing and expressing the pyruvate decarboxylase (pdc) and alcohol dehydrogenase (adhB) coding genes of *Z. mobilis (*Inui *et al.*, 2005*)*. Oxygen deprived growth-arrested cells of recombinant ethanologenic *C. 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 NADH concentrations in *C. glutamicum* are correlated to oxygen-deprived metabolic flows (Inui *et al.*, 2005). *Scheffersomyces stipitis*, an excellent ethanol producer from lignocelullosic hydrolysates, has been reported to successfully produce ethanol (YP/S 0.38 g g^{-1} and QP 0.23 g L^{-1} h^{-1}) from detoxified cellulosic hydrolysate (Dussan *et al.*, 2014). Future process improvements requiring deeper understanding of cellular and metabolic activities will combine key process steps, thus reducing 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 function near theoretical limits and modified energy plants will serve as improved substrates for the new enzymes or will harbor genes encoding enzymes necessary for self-deconstruction, which are activated before harvest or at normal conclusion of the growth cycle (Himmel *et al.*, 2007). Melin *et al.*, (2016) reported about enhanced biofuel production utilizing separate lignin and carbohydrate processing of lignocellulose to produce ethanol and methane via syngas from biomass.

Glucose derived from cellulosic paper sludge by acid hydrolysis (Arkenol, USA) has also been successfully used by the author Dr. Savas G. Anastassiadis (USA) to produce lactic acid by a new developed continuous fermentation process reaching continuously more than 100 g/l, which was slightly better than using pure dextrose powder.

Carbon sources for third-generation biofuels: The hotly debated globally oil seeds, waste cooking oil and animal fat, mostly used for renewable energy production in recent years, are unable to target efficient biofuel production, climate change mitigation and economic growth, without intervening with food supply (Patil *et al.*, 2008; Mata *et al.*, 2010). Despite concerns over food prices, land use, deforestation etc., first generation biofuels have essentially led to the establishment and development of infrastructure, policies, and knowhow (Scott *et al.*, 2010) of a new challenging scientific field of biofuels, as often happens historically in pioneering sciences. Multidisciplinary developed by biologists, agronomists, chemical engineers, fuel specialists, and social scientists, locally sustainable systems could produce biofuels in an environmentally responsible way from dedicated energy crops, separately and distinctly from food, to supply about 30% of global demand without affecting food production (Koonin, 2006). The highly promising biorefining concept created a biomass-based industry, which processes biomass to obtain energy, biofuels and high value products (Gonzalez-

Delgado and Kafarov, 2011).

According to Kevin Hicks, third-generation biofuels are broadly defined as new and hybrid processing technologies converting organic materials into useful bioproducts, while in the meantime speeding up natural processes turning organic material into oil or coal over millions of years is one of scientific aims (Ruth, 2008). Gao and McKinley, (1994) reported about the great potential of macroalgae for environmentally friendly biomass production for biofuels and CO_2 bioremediation, without to compete with terrestrial crops for farm land in contrast with land plants, driving next generation of economic opportunities and pollution abatement. Increased attention have recently received the very promising 3rd generation biofuels, such as biodiesel, bioethanol, biomethane, biohydrogen and biomass-to-liquid biofuel, derived from sustainable micro- and macroalgal biomass (Schenk *et al.*, 2008; Dragone *et al.*, 2010; Méndez-Vilas, 2010; Kröger and Müller-Langer, 2012; Konda *et al.*, 2015) called as 3rd generation biomass (Xu *et al.*, 2014). Marine based macroalgal biomass (marine seaweeds) is an abundant source of renewable sugars, not requiring arable land, fresh water or intense care for its production, among other benefits (Konda *et al.*, 2015). Microalgae reemerged recently as one of most promising alternative and abundant feedstock sources for next generation of food, feed, cosmetics, renewable energy, biofuels, valuable chemicals, natural and other products (Stephens *et al.*, 2010; Rumin *et al.*, 2015). Invasive marine brown seaweed *Sargassum muticum*, which is unsuitable for biofuel production due to high ash and water content, has naturally a high content of pharmaceutically relevant materials, such as antioxidants, carotenoids and phenols, and the well-known anti-cancer compound fucoxanthin (Rumin *et al.*, 2015). Grown away from farmlands and forests and yielding much higher oil amounts than traditional oilseeds, they have emerged as one of most promising feedstocks for biodiesel production, replacing petrodiesel and biodiesel from edible oil crops and reducing land demand (Francisco *et al.*, 2009). Microalgae are very small aquatic plants presenting one of few technologies for capturing and utilization of CO_2 emitted by stationary industrial and power plants. Recently attracting attention for biofuel production appear to be the only source of renewable biodiesel capable to meet and cover global demand for transport fuels (Patil *et al.*, 2008; Francisco *et al.*, 2009; Ogawa *et al.*, 2015). Microalgae belong to most productive biological systems for capturing and sequestering carbon and generating biomass, which can be completely harvested (Sayre, 2010). Unicellular microalgae are more efficient and sustainable for biofuel-production, and even superior to most productive vascular oil crops such as soya and oil palm, and similarly sugarcane used for bioethanol production, whereas consistently productive strains under a variety of environmental conditions are more desirable (Chisti, 2008; Hildebrand *et al.*, 2012; Vanthoor-Koopmans *et al.*, 2013). Algal and microalgal biomass, reaching a very distinctive growth yield as compared with classical second lignocellulosic biomass, is one of most sustainable feedstocks for lipid-based third-generation biodiesel production (Brennan and Owende, 2010; Lee *et al.*, 2014).

Autotrophic microalgae convert solar energy very efficiently into biomass and oil and are more flexible in land and water quality, greatly mitigating "food-versus-fuel" concern (Davis *et al.*, 2011). They grow in salt water and waste streams, utilize marginal and infertile land and solar light as well as CO_2 gas as nutrients (Saifullah *et al.*, 2014). Microalgae grow and produce biomass more efficiently and sustainably producing 10-100 times, higher oil than oleaginous crops (e.g. soya and oil palm) with high lipid content and they do not compete for arable land. Microalgal growth, harvest and oil extraction are easier and more economical, but we have to overcome scientific and technical barriers and necessarily use optimally all produced compounds, to realize economically feasible large-scale production of microalgal biofuels (Vanthoor-Koopmans *et al.*, 2013; Srivastav *et al.*, 2014; Rumin *et al.*, 2015). Capital cost reduction can furtherly reduce production cost more than operating cost. Faster growing and more importantly higher lipid containing microalgal strains, as well as bioengineering can potentially reduce production costs (Davis *et al.*, 2011). There is still some way from realizing the undoubted potential offered by algal biodiesel in terms of positive energy balance and global warming potential (Scott *et al.*, 2010). Third-generation biofuels are advanced and viable biofuels, specifically derived from single-celled photosynthetic microorganisms, such as microbe and microalgae. They grow in different environments under a wide range of temperatures, in warm, tropical, and subtropical climates, pH and nutrient availability (González-Delgado and Kafarov, 2011). Selection of high lipid producing strains optimized to regional climate conditions and to large-scale production of algae biomass, and preferably amenable to metabolic engineering, will be crucially important (Scott *et al.*, 2010). Hydrogen (H_2) has been produced from microalgal biomass (dark fermentation) and methane (CH_4) from the residues of H_2 production in a combined two-stage fermentation process, while H_2 production increases seven-fold applying enzymatic pre-treatment (Wieczorek *et al.*, 2014). Microalgae are also a promising future source for sustainable edible oils, proteins, lipids and carbohydrates for the food/feed and biofuel industry, whereas metabolic engineering techniques and cultivation strategies can make microalgal oil a cost-effective alternative for common vegetable oils (Vanthoor-Koopmans *et al.*, 2013; Klok *et al.*, 2014).

Microalgae is one of planet′s oldest living organisms growing either as individually existing unicellular algae, or in chains or groups forming the base of alimentary chain in seas and rivers, also known as 'plankton' (Satyanarayana *et al.*, 2011). Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO_2, N, P, and K), tolerating high salts concentration and allowing the use of any type of water and innovative compact photobioreactors. They are a potential source of biomass with a great biodiversity and consequent variability in biochemical composition, which produces lipids, proteins and carbohydrates in large amounts over short periods, processed into biofuels and other valuable co-products (Brennan and Owende, 2010; Satyanarayana *et al.*, 2011). Growth characteristics and microalgae composition knowingly depend significantly on cultivation conditions (Chojnacka and Marquez-Rocha, 2004; Chen *et al.*, 2011), particularly light density which decreases exponentially in water, especially in northern waters with more plankton and suspended material (Adams *et al.*, 2009). About half of dry weight of microalgal biomass is carbon (Mirón *et al.*, 2003), which is typically derived from carbon dioxide. Producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide (Chisti, 2008). Mirón *et al.*, (2003) reported about an average elemental biomass composition comprising 49.2% C, 6.3% H, 0.8% N, and 1.3% S, whereas chlorophylls, carotenoids and pigments content of biomass change in dependence on irradiance.

