GREEN FUELS: SUMMARISED NOTE ON MICROBIAL CONSORTIA OPTIONS AND ITS SIGNIFICANCE

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Abstract. Consolidated bioprocessing (CBP) is one of the recent cost-effective biofuel production technologies that involves microorganisms capable of performing enzymatic saccharification of lignocellulosic substrates and fermentation of the converted sugars into biofuel in a single step. This process demands the need for an efficient microbial strain/microbial consortium with complementary metabolic functions. This review highlights the importance of microbial consortia in the production of green fuels as a sustainable alternative to fossil fuels and highlights the significance of utilizing microbial communities for biofuel production due to their diverse metabolic capabilities and synergistic interactions. By harnessing the power of microbial consortia, researchers aim to overcome the limitations of traditional biofuel production methods and enhance the efficiency and sustainability of green fuel production. Furthermore, this review emphasizes the need for technological breakthroughs in biochemical and genetic engineering to optimize the production of biofuels from renewable sources. The integration of synthetic biology approaches and microbial consortia engineering holds great promise for advancing the field of green fuel production. Through the design and construction of synthetic microbial consortia, researchers can tailor metabolic pathways for enhanced biofuel synthesis and improve overall process efficiency. Overall, the abstract underscores the critical role of microbial consortia in the transition towards a more sustainable energy future. By leveraging the metabolic diversity and cooperative behaviors of microbial communities, scientists are paving the way for innovative solutions in green fuel production that are environmentally friendly and economically viable.

Keywords: *green fuel, microbial consortia, biobutanol, bioethanol, biohydrogen, biomethane and biodiesel*

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Introduction

The demand for alternate energy options are increasing day by day with new ventures on finding clean and green fuels. This demand has led a major breakthrough on utilization of waste biomass for fuel production that conserves environmental sustainability. This energy conversion from wastes is feasible by physicochemical or thermochemical or biological processes or combination of these such as bioethanol, biobutanol, biohydrogen, biomethane are generated from agricultural residues and biodiesel produced from algal biomass. Recently, consolidated bioprocessing (CBP) is an economically efficient technique that encompasses biofuel production from polysaccharides by merging the production of lignocellulose degrading enzymes, lignocellulose hydrolysis and fermentation in a single step. This process demands the need of an efficient microbial strain/ microbial consortia with complementary metabolic functions. A microbial consortium is typically defined as a collection of several microorganisms with the ability to cooperate as a community. Generally, we have 3 types of mcrobial consortia viz., Natural Microbial consortia (NMC), Artificial Microbial Consortia (AMC) and Synthetic Microbial Consortia (SMC).

In Natural microbial consortia (NMC), microbes come from a single source, may interact naturally and typically obtained through one or successive enrichment cultures. Artificial microbial consortia (AMC) are composed of microorganisms isolated from different sources and combined. In Synthetic microbial consortia (SMC) the proportion of microbial members are carefully designed and chosen to achieve the desired goal. Formulation of microbial consortia inclusive of lignocellulose degrading strains, enzyme producing strains and fermentative strains exhibit superior advantages in clean and quick energy production. The development of synthetic microbial consortia emerges from a new field of systems biology in which microbes are manipulated genetically for controlled production of end products including green fuels.

This review will focus on

1. Green fuels and their significance.
2. Microbial consortia for bioethanol and biobutanol production.
3. Microbial consortia for biomethane production.
4. Microbial consortia for biohydrogen production.
5. Microbial consortia for biodiesel production.
6. Emerging field of synthetic microbial consortia for green fuels.
7. Conclusion.

Green fuels and its significance

The energy crisis in the world is mounting to a peak due to the exploitation of depleting natural fossil fuels. The ever-increasing demand and usage of non-renewable fossil fuels leads not only to energy exhaustion but also to an alarming environmental degradation by the emission of greenhouse gases (Chakraborty and Mukhopadhyay, 2020). Moreover, it is crucial to minimize the use of non-renewable fossil fuels to save our environment from global warming, ozone layer depletion and also energy balance which necessitates the search for alternative and renewable fuels. It is worth to search and harness various waste sources or raw materials for the production of variety of clean and green fuels. Hence, the switching over