Microalgal metabolism supports phototrophic, heterotrophic, mixotrophic combining heterotrophy and autotrophy by photosynthesis, photoheterotrophic using light as energy source, and chemoheterotrophic algae growth oxidizing organic compounds for energy, enabling efficient future developments and commercially viable technologies for microalgae-based biodiesel production (Dragone *et al.*, 2010) (Chen *et al.*, 2011). Autotrophs can be photoautotrophic using light as energy source, or chemoautotrophic oxidizing inorganic compounds for energy, while heterotrophic microalgae use organic compounds for growth (Schenk *et al.*, 2008; Dragone *et al.*, 2010). Some microalgae can metabolize simultaneously or independently inorganic and organic carbon sources, while light and/or an organic carbon source supply the energy. Grown in circulated ponds on non-arable land, microalgae produce natural vegetable oils potentially at 50-100 times greater yields than soybeans, sustainably returning high-energy with little impact on food production and prices (Gallagher, 2011). More than 10^5 types of microalgae can be used to produce biodiesel. Furthermore, they are known as essential components of coral reefs, while, in addition to being exceptionally diverse, they represent highly specialized group of organisms able to adapt to various ecological habits (Satyanarayana *et al.*, 2011). Microalgae, very small aquatic plants presenting one of few technologies for capturing and utilization of CO_2 emitted by power plants and other sources appear to be the only source of renewable biodiesel capable to meet the global demand for transport fuels (Chisti, 2007). Microalgal biodiesel is the only renewable biofuel, which can potentially displace petroleum-derived transport fuels completely without adversely affecting supply of food and other crop products (Chisti, 2008). Algal biomass is considered as a potential source of lipids for biodiesel production mitigating CO_2 emission, generally referred to as third-generation biofuels (Chen *et al.*, 2011; Lee *et al.*, 2014). Algal biofuels production relies on microorganism's lipid content. Usually, species such as mixotrophic *Chlorella* (*C. vulgaris*) reach high lipid content of around 60-70% (Liang *et al.*, 2009) and high productivity, of e.g. 7.4 g/l/d for *Chlorella protothecoides* (Chen *et al.*, 2011). Oil content of some microalgae exceeds 80% of dry weight of algae biomass (Chisti, 2007; Chisti, 2008). Autotrophic growth of mixotrophic *C. vulgaris* provides higher cellular lipid content (38%), lipid productivity is however much lower compared with heterotrophic growth on acetate, glucose (1%, w/v), or glycerol (Liang *et al.*, 2009). Unicellular phytoflagellate *Euglena gracilis* is an attractive feedstock for efficient biomass and biodiesel production and large amounts of wax esters by genetically modulating photosynthetic capacity (Ogawa *et al.*, 2015). Diatoms (Stramenopile or heterokont class of algae), which are highly different and have a more complex evolutionary history than green algae and vascular plants, have tremendous ecological superiority typically accumulating higher lipid quantities than other algal classes, and own beneficial attributes for potential biofuels production (Hildebrand *et al.*, 2012). Commercial algal productivity of large aquatic microalgae farms (ponds) can be enhanced by directly adding CO_2 from fossil-fueled power plants and other high carbon emitting facilities. Large amounts of biodiesel feedstock are produced, simultaneously recycling waste CO_2 and reducing CO_2 emissions to the atmosphere (Gallagher, 2011). An elevated atmospheric CO_2 concentration has been reported to evidently suppress partially higher plant respiration. Combustion-based atmospheric CO_2 is taken up by the oceans and plays an important role in oceanic carbon processes. Macroalgae have also been used for pharmaceutical, chemical and food purposes, as well as in waste-water treatment, recycling of nutrients and as biofilters, attracting attention as sustainable producers of lipid-containing biomass for food, animal feed, and biofuels (Ota *et al.*, 2016).

Meanwhile, the whole genome and transcriptome of unicellular green alga *Parachlorella kessleri* (Trebouxiophyceae), a high-biomass lipid-rich and starch producing green alga related to Chlorella, has been sequenced. Lipid accumulation under sulfur depletion is associated with the transcriptomic induction of enzymes involved in sulfur metabolism, triacylglycerol (TAG) synthesis, remodeling of light-harvesting complexes, and autophagy (Ota *et al.*, 2016).

Novel nanostructured materials have been developed capable of absorbing photonic energy from sun turning it into chemical or electrical energy via heterogeneous photocatalysis (Green Chemistry), e.g. biomass conversion into oxygenated hydrocarbons or hydrogen (Colmenares *et al.*, 2009).

Land use management: Since the beginning of civilization, humans have deliberately managed and converted landscape to derive valuable natural resources such as food, fiber, fresh water, and pharmaceuticals (Goldewijk and Ramankutty, 2004). Large-scale changes in land use and land cover have had significant biophysical and biogeochemical consequences for global and regional climates, global biogeochemical cycles such as carbon, nitrogen, and water, biodiversity, etc. (Goldewijk and Ramankutty, 2004). Land-use and land-cover, altering continuously in response to evolving economic, social, and biophysical conditions changes, affect local, regional, and global climate, in a comparable degree as other well-known anthropogenic climate forcings (Brown *et al.*, 2014). Expansion of agricultural land and intensification of cultivation were among the most predominant human alterations to global environment in 20th century (Matson *et al.*, 1997). Agricultural systems are concurrently degrading land, water resources, biodiversity and climate on global scale (Foley *et al.*, 2011). Crop cultivation replaced much of natural needleleaf evergreen, broadleaf deciduous and mixed forests of Eastern United States and to a lesser extent the natural grasslands in Central United State. Agriculture occupies about 38% of Earth′s terrestrial surface, while between 1985 and 2005, world′s croplands and pastures expanded by 154 million hectares (about 3%), increasing substantially global crop production including cereals, oilseeds, fruits and vegetables by 47%, meaning 25% increase in yield (Foley *et al.*, 2011). Agricultural intensification has contributed substantially to tremendous increases in food production over past decades (Matson *et al.*, 1997). Global agriculture is facing an increasing demand for food, bio-based energy and fiber products (Gutzler *et al.*, 2015), as well emerging challenges relating to food security and its impact on global environment, raising questions about production capacities of various farming system types such as organic, intensive, integrated, etc. (Makowski *et al.*, 2014). Farmers may respond multifunctionally through the choice of crops, crop rotations, utilization of crops, and intensification of production (Gutzler *et al.*, 2015), whereas concerns have developed over long term sustainability and environmental consequences of agricultural intensification, which can have negative local and global consequences, including impacts on atmospheric constituents and climate (Matson *et al.*, 1997). Globally, only 62% of crop production (on a mass basis) is allocated to human food, 35% to animal feed and 3% for bioenergy, seed and other industrial products (Foley *et al.*, 2011). Agricultural management takes place at farm scale, may however well impact upon regional sustainability (Gutzler *et al.*, 2015). Land use intensification in agriculture and forestry is irrefutably the main cause of global change and biodiversity loss (Tscharntke *et al.*, 2005). Agricultural expansion, dramatic agricultural intensification of land use and land conversion, along with intensive applications of fertilizers, pesticide and water, have had tremendous impacts on environment and natural streams, habitats, biodiversity, carbon storage and soil conditions in recent decades. They affect large parts of terrestrial area, alter biotic interactions and patterns of resource availability in ecosystems and can have serious local, regional, and global environmental consequences, such as pollution of ground water and eutrophication of rivers and lakes, while their contribution to biodiversity is critical for successful conservation. Seventy percent of global freshwater withdrawals are devoted to irrigation, while fertilizer use, manure application, and nitrogen fixing leguminous crops have dramatically disrupted global nitrogen and phosphorus cycles (Matson *et al.*, 1997; Schenk *et al.*, 2008; Elshahed, 2010; Foley *et al.*, 2011).

First-generation biofuel feedstocks essentially require inorganic phosphorus (P, ~2%) depleting slowly its reserves, so necessitating P recycling (Hein and Leemans, 2012). Agricultural mechanization utilizing fossil fuels, along with irrigation, biological improvements and chemical input enhanced agricultural production during past decades. Reduced tillage or no-tillage would reduce energy consumption compared with conventional farming, contributing in significant fuel savings (Cavalaris *et al.*, 2013). Nevertheless, a considerable potential for agricultural intensification exists, which is accompanied by adverse environmental and socio-economic impacts (Gutzler *et al.*, 2015). Agriculture management is influencing quality of water, air, soil as well as biodiversity at global scale. Upscaled crop model simulations can estimate potential yields and assess the effect of climate change and resource scarcity at global scale (Makowski *et al.*, 2014).

Various critical social, economic, environmental and technical issues emerged, relating to large-scale biofuels′ production and use. They comprise the effect of moderating oil prices, the "food vs. fuel" debate, poverty reduction potential, carbon emissions levels, sustainable biofuel production, land availability, land change, indirect land use change, deforestation and soil erosion, loss of biodiversity, and impact on water resources (Meyer, 2010; Havlík *et al.*, 2011). An active debate has also arisen around greenhouse gas emissions due to indirect land use change (iLUC) of expanding agricultural areas, dedicated to biofuel production (Havlík *et al.*, 2011) claiming that cropland producing biofuels in one place can somehow lead to greenhouse gas emissions in another, undefined and unknown place. Bioenergy involves direct use of land, henceforth, increased bioenergy demand would cause dramatic land use changes (LUC), to produce harvest residues, crops or forestry, severely implicating economic, social and environmental sustainability of bioenergy (Ahlgren and Di Lucia, 2014). Consequently, the issue of fuel-specific indirect land use changes (ILUC), caused by the promotion of transport biofuels, has been officially suggested by policy makers to deal with the ILUC of biofuels and has attracted considerable attention in recent years. LUC directly associated with a bioenergy project refers to as DLUC, converting one type of land use to a bioenergy plantation, whereas indirect LUC (ILUC) means changes in land use taking place because of a bioenergy project, but is geographically disconnected to it (Ahlgren and Di Lucia, 2014). Modelling efforts had initially focused on conventional biofuel technologies, with limited interest on advanced technologies. Economic equilibrium models are complex optimization models, studying the entire global economy (general equilibrium models), or a specific sector such as agriculture (partial equilibrium models) (Di Lucia *et al.*, 2012). Predictions of future changes in complex natural and sociotechnical systems are intrinsically uncertain. Future ILUC will depend not only on economic reactions, but also on other unforeseen factors, such as agricultural and trade policies in different parts of world (Ahlgren and Di Lucia, 2014). Using GLOBIOM, an economic partial equilibrium model of global forest, agriculture, and biomass sectors, Havlík *et al.*, (2011) indicated that advanced second generation biofuel production fed by wood from sustainably managed existing forests would lead to a negative iLUC factor, meaning that overall emissions will be 27% lower compared to "No biofuel" scenario by 2030. Using a worldwide agricultural model to estimate emissions from land-use change, Searchinger *et al.*, (2008) determined that corn-based ethanol, instead of producing 20% savings, nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years. Biofuels derived from switch grass grown on U.S. corn lands increase emissions by 50%, raising concerns about large biofuel mandates and highlighting the value of using waste products (Searchinger *et al.*, 2008). Land use optimization models based on linear programming can access and approach global issues, exploring land-use allocations to optimize agricultural, economic or environmental objectives at local and global level (Makowski *et al.*, 2014). Moreover, ecologically based management strategies can increase sustainability of agricultural production while reducing off-site consequences (Matson *et al.*, 1997).

Forest management: Over the last three centuries, large areas of forests, woodlands and grasslands have been cleared (Goldewijk and Ramankutty, 2004). Prevention of deforestation releasing carbon dioxide (CO_2) to atmosphere, and afforestation are strategies to slow global warming. Biophysical effects such as changes in land surface, evapotranspiration, and cloud cover also affect climate (Bala *et al.*, 2007). Forest harvest residues are plentiful raw materials for bioenergy production, indirectly emitting CO_2 into atmosphere through combustion, in opposite to slow release at harvest sites by decomposition (Repo *et al.*, 2011). Sufficient and comprehensive understanding of full complex system of C impacts is essential for taking maximum challenging advantages, in attempting to mitigate net emissions of CO_2 into atmosphere. There has also been an increasing interest in using forest management for this purpose. Forest ecosystems, with most productive the tropical, and forest management play an important role in global carbon cycle, while deforestation affects climate change by releasing carbon stored in plants and soils (Malhi, 2002; Aguilar *et al.*, 2007; Bala *et al.*, 2007). Tropical rain forests characterized by a water-rich environment exert a significant influence on global hydrological cycle, generating a major part of global land surface evaporation (Malhi, 2002). Although global forest reduction has accompanied human history since the dawn of agricultural revolution 8000 years ago, only ca 7% of global forest area had been lost by 1700 and ca 20-30% of original forest area had been lost by 1990 (Goldewijk, 2001; Malhi, 2002; Millar *et al.*, 2007).

Humans have significantly transformed Earth's environment, primarily through the conversion of natural ecosystems to agriculture. People use, and manage forests depending on socio-economic and socio-cultural environment, age and gender(Aguilar *et al.*, 2007). Reforestation and afforestation, entailing converting non-forested land to forested land through planting, seeding and/or promotion of seed banks and sources, have been both integrated into international climate change regime, i.e. the Kyoto Protocol (Aguilar *et al.*, 2007). Evolution of climate change and managing forests and forested ecosystems in coming future is rather uncertain, while quantitative models can rarely predict environmental changes and forest responses accurately and precisely(Millar *et al.*, 2007).

Human civilization has always influenced local carbon cycle through fire management and clearing of natural ecosystems, however humans caused a significant global effect since the 18th century (Malhi, 2002). Earth has entered a new geological era of extraordinary environmental changes without any precedent in the past(Millar *et al.*, 2007), the Anthropocene, with a permanent future imprint of human activity, inevitably altering atmosphere and climate fundamentally (Crutzen 2002 (Malhi, 2002). Human activities have increased the atmospheric concentrations of most important greenhouse gas CO_2 by about 31% or 40%, since the preindustrial era of late 18th from about 280 ppm to 368 or 370 ppm in 2000 or 2002, and more than doubled the available to ecosystems amount of nitrogen (Malhi, 2002; Malhi *et al.*, 2002; Brown *et al.*, 2014). CO_2 is an important ecumenical greenhouse gas with a short residence time of ~4 yrs., potentially regulating climate, as either a climate driver or an important amplifier, over a vast range of timescales from years to millions of years and the primary driver for global warming last 100 years (Shackleton, 2000; Royer *et al.*, 2004). Residence times for carbon in lithosphere are millions of years, ca. 400 years in the oceans (CO_2) and only 3–4 years in the atmosphere (Malhi, 2002). Periodic cosmic ray flux fluctuations may be of some climatic significance, but are likely of second-order importance on a multi-million year timescale (Royer and Berner, 2003). CO_2 is also exchanged with the oceans, primarily by simple physical dissolution forming to 99% bicarbonate, HCO_3^- and carbonate CO_3^{-2} ions (Malhi, 2002). Volcanoes affect climate change, injecting during major explosive eruptions huge amounts of volcanic gas like Sulphur dioxide that can cause global cooling and greenhouse gas CO_2 that potentially promotes global warming, aerosol droplets, and ash into the stratosphere. Anthropogenic CO_2 emissions dwarf global volcanic CO_2 emissions.

CO_2 concentration, global warming and climate changes have never exceeded current levels, possibly during the past 20 million years(Malhi, 2002). Global carbon dioxide emissions are most significant driver of human-caused climate change. Human-accelerated cycles of nitrogen, phosphorus, and sulfur also influence directly or indirectly climate (Brown *et al.*, 2014). This has fundamentally altered many of Earth's biogeochemical cycles in recent centuries, most prominently the global carbon cycle by the dramatic release of carbon that had been trapped by prehistoric ecosystems (i.e. fossil fuel) (Malhi *et al.*, 2002). Altered biogeochemical cycles, combined with climate change, increase vulnerability of biodiversity, food security, human health, and water quality (Brown *et al.*, 2014).

Date	**CO_2 ppm**
late 18th century	280
1987	350
2002	370
2015	400.99
2100	500-1000

Table 4: CO_2 concentrations (Malhi, 2002).

Understanding and managing the disruption of atmospheric composition and global climate will be likely amongst most pressing issues of 21st century(Malhi, 2002). According to global climate model simulations, large-scale deforestation of Amazonia would increase regional surface temperature between zero and 2°C, and reduce evaporation and precipitation by about 25% (Malhi *et al.*, 2002).

Sustainability and sustainable forest management (SFM) are old concepts, even though new light was shed on this issue in more recent times, while the "Forest Principles" was adopted at the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro in 1992, based on general international understanding of sustainable forest management (United Nations, http://www.fao.org/forestry/sfm/en/). Critical elements are solar energy and photosynthetic processes, providing a feasible route to remove atmospheric CO_2, to collect it on a large area of collectors and most advantageously store or recycle, once it has been collected (Marland and Schlamadinger, 1997). Mitigation of atmospheric CO_2 accumulation can be accomplished either by the collection and storage of C in growing trees, i.e. reforestation (replanting) or afforestation, or by displacing fossil-fuel combustion through renewable biomass fuels, i.e. by recycling C through biomass fuels (Marland and Schlamadinger, 1997). There are two available alternatives in using unused or degraded land for a forest plantation, namely (1) afforestation protecting and allowing trees to grow and store C away from the atmosphere indefinitely and (2) development of a short-rotation energy crop (perennial herbaceous crop) instead of a fossil fuel, basically expecting regular harvest at appropriately chosen rotation time and fuel usage (Marland and Schlamadinger, 1997). Relative benefits of using land to grow woody crops as opposed to simply afforesting land, with the intent to store C in standing trees, have been described by Schlamadinger and Marland, (1996), with respect to net impact on CO_2 emissions into atmosphere. Carbon content of dry wood is about 50%, meaning that carbon atom (C) is most important and abundant constituent of biological mater and biomass (50% of dry biomass). C amount stored in biosphere and in forest products reaches a steady state over long time intervals, while continuing mitigation of CO_2 emissions depends on the extent to which fossil fuels is displaced by bioenergy and wood products (Schlamadinger and Marland, 1996). Henceforth, wood materials can be recycled, placed in a landfill or used to generate energy at the end of their useful lives (Marland and Schlamadinger, 1997).

Energy and water are to a large extent interdependent valuable resources underpinning human prosperity. Water, a polar agent and most abundant solvent for salts and organic compounds, is the bloodstream of biosphere and most precious agent to mankind, constituting the key for life, determining the sustainability of living systems, connecting ecosystems across landscape, and shaping our planet′s face (Ripl, 2003; Viala, 2008). Solar-driven evaporation, condensation and precipitation, crystallization and dissolution, runoff, infiltration, and transpiration, as well as water in biological cell and disintegration and recombination of water molecule (carbon fixation and respiration) form a water cycle almost without loss of matter, which cools hot spots and warms cooler areas (Ripl, 2003). 70% of water is withdrawn by agriculture, 20% by industry and 10% by municipalities (Viala, 2008). Water is a plentiful resource, but not always available for human use in the quantities or at the quality, time and place required. 2.5% of world′s water is freshwater, from which less than 1% is accessible via surface sources and aquifers and the rest is locked up in glaciers and ice caps or is deep underground. Surprisingly, about 3,000 liters of water converted from liquid to vapor, meaning about 1 liter per calorie, are required to produce enough food to satisfy a person′s daily dietary needs, while only about 2-5 liters of water are required for drinking (Viala, 2008). A large-scale expansion of energy crop production would lead to a large increase in evapotranspiration appropriation for human uses, potentially as large as present 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). About 80% of agricultural evapotranspiration (when crops turn water into vapor) comes directly from rain, and about 20% from irrigation, while irrigated area doubled and water withdrawals tripled since 1950 (Viala, 2008). World has enough freshwater to produce enough food over the next half century on condition of better water management along with non-miraculous changes in policy and production techniques and world leaders will take action before the opportunities are lost (Viala, 2008).

Carbon resources for biotechnology: Another sector competing for renewable carbon sources and agro-industrial wastes is industrial microbiology and biotechnology.

Biotechnology is exploration of living matter to produce a great variety of useful products since ancient times. Various metabolites can be produced via biotechnological processes through microbial fermentations from various carbon sources, including sugars, carbohydrates, vegetable oils and lignocellulosic matter. Global energy as also carbon source demand, production and availability influenced strongly and oriented directly the economy and development of biotechnology and industrial microbiology during the 20th and ongoing 21st century, in recent times and will continue to do so in far future. Since the beginning of 20th century, various carbon sources derived from plants, animals and microorganisms or fossils have been used for microbial production of food and feed ingredients, commodity, bulk and fine chemicals and antibiotics, as well as biofuels, historically reflecting global geopolitical and socio-economic developments, needs and continuity. They include sucrose, molasses, glucose hydrolysates, hydrocarbons, ethanol, proteins, fatty acids, plant and animal oils, glycerol etc.

Microorganisms are used since ancient times to obtain different types of food, such as beer, wine, bread, cheeses, and fermented milk, while industrial microbiology expanded in 20th century even more, giving new possibilities for obtaining a large variety and quantity of products by fermentative processes (Gonçalves *et al.*, 2014). Biotechnological processes are particularly attractive since microorganisms can utilize renewable feedstocks towards valuable products, only producing few toxic by-products (Cheng *et al.*, 2013). Fermentation is an advantageous process, applying renewable sources and waste to produce useful products at very mild environmental conditions consuming less energy (Angumeenal and Venkappayya, 2013). Raw material cost is currently limiting fermentation processes, necessitating moving towards cheaper and sustainable raw materials along with the shift from first to second-generation (Gavilà *et al.*, 2015) or third generation biofuels.

Non-conventional yeast *Y. lipolytica*: Non-conventional yeasts have increasingly attracted interest in recent years due to their biochemical characteristics and potential applications (Liu *et al.*, 2015). Oleaginous strictly aerobic yeast *Yarrowia lipolytica*, a production host for a large variety of biotechnological applications, and with pronounced lipolytic and proteolytic activities, primarily found in foods, particularly in fermented dairy products and meat, is a "safe-to-use" organism (Groenewald *et al.*, 2014; Liu *et al.*, 2015). Dimorphic yeast *Yarrowia lipolytica* displays several versatile important characteristics for basic biological, environmental and industrial applications. They include salt tolerance, bioremediation (e.g. aliphatic and aromatic compounds, organic pollutants, 2,4,6-trinitrotoluene, and metals), heterologous protein expression, production of unique inherent enzymes (inulinases, α-mannosidases), synthesis of β-hydroxy butyrate, l-dopa, and emulsifiers, lipid accumulation, as well as lipase and biofuel production, and cheese ripening contributing to superior organoleptic characteristics (Bankar *et al.*, 2009; Darvishi *et al.*, 2009; Groenewald *et al.*, 2014; Liu *et al.*, 2015). Non-conventional yeast *Y. lipolytica* with specific characteristics as well as potential physiological and metabolic capabilities assimilates many different carbon sources, including typical hydrophilic and hydrophobic materials. *Y. lipolytica* degrades very efficiently hydrophobic substrates, including *n*-alkanes, fatty acids, fats, and renewable low-cost substrates such as various plant oils as sole carbon source, to produce organic acids, single-cell oil, lipases, and so forth (Bankar *et al.*, 2009; Darvishi *et al.*, 2009; Liu *et al.*, 2015). *Y. lipolytica* and other *Candida* yeast strains produce a wider range of acids, including citric acid (CA), isocitric acid (ICA), 2-ketoglutaric acid, pyruvic acid as well as succinic acid, from a greater variety of carbon sources than fungi. They utilize hydrophobic substrates like triglycerides, oils and fatty acids, edible oils and fats, glucose, ethanol, molasses, starch hydrolysates etc., and pure or raw glycerol from biodiesel industry (10%) nowadays, a byproduct of biodiesel production process (Darvishi *et al.*, 2009; Kamzolova *et al.*, 2013; Jost *et al.*, 2015). Nonconventional, nonpathogenic, dimorphic aerobic *Y. lipolytica* and its recombinants owing multiple biotechnological applications produces under certain limitation conditions high amounts of several organic acids such as pyruvic, alpha-ketoglutaric, succinic acid, as well as simultaneously citric and isocitric, single-cell oil, among most important industrial enzymes lipases (EC 3.1.1.3), and so forth. The ratio between citrate and isocitrate depends on strain, carbon source, and fermentation medium composition (e.g. type of carbon source and concentration, nitrogen limitation, trace element, especially iron concentration) and cultivation conditions e.g. pH, temperature, agitation, dissolved oxygen. Various carbon sources have been historically used for the production of CA and ICA, and other organic acids by yeasts from different carbon sources, such as acetate, *n*-alkanes, glucose, ethanol, sucrose, fatty materials, vegetable oils (sunflower oil, rapeseed oil), and pure or crude (~10% w/w of biodiesel production) glycerol (Anastassiadis *et al.*, 2002; Anastassiadis *et al.*, 2008; Darvishi *et al.*, 2009; Gonçalves *et al.*, 2014; Jost *et al.*, 2015).

Metabolite formation in *Y. lipolytica* depends on growth conditions such as type and concentration of carbon and nitrogen source, and fermentation parameters such as pH, temperature, agitation and aeration (oxygenation) (Gonçalves *et al.*, 2014; Kamzolova *et al.*, 2015; Morgunov and Kamzolova, 2015). Commercial citric acid production by yeasts tolerates higher initial sugar concentration than fungus *A. niger*, is insensitive to metals and reaches higher fermentation rates (Anastassiadis *et al.*, 2008). Fermenter pressure and external addition of CO_2 (supplementary carbon source, auxiliary carbon source) also strongly influence continuous and discontinuous citric acid production by yeast *Candida lipolytica* from glucose as carbon source under controlled dissolved oxygen conditions, increasing citric acid concentration.

Citric and isocitric acid production, single cell production: Citric acid is a multifunctional mainly produced by fermentation organic acid, which is widely used in food and beverage industry as a flavoring and preservative (~70%) denoted as E330, in washing powders (~20%), and in chemical and pharmaceutical industries (~10%) (Anastassiadis *et al.*, 2008; Rymowicz *et al.*, 2010). Nature of carbon source has a strong effect on citric acid fermentation, whereas easily metabolized carbohydrates are essential for the successful production of citric acid. Various sources of carbohydrates have been historically utilized for the legendary fermentative production of citric acid by *Aspergillus niger* or *Candida* strains and genetically modified microorganisms. They include molasses (cane or beet sugar, 40-55% sugar content), starch or sucrose based media, dextrose syrups and crystallized dextrose, and hydrocarbons. Various low cost renewable carbon sources and agro-industrial residues have also been applied, such as apple pomace, cassava bagasse, coffee husk, wheat straw, pineapple waste, sugar beet cosset, kiwi fruit peel, sugar rich plant and fruit sources including plant biomass due to its abundant availability, crude glycerol etc. Citric acid is the most important organic acid, extensively used in food and pharmaceutical industries, and the main intermediate of microbial carbohydrate metabolism. Submerged fermentation processes (80% of world production) and to less extend surface and solid state fermentations (agricultural residues) are applied abroad, using chemically defined media or molasses as raw material (waste of sugar industry) using *Aspergillus niger* or *Yarrowia lipolytica* (Anastassiadis *et al.*, 2008; Swain *et al.*, 2011; Angumeenal and Venkappayya, 2013; Jost *et al.*, 2015; Kamzolova *et al.*, 2015). Beet and sugar cane molasses and glucose syrups are the main carbon sources utilized in industry with *A. niger*. Various carbon sources have been historically used for the production of citric (CA) and isocitric acid (ICA) by yeasts from different carbon sources, such as *n*-alkanes, glucose, ethanol, fatty materials, vegetable oils, glycerol etc. (Ermakova *et al.*, 1986; Darvishi *et al.*, 2009; Holz *et al.*, 2009; Rymowicz *et al.*, 2010; Morgunov and Kamzolova, 2015). Disadvantageously, CA production on plant oils is accompanied with the same amount of ICA (Darvishi *et al.*, 2009). World oil crisis of 1973-74 ended the historical exploitation of *n*-alkanes for industrial citric acid production and single cell protein by yeasts, especially *Candida* or *Yarrowia* species, followed by the use of carbohydrates as carbon source, especially glucose (Anastassiadis *et al.*, 2002; Anastassiadis *et al.*, 2008). Up to 250 g/l of citric acid has been produced from glucose by selected *Candida* (*Yarrowia*) *lipolytica* from glucose in continuous mode of repeated fed batch operation.

Using natural sources can minimize environmental problems (Angumeenal and Venkappayya, 2013). Glycerol and glycerol-containing materials, a byproduct of biodiesel and other industries, became of great practical interest for various microbial transformations in recent years, while petroleum hydrocarbons has been considered as main relatively low cost carbon and energy source for microbial biotechnology in 20th century (Rymowicz *et al.*, 2010). *Y. lipolytica* N15 produced CA from pure glycerin and glycerol-containing waste, reaching 98 g/L of citrate and only 3.3 g/L of ICA (CA:ICA ratio of 30, Y_{CA} of 0.9 g/g), and 71 g/L citrate and 5.6 g/L ICA (CA:ICA ratio of 12.7) were produced after 144 h, respectively (Rymowicz *et al.*, 2010). Mutant *Y. lipolytica* NG40/UV7 utilized concurrently glycerol and fatty acids during growth on glycerol-containing waste (from Greek Biodiesel industry, Pythia Institute of Biotechnology, Greece). Glycerol was utilized at a higher rate than fatty acids, synthesizing 122.2 g/l CA at a citrate-to-isocitrate ratio of 53:1 and a yield of 0.95 g/g in fed-batch operation (Kamzolova *et al.*, 2015; Morgunov and Kamzolova, 2015). Kamzolova *et al.*, (2015) reported about the production of technical-grade sodium citrate from glycerol-containing biodiesel waste by *Yarrowia lipolytica*, operating batch wise for 144 h and fed-batch wise for more than 500 h, reaching 79–82 g/L citrate, which was isolated from fermentation medium by the addition of a small amount of NaOH. DS-threo-isocitric acid or citric acid (175 g/l, 1.5 g/g oil) have been produced from rapeseed oil using mutants of *Y. lipolytica* after the reduction of droplet size of hydrophobic substrates by bio-surfactants, the hydrolysis of rapeseed oil to glycerol and fatty acids by extracellular lipases, and then the degradation of fatty acids by β-oxidation (Kamzolova *et al.*, 2013; Gonçalves *et al.*, 2014). *Y. lipolytica* strains produced citric acid from glucose-enriched olive mill wastewater from Greece (Sarris *et al.*, 2011). Other organic acids are also produced by fermentation such as lactic, gluconic, itaconic acid and acetic acid.

Succinic acid production: Succinic acid (amber acid or butanedioic acid, $C_4H_6O_4$) is a versatile four-carbon dicarboxylic acid ecumenically found in plants, animals and microorganisms as an intermediate metabolite of tricarboxylic acid cycle, or one of mixed-acid fermentation products of anaerobic metabolism. It is an environmentally advantageous greenhouse gas CO_2 fixation based carboxylation metabolite of C3 energy metabolism, towards C4 metabolites, contributing to the reduction of CO_2 emissions. Succinate has multiple applications as deicing agent, as an important valuable platform chemical and C4 building-block, for multiple potentially useful applications, such as γ-butyrolactone, tetrahydrofuran, and 1,4-butanediol synthesis, precursor for biodegradable polymers (poly-butylene succinate) and fibers, manufacture of synthetic resins, additive in paints etc. Industrial scale production of succinate bases on petrochemical based maleic anhydride oxidation (Lee *et al.*, 2006; Borges and Pereira, 2011; Pereira *et al.*, 2012; Cao *et al.*, 2013; Cheng *et al.*, 2013; Carvalho *et al.*, 2014; Tajima *et al.*, 2015). Annual global production of succinic acid fluctuates between 30,000 and 50,000 tons per year, which is expected to grow at a rate of 18.7% from 2011 to 2016 (Cao *et al.*, 2013). A sustainable and economically attractive biobased fermentative succinate production from a variety of renewable carbon resources, obtained through improvements in microbial strains, fermentation engineering, and downstream processing, would prospectively open a new path in industrial manufacturing of bulk chemicals, replacing the traditional petrochemical processes in near future (Lee *et al.*, 2006; Wang *et al.*, 2011; Cao *et al.*, 2013). Various microorganisms produce succinic acid from glucose and renewable raw materials like corn stover, straw, corn fiber hydrolysates and glycerol like *Mannheimia succiniciproducens*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Basfia succiniciproducens* DD1. Metabolic engineered overproducers have also been frequently reported like *Escherichia coli*, *Corynebacterium glutamicum*, *Yarrowia lipolytica* or *Saccharomyces cerevisiae* (Lee *et al.*, 2002; Lee *et al.*, 2006; Song and Lee, 2006; Raja and Dhanasekar, 2011; Cao *et al.*, 2013; Carvalho *et al.*, 2014; Kongruang and Kangsadan, 2015). Nature of carbon source is a significant factor directly affecting metabolic fluxes, succinic acid yields and byproduct formation, as also influencing biotechnological process economics. Metabolic engineering produces industrial *A. succinogenes* strains, redirecting carbon flux towards succinate pathway instead of acetate and formate (Rafieenia, 2014). Mixed substrates, e.g. xylose and glucose (Rafieenia, 2014), or fructose and glucose (Wang *et al.*, 2011) for succinate, have often a positive influence on microbial production, basing on the principle of auxiliary carbon. *Actinobacillus succinogenes* overproduces succinic acid, from cheap feedstock glycerol using an external electron acceptor, e.g. dimethyl sulfoxide, opening new perspectives for the use of glycerol (Carvalho *et al.*, 2014). Kongruang and Kangsadan, (2015) reported about succinic acid production from crude glycerol by encapsulated *Anaerobiospirillum succinicproducens* ATCC 29305, reaching 34.8 g/l of succinic acid at 87% yield in shake flasks (150 rpm, 39°C, pH 6). (Borges and Pereira, 2011) reported about succinic acid production by *Actinobacillus succinogenes* using hydrolysate of sugarcane bagasse hemicellulose, yielding under optimized conditions (37°C, pH 7, and 150 rpm) 22.5 g l^{-1} within 24 h, adding CO_2 into fermenter. Shen *et al.*, (2015) reported about succinic acid production by *Actinobacillus succinogenes* GXAS137 using low-cost carbon source sugarcane juice (~70 g/L glucose content), and corn steep liquor and peanut meal as nitrogen sources (3:1 ratio), reaching 57.06 g/L of succinic acid in batch (37°C, pH 6.5-7, 100 rpm, 0.3 vvm CO_2) and 62.06 g/L in fed-batch operation. Ultrasonically pretreated for 40 min of lignocellulosic sugarcane bagasse hemicellulose, yielding 29.5% higher total sugar compared with the diluted sulfuric acid treatment (43.9 g/L from non-detoxified total sugar), resulted in 20.9 higher succinic acid production (23.7 g/L SA from 30 g/L and 20.2% higher yield reducing medium yeast extract by 60% (Xi *et al.*, 2013). Enhancement and introduction of carboxylation pathways (Fig. 2) from phosphoenolpyruvate (PEP) and pyruvate to oxaloacetate (OAA) is a general strategy to stimulate effectively succinate synthesis (Tajima *et al.*, 2015).

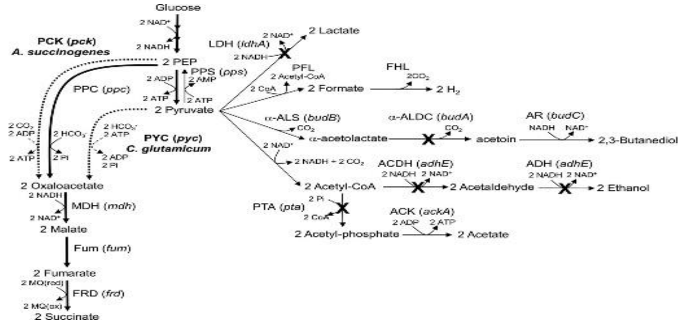

Fig. 2. Metabolic pathways in *E. aerogenes* (Tajima *et al.*, 2015).

Among the CO_2-fixing sequences Phosphoenolpyruvate (PEP) carboxykinase, PEP carboxylase, and malic enzyme, PEP carboxykinase (PCK), forming ATP, is the most important for anaerobic growth of *M. succiniciproducens* and succinic acid formation through oxaloacetate, sequentially catalyzed by malate dehydrogenase, fumarase, and fumarate reductase (Lee *et al.*, 2006). Tajima *et al.*, (2015) described recombinant strain *Enterobacter aerogenes* ES04/PCK+PYC with inactivated ethanol, lactate, acetate, and 2,3-butanediol pathways and coexpressed PCK and *Corynebacterium glutamicum* pyruvate carboxylase (PYC). ES04/PCK+PYC generated under weakly acidic conditions (pH <6.2) majorly succinate from glucose at over 70% yield, without any measurable ethanol, lactate, or 2,3-butanediol(Tajima *et al.*, 2015). Metabolically engineered favor strain LPK7 for industrial production of succinic acid produced 52.4 g/liter of succinic acid from glucose at a molar yield of 1.16 mol, 1.8 g/liter/h productivity and at very low or without any acetic, formic, and lactic acid(s) formation (Lee *et al.*, 2006). Metabolic engineering elimination of pyruvate node reactions involved in lactate, acetate, and 2,3-butanediol synthesis from *E. aerogenes* ΔadhE/PCK reduces by-product formation, without to correlate with increased succinate production in PYC or PCK overexpressing strains. Strain ES04 (ΔadhE ΔldhA Δpta ΔbudA)/PCK+PYC produced succinate with low by-production and over a 70% yield at pH 5.5 (Tajima *et al.*, 2015). Metabolic engineering created further recombinant microbial strains with improved characteristics regarding high yielding succinate production close to maximum theoretic values with minimal byproduct formation and enlarged spectrum of suitable carbon sources, including strains of *Escherichia coli*, *Corynebacterium glutamicum* (Kim *et al.*, 2004; Wang *et al.*, 2011). Oxygen supply influenced byproduct formation of formate and acetate, resulting therefore in lower succinate yield in recombinant *E. coli* (Wang *et al.*, 2011). Overexpression of phosphoenolpyruvate carboxykinase (PEPCK) of *A. succinogenes* in *Escherichia coli* K-12 had no effect on succinate fermentation, in contrast to phosphoenolpyruvate carboxylase mutant *E. coli* strain K-12 ppc::kan, where PEPCK overexpression increased succinate production 6.5-fold (Kim *et al.*, 2004). Kamzolova *et al.* (2012) reported about α-ketoglutaric acid production reaching 88.7 g/L by thiamine-auxotrophic *Yarrowia lipolytica* VKM Y-2412 grown on ethanol, which was subjected to chemical treatment with hydrogen peroxide to produce significant quantities

(71.7 g/L) of succinic acid. Further direct esterification of succinic acid with excess absolute ethanol yielded diethyl succinate.

Production of other metabolites: Additional products are also produced by *Y. lipolytica* at various subjected growing conditions, including erythritol a four-carbon polyol (natural sweetener extensively used in food and pharmaceutical industries, and from diabetics), single cell protein, bio-surfactants (made predominantly of glycolipids), γ-decalactone (peach-scented compound), enzymes (acid or alkaline proteases, lipases, and RNase) and intracellular lipids and microbial oil for biodiesel (Tai and Stephanopoulos, 2013; Gonçalves *et al.*, 2014; Mirończuk *et al.*, 2015).

Erythritol production: (Mirończuk *et al.*, 2015)described a two-stage process for the production of erythritol utilizing industrial raw molasses and glycerol by recombinant *Y. lipolytica*, expressing the *Saccharomyces cerevisiae* SUC2 gene, producing 52-114 g/l of erythritol. Mutant *Y. lipolytica* MK1 received from *Y. lipolytica* Wratislavia K1 by classical mutagenesis (ultraviolet) produced in batch operation up to 82.2 g/l erythritol with 0.55 g/g yield and 0.84 g/l h productivity along with less than 5% of byproducts. 224 g/l with 0.77 g/g yield and 0.54 g/l h productivity were achieved in repeated batch process and below 2.3% of by-products from glycerol.

Production of biosurfactants/bioemulsifiers

Growth of a variety of prokaryotic (certain bacteria like *Arthrobacter* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*) and eukaryotic microorganisms (yeasts like *Candida lipolytica*, *Y. lipolytica*, *Torulopsis bombicola*, *Rhodotorula* sp., and filamentous fungi) on water-immiscible substrates, such as carbohydrates like alkanes and oils such as vegetable oils (e.g. corn, soya bean, safflower, and sunflower oils), is often associated with the production of surface active amphiphilic compounds (biosurfactants/bioemulsifiers). They are mainly produced during the late exponential phase of growth. They include a wide variety of chemical structures, such as glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids, as well as polysaccharide-protein and polysaccharide-protein-fatty acid complexes (Cirigliano and Carman, 1984; Sarubbo *et al.*, 2001; Banat *et al.*, 2014; Csuka *et al.*, 2015). Surface-active chemicals and biosurfactants produced by yeast species belonging to *Candida*, *Rhodotorula* and *Yarrowia* genera find main applications in various fields and industrial sectors, such as bioremediation, biodegradation (increasing bioavailability of organic pollutants), enhanced oil recovery, pharmaceutics, food processing, therapeutics, and cosmetics among many others. They own therefore a huge and ever increasing market and demand (Banat *et al.*, 2014; Antoniou *et al.*, 2015; Csutak *et al.*, 2015). Large-scale production and commercialization remains economically challenging for many types of these products. Renewable low cost and abundant raw carbon resources, such as agro-industrial waste, sugars, molasses, plant oils, oil wastes, oil industries, animal fat, starchy substances, lactic whey, distillery wastes, and molasses as carbon sources, and developments in fermentation technology are necessary to reduce production cost of biosurfactants/emulsifiers. They may replace the accumulative non-biodegradable and toxic petroleum based chemically synthesized compounds (Banat *et al.*, 2014). Hemiascomycetous *Y. lipolytica* CMGB32, conditionally forming pseudomycelium or true mycelium, showed a high multiplication rate cultivated on Yeast Peptone media with 1% petroleum, forming elaborate pseudohyphae and petroleum mini-droplets after 72 hours, and good lipolytic activity in the presence of 1% Tween 80, offering a biotechnological potential for biosurfactant production (Csutak *et al.*, 2015). High emulsification index values (E24) have been shown against toluene (52%), *n*-hexadecane and *n*-dodecane, while biosurfactant's activity increased at 4°C and decreased significantly adding a 10% NaCl solution, and had no effect against seven tested potential pathogen yeast strains (Csutak *et al.*, 2015). *T. bombicola* produced 70 g/liter of biosurfactant at a selectivity of 35% in a complex mixed carbon source medium containing a carbohydrate and a vegetable oil (Cirigliano and Carman, 1984). *Y. lipolytica* degrades a variety of organic compounds, including aliphatic and aromatic hydrocarbons, always accompanied by the formation of bio-surfactants (increase contact surface), predominantly made up of glycolipids (Gonçalves *et al.*, 2014; Csutak *et al.*, 2015). *Y. lipolytica* CMGB32 had shown good lipolytic activity in the presence of 1% Tween 80 (Csutak *et al.*, 2015). *C. lipolytica* has shown thermostable emulsification activity with maximum activity at pH between two and five, after entering the stationary phase of growth in hexadecane-supplemented cultures, and only negligible emulsification activity grown on glucose (Cirigliano and Carman, 1984). *C. lipolytica* IA 1055 produced grown on varying glucose concentrations in absence of extracellular hydrocarbons a complex bio-surfactant biopolymer, constituting proteins, carbohydrates, and lipids. Maximum production of emulsification activity has been detected at 27°C in stationary growth phase reaching a low pH (Sarubbo *et al.*, 2001).

Lipase production: Olive oil (55–83% oleic acids) proved to be most suitable, among soy bean, canola, castor, sesame, wheat bud, sweet almond, bitter almond, walnut and coconut oils, for lipase formation, due to high content of oleic acids, acting as stabilizer/activator for extracellular lipase (Darvishi *et al.*, 2009). Canola oil with 55% oleic acid contents is the next good candidate for extracellular lipase production after olive oil. Plant oils with high content of oleic acid are also suitable for biomass and single-cell protein production (Darvishi *et al.*, 2009). The simultaneous production of biotechnologically important lipase and CA could be economically desirable. Lazar *et al.*, (2011) reported about simultaneous production of citric acid (CA) and invertase by *Yarrowia lipolytica* A-101-B56-5 (SUC+ clone) grown on sucrose and other carbohydrates, such as glucose, fructose, and glycerol, reaching 57.15 g/L CA from glycerol (Y = 0.6 g/g) and 45 g/L from sucrose ($Y_{CA/S}$ = 0.643 g/g).

Production of bioethanol and advanced higher alcohols: Microbial processes are widely advantageously superior for biofuel production over conventional chemical processes, being renewable, sustainable, and carbon neutral. Bioethanol can be produced from different biomass sources, basically from first generation feedstocks, including some cereals and legumes, such as corn, sugar beet, sugar cane, molasses, fruits, wheat and barley, also used for food source, or second generation feedstocks such as lignocellulosic materials like waste or forest residue (Özçimen and İnan, 2015). Current economic competitiveness and wider implementation of bioethanol redirects bioethanol fermentation towards integration with anaerobic digestion, defined as biological degradation of organic matter into biogas (methane and CO_2), in a biorefinery concept, to produce simultaneously bioethanol along with biogas (heat and electricity) (Cesaro and Belgiorno, 2015). Alternatively, the combination of bioethanol fermentation and anaerobic digestion under different operating conditions, utilizing the anaerobic effluent (digestate) for ethanol production, is another viable option. This is a mixture of partially degraded organic matter, microbial biomass and inorganic compounds, characterized as a potential fertilizer with high contents of nitrogen, phosphorous, potassium and micronutrients, with the risk of containing also potentially toxic compounds (García-Olivares, 2015). High global demand of ethanol, climate change, fuel security and economics necessitate the exploration of new alternative renewable biomass sources like microalgae and certain algae. They have a shorter growth cycle as compared to plants and are a promising alternative source for lipids and carbohydrates of various forms for third generation biodiesel and ethanol production (Adams *et al.*, 2009; Chaudhary *et al.*, 2014), they don't occupy agricultural lands and they needn't any fertilizer for cultivation (Özçimen and İnan, 2015). Marine 3rd generation macroalgae biomass has many advantages over terrestrial plant biomass as a prospective feedstock for producing biofuels and chemicals by natural or engineered microbial cells (Wei *et al.*, 2013; Konda *et al.*, 2015). Irradiance, temperature, nutrients, grazing and stand density are most important parameters affecting sea biomass production (Gao and McKinley, 1994). Marine brown macroalgae are attractive alternative substrate for fermentation-derived biorefinery products and biofuel production due to high content of carbohydrates (~2/3 of dry biomass) and near or full lignin absence, while the rest is ash/salt (Mazumdar *et al.*, 2014; Konda *et al.*, 2015). Commercial viability of macroalgae-based biorefineries depends on economic processes performance of industrial scale macroalgae conversion to biofuels and chemicals (Konda *et al.*, 2015). Aquatic macroalgae biomass of *Laminaria japonica* (*Saccharina japonica*, mainly consisting of algal polysaccharides carbohydrates mannitol and alginate, has been utilized to produce lipids with a similar composition of fatty acids with vegetable oils by fermentation applying the oleaginous yeast *Cryptococcus curvatus* (Xu *et al.*, 2014). Most productive oleaginous yeasts *Yarrowia lipolytica*, *Cryptococcus curvatus*, and *Rhodosporidium toruloides*, produce lipids reaching from 40% to 70% of biomass (Ratledge and Cohen, 2008). *C. curvatus* accumulates up to 60% oils by dry cell weight (DCW) using economical carbon source. Lipid accumulation in oleaginous microorganisms is a non-growth associated process, occurring after the exhaustion of nitrogen or to a lesser extent of other essential nutrients like phosphorus or sulfate from culture medium, leading to a rapid decrease of cellular AMP concentration and alteration of Krebs cycle function towards intra-mitochondrial citric acid accumulation. Reserve lipid breakdown (lipid turnover) takes place when extra-cellular carbon cannot satisfy the metabolic requirements (Papanikolaou and Aggelis, 2011; Papanikolaou, 2012). Yeast oils, the cheapest producible by heterotrophic microorganisms, are too expensive alternatives to major commodity plant oils for biodiesel production (Ratledge and Cohen, 2008). Macroalgae biomass composition varies throughout the year (Adams *et al.*, 2009). Algal oils are similarly unlikely to be economic within the next 10 to 15 years with the current prices of the major plant oils and crude oil, it would however be more prospective to focus on algae as sources of polyunsaturated fatty acids (Ratledge and Cohen, 2008). Macroalgal hydrolysates of *Saccharina latissimi* have been used for the novel microbial production of pinene without any further treatment and/or purification (Scullin *et al.*, 2015). Cho *et al.*, (2015) applied thermal acid hydrolysis and enzymatic saccharification on third-generation lignin free biomass of highest carbohydrates containing red alga *Gelidium amansii* among seaweeds towards monomeric galactose (25.5 g/l) and glucose (7.6 g/l), to produce ethanol by acclimated to high galactose concentration strains of *Pichia stipitis* or *Saccharomyces cerevisiae* with reduced glucose-induced repression on galactose transport. Autoclave-treated *Gelidium amansii*, a popular source of food and chemicals due to high galactose and glucose content, has also been used to produce ethanol, applying either separate hydrolysis and fermentation or simultaneous saccharification and fermentation processing (Kim *et al.*, 2015). Freshwater chlorococcal alga *Chlorella* sp., a high starch producer characterized by a very high growth rate (mmax¼0.20/h) and tolerance to high temperature (40°C), might substitute for starch-rich terrestrial plants in bioethanol production. Under macroelement growth limiting conditions it reached 55% starch of DW by phosphorus, 38% by nitrogen or 60% by sulfur limitation, and 60% of DW by cycloheximide-treated (specific inhibitor of cytoplasmic protein synthesis) cells. Different species of algae can be converted directly into various valuable biofuels, such as biodiesel, bioethanol and biomethanol. Recombinant ethanologenic strains, e.g. *Rhodobacter* sp., have also been developed, to redirect carbon from Calvin cycle to ethanol by introducing the ethanol-producing genes (pyruvate decarboxylase and alcohol dehydrogenase) (Chaudhary *et al.*, 2014). (Mazumdar *et al.*

(2014) reported about L-lactate production from brown seaweed *Laminaria japonica* hydrolysate by engineered *Escherichia coli*, utilizing glucose and mannitol under micro-aerobic condition reaching 37.7 g/L of high optical purity L-lactate at 80% of maximum theoretical value. Judd *et al.* (2015) describe various algal open or closed reactor systems operating at various configurations, generally trending in increasing intensivity and design complexity, aiming in the biological fixation of CO_2 from gaseous effluent streams, and the removal of nutrients from wastewaters. Molasses, a low cost waste product of sugar industry, is usually used to produce alcohol, organic acids and single cell proteins by fermentation (Angumeenal and Venkappayya, 2013). Excessive consumption of glucose is a threat to global food security; therefore, low-cost nonfood feedstocks, e.g. glycerol, lignocellulosic xylose and other second-generation feedstocks, and development of a suitable microbial strain are crucial for successful succinate production (Cao *et al.*, 2013). Glycerol obtained as a by-product from biofuel industry and other renewable raw materials served as carbon and energy source for microbial growth and production in industrial microbiology with many possible applications in industrial fermentation (Da Silva *et al.*, 2009; Carvalho *et al.*, 2014). Fermentative utilization of abundant and cheap versatile carbon source crude glycerol to higher value multifunctional products, such as 1,3-propanediol (1,3-PDO), xylitol, dihydroxyacetone, butanol, co-production of ethanol and hydrogen or ethanol-formate, succinic acid, citric acid, pigments, biosurfactants, etc. can establish and implement biorefineries to improve economic viability and competitiveness of biofuels, especially biodiesel. Due to higher degree of reduction, glycerol advantageously reaches higher yields of fuels and reduced chemicals over sugars (Biebl *et al.*, 1998; Yazdani and Gonzalez, 2007; Da Silva *et al.*, 2009; Clomburg and Gonzalez, 2013; Carvalho *et al.*, 2014). Bio-butanol has received growing interest as an advanced biofuel over fuel ethanol in recent years, yet main challenges are high energy demanding downstream product recovery by distillation and low butanol titer. Xue's group at the Dalian University of Technology developed an integrated process, comprising ABE fermentation with in situ gas stripping-pervaporation, recovering concentrated butanol directly from fermenter.

Advanced microbial biofuels: Finite reservation and cumulative concerns about inevitable depletion of fossil resources, as well as environmental and climatic problems (global warming) and energy crises are urging us to develop renewable biofuels and energy from renewable biomass for the future bio-era, independently of fossil resources. They include the microbial production of hydrocarbons and higher alcohols, including 1-propanol, 1-butanol, isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol, as alternatives to low energy density ethanol. They possess properties more similar to those of petroleum-based fuel (Choi *et al.*, 2014). Microbial biofuel production is advantageously superior over conventional chemical processes in terms of renewability, sustainability, and carbon neutralization, suffers however under low yields to compete with and replace fossil fuels. Advanced microbial biofuels have similar properties to petroleum-based fuels; require however a complex multilevel engineering of microorganism's metabolism to reach suitable yields towards commercialization (Peralta-Yahya *et al.*, 2012). Design, bioengineering, systems biology, synthetic biology, metabolic and genome engineering, modulation and optimization of cellular metabolism's energy balance of high-yielding microbial factories for biofuel manufacturing requires to deeply understanding bacterial life and metabolic pathway coordination and metabolic networks under different environmental conditions. Recent advances in synthetic biology, encompassing systems biology, synthetic biology and metabolic engineering, enable us to engineer microorganisms to produce alternative biofuels and other products and significantly improve limitations of microbial production, product recovery, or need of pretreatment of raw substrates (Cheng *et al.*, 2013). These technologies will expand the spectrum of utilizable carbon sources (Mazumdar *et al.*, 2014) and provide new future opportunities for potential applications, especially of *Y. lipolytica* (Liu *et al.*, 2015). Advances in synthetic biology encompassing systems biology and metabolic engineering enable to design, engineer and/or create tailor made microorganisms to produce efficiently future bio-era alternative biofuels, i.e. butanol, hydrocarbon, alkanes, H_2, maximizing performance of cellular metabolisms during energy flow, gaining novel metabolic functions for bioenergy of platform cells, and forwarding to minimization and reverse engineering. Strain tolerance to toxic metabolites, such as solvents as biofuels or commodity chemicals is an important trait for biotechnological applications (Zingaro and Papoutsakis, 2012). Suitable high yielding microbial systems for biofuel production must tolerate end-product toxicity and fermentation inhibitors, proposing many improvements in feedstock and fuel (Fischer *et al.*, 2008). Improving solvent tolerance, to withstand final product inhibition during microbial production of advanced solvent-like biofuels and bulk chemicals, or to stand feedstock impurities and toxicity of desired product and undesired side products, is becoming another essential aspect of engineering complex phenotype microbial production strains, requiring the coordinated and tuned expression of several genes, e.g. overexpressing autologous heat shock proteins (Zingaro and Papoutsakis, 2012; Mukhopadhyay, 2015). Metabolic engineering has delivered new microbial cell factories with powerful high-flux pathways and processes for the production of different compounds, including biofuels, (di)carboxylic acids, alcohols, and amino acids, most of which are aerobic with few exceptions, e.g., alcoholic fermentation (Cueto-Rojas *et al.*, 2015). Significant advances have recently become in enhanced bio-based production of wide use industrial chemicals via various biosynthetic pathways from diverse inexpensive and abundant renewable carbon resources, such as corn stover, straw, and woody waste yielding C5 and C6 substrates, employing metabolically engineering on *Clostridium* strains (Cho *et al.*, 2015). They produce naturally a variety of chemicals, such as acetic acid, butyric acid, ethanol, isopropanol, butanol, 1,3-propanediol, 2,3-butanediol, and acetone or non-naturally isobutanol and high selectively 1-butanol reducing formation of acetate, butyrate, and acetone (Choi *et al.*, 2014; Cho *et al.*, 2015). *Klebsiella pneumoniae* converts glycerol to 1,3-propanediol, 2,3-butanediol and ethanol under conditions of uncontrolled pH reaching a maximum yield at pH 5.5, while excess glycerol in the culture enhanced butanediol yield (Biebl *et al.*, 1998). Engineered avirulent strain *K. pneumoniae* (ΔwabG ΔldhA ΔpflB) produced promising platform chemical 2,3-butanediol with various industrial applications, in a minimal medium at a yield of 0.461 g/g glucose, corresponding to 92.2% of theoretical maximum, significantly reducing by-product formation (Jung *et al.*, 2014). Okino *et al.*, (2008) described a fed batch process for succinic acid production applying recombinant strain *Corynebacterium glutamicum* (ΔldhA-pCRA717), with overexpressed pyruvate carboxylase encoding gene pyc and disrupted ldhA gene encoding l-lactate dehydrogenase. A high-cell density under oxygen deprivation and 146 g/l of succinic acid were obtained within 46 h at a yield of 0.92 g/g (intermittent addition of sodium bicarbonate and glucose) and 0.10 g/g yield for acetic acid (Okino *et al.*, 2008).

Fossil fuel energy generation has led to large-scale industrial development, as also to significant depletion of natural geologic deposits and to negative effects on global warming and climate, with the emissions of greenhouse gases (greenhouse effect) along with the depletion of fossil fuels. Accordingly, the interest in last decades has been redirected to the search for nonpolluting and alternative renewable clean high-energy carriers, as biohydrogen (H_2), leaving behind H_2O as major by-product after combustion, in order to replace the slowly exhausted fossil fuels (Cesaro and Belgiorno, 2015; Chandrasekhar *et al.*, 2015). H_2 is primarily produced from fossil fuels through steam reformation of natural gas or methane (CH_4) (Chandrasekhar *et al.*, 2015), exceeding currently 1 billion m^3/day worldwide, of which 48% is produced from natural gas, 30% from oil, 18% from coal, and 4% from H_2O-splitting electrolysis (Chandrasekhar *et al.*, 2015). H_2 is primarily produced from fossil fuels through steam reformation of natural gas or methane (CH_4), resulting in increasing emissions of GHGs (Chandrasekhar *et al.*, 2015). Fermentative hydrogen production from wastes by concentrated by biomass retention anaerobic mixed cultures prevails over chemical methods, and is sustainable and eco-friendly. A variety of biological routes for H_2 production exist including photobiological fermentation, anaerobic fermentation, enzymatic and microbial electrolysis, and a combination of these processes (Chandrasekhar *et al.*, 2015). Biohydrogen production, e.g. by dark fermentation, exhibits a very promising alternative potential to replace fossil fuels (Tapia-Venegas *et al.*, 2015) while metabolic engineering is applied to provide metabolic energy exceeding thermodynamic limitations (Chandrasekhar *et al.*, 2015). Tapia-Venegas *et al.*, (2015) overviewed H_2-production by dark fermentation, which is integrated or sequentially coupled with other biological processes, such as anaerobic digestion towards biohythane production (H_2/CH_4), and its integration into environmental biorefinery concept as an alternative fuel. Various parameters are listed, such as bioreactor development including new solid-state fermentation processes, parameter optimization, process modeling and simulation, exploitation of local waste and cheaper raw materials, combined dark-fermentation with photo-fermentation, and the coupling of hydrogen purification with the production process (Tapia-Venegas *et al.*, 2015). Substrate is the main cost in fermentative hydrogen production, while complex lignocellulosic biomass represents the most attractive low-cost feedstock, and its bio-augmentation to alternative H_2 via synergistic co-cultures is more efficient (production and yield) than with monocultures. Continuous stirred tank reactor (CSTR) also applying biomass retention is widely used for optimizing continuous fermentative hydrogen production from various substrates and other microbial metabolites, along with fermenters with integrated biomass retention by microfiltration (Fig. 3 and 4).

Alternatively to photoautotrophic organisms such as algae which produce fuels and chemicals directly from CO_2 and sunlight, a great interest emerged in non-photosynthetic conversion of CO_2 to fuels and chemicals, e.g. isobutyric acid or heptadecane formation by genetically modified obligate chemolithoautotrophic bacteria like *Acidithiobacillus ferrooxidans* used in mining operations, deriving metabolic energy from oxidation of iron (Fe^{2+} to Fe^{3+}) or sulfur species at low pH. The inorganics can be reduced using renewable electricity obtained by wind turbines or solar panels (Kernan *et al.*, 2016). Recent metabolic engineering designed microbial

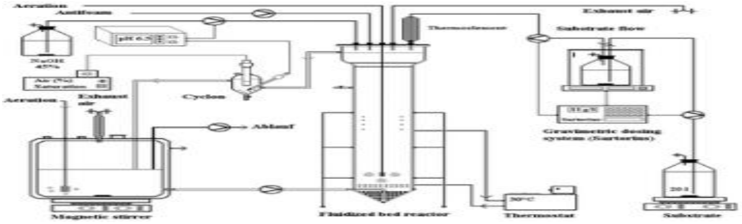

Fig. 3: Fluidized bed reactor.

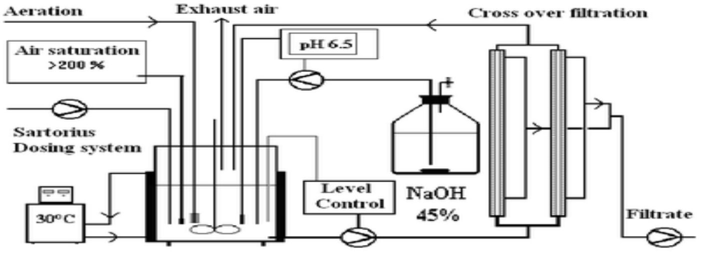

Fig. 4: Fermenter with integrated biomass retention by microfiltration.

cell factories of *Saccharomyces cerevisiae* and *Escherichia coli*, redirecting cellular metabolism towards desired products, such as several sesquiterpenes with most prominent examples the sesquiterpene farnesene, which fulfills various functions in plants and can serve as diesel substitute in its hydrogenated form farnesane that can be obtained efficiently from yeast fermentation (Tippmann *et al.*, 2016).

Other biotechnological products: More valuable microbial products can be produced from glycerol, such as recombinant proteins and enzymes, medicinal drugs, antibiotics and fine chemicals (Da Silva *et al.*, 2009). β-carotene production in batch cultures by *Blakeslea trispora* has been reported, applying glycerol as a supplementary carbon source to glucose (Mantzouridou *et al.*, 2008). Natural bioactive agents with antimicrobial and/or anticancer activity, especially produced by fungi and microalgae, gained interest in pharmaceutical and medical biotechnology for developing new therapeutic agents (Sayegh *et al.*, 2015). Fungus *Thamnidium elegans* grown on raw glycerol and autotrophic microalga *Nannochloropsis salina* produce polyunsaturated fatty lipids with antimicrobial activity, offering an alternative solution to increased microbial resistance to traditional antibiotic drugs (Sayegh *et al.*, 2015). Trindade *et al.*, (2015) reported about the production of exopolysaccharides (used in food, chemical and pharmaceutical industry, among others) by various bacteria including *Xanthomonas campestris* pv. *mangiferaeindicae* IBSBF 1230, *Pseudomonas oleovorans* NRRL B-14683, *Sphingomonas capsulata* NRRL B-4261 and *Zymomonas mobilis* NRRL B-4286 from raw glycerol, a byproduct from biodiesel synthesis, instead of commonly used glucose, directly impacting production costs and limiting market potential. Crude glycerol from Greek Biodiesel industry has been supplementary used as single carbon source or along with other carbohydrates at Pythia Institute of Biotechnology (Greece and Bulgaria) to produce by advanced new generation fermentation a novel superior organic soil conditioner named EcoPlant, which is being used with great success for all kinds of plants in Greece, Bulgaria and other countries. EcoPlant protects plants from freezing, e.g. Greenhouse lettuce at -18°C (Montana, Bulgaria 2015) and outdoor lettuce at -8 °C (Drama, Greece 2015), as also high temperatures, e.g. Zucchini at -45°C for 45 consecutive days (Iraklion, Crete 2013). It accelerates growth, enhances production and resistance to diseases and prolongs the life and duration of production of all plants (e.g. tomatoes). Moreover, it reduces semantically the use of fertilizers and agrochemicals and total production cost (Anastassiadis 2015, www.pythia-biotechnology.bg, www.pythia-biotechnology.com). 504 g/l of gluconic acid were produced in fed batch operation and up to 400-450 g/l in continuous fermentation from glucose by isolated wild strains of yeast-like fungus *Aureobasidium pullulans* (Anastassiadis *et al.*, 1999). *Zymomonas mobilis* NRRL B-4286 from raw glycerol, a byproduct from biodiesel synthesis, instead of commonly used glucose, directly impacting production costs and limiting market potential. Crude glycerol from Greek Biodiesel industry has been supplementary used as single carbon source or along with other carbohydrates at Pythia Institute of Biotechnology (Greece and Bulgaria) to produce by advanced new generation fermentation a novel superior organic soil conditioner named EcoPlant, which is being used with great success for all kinds of plants in Greece, Bulgaria and other countries. EcoPlant protects plants from freezing, e.g. Greenhouse lettuce at -18°C (Montana, Bulgaria 2015) and outdoor lettuce at 8°C (Drama, Greece 2015), 504 g/l of gluconic acid were produced in fed batch operation and up to 400-450 g/l in continuous fermentation from glucose by isolated wild strains of yeast-like fungus *Aureobasidium pullulans* (Anastassiadis *et al.*, 1999). Expansion of industrial microbiology during 20th century and the development of recombinant and molecular biology techniques in 1970's perceived new possibilities for techniques in 1970's and new possibilities for obtaining a large variety and quantity of products by industrial scale biotechnological processes, including solvents, antibiotics, enzymes, vitamins, amino acids, and polymers, among many others (Gonçalves *et al.*, 2014). A feasible way to sustain energy and combat climate change is to build a 100% renewable global energy mix, while sustainable alternatives based on electricity, charcoal, biogas and hydrogen can replace principle economic processes (García-Olivares, 2015). Current falling oil prices may have a negative impact on the investment on research and development and the production of biofuels, especially second generation ethanol, without to considerably implement a public/state subsidy (Reboredo *et al.*, 2016).

Conclusions and future developments: Carbon sources is the basis of known life on Earth initially originating from atmospheric carbon dioxide, which is being fixed via photosynthetic organisms during photosynthesis applying solar energy. CO_2 and carbon sources are steadily recycled through biogeochemical carbon cycle or captured for millions of years in form of various fossils, whereas overproduction and accumulation of CO_2 caused global warming and semantic climate changes in recent times. Energy is the lifeblood of modern societies and essential for socioeconomic and sustainable human development. The steadily rising world population and the increasing energy demand requires the search and development of new alternative carbon and energy sources including renewable biomass, to replace the slowly exhausted fossil fuels. Biomass is used along various competing industrial sectors for the production of food and feed, biofuel energy, biomaterials, biochemicals and bioproducts, etc. A competition for biomass, conventional energy, alternative energy and renewable bioenergy exists due to depleting fossil on different levels, including various industry sectors (Chemistry, biotechnology, food industry, and energy industry), countries with different economy power and population, and organized country unions (US, EE, United Nations etc.). Expansion of industrial microbiology in 20th century and recombinant and molecular biology perceived new possibilities for industrial scale biotechnological production of a large variety and quantity of products. Competition for useful land and territories, used for housing and living, agriculture, livestock, production of goods, materials and energy and other activities, led to antagonisms between groups of people and States and in the worst case in confrontations and wars since ancient times and possibly will continue so in coming times. It is necessary a mutual research, development and reasonable understanding and cooperation between countries and people to overcome those global issues in coming generations of human history.

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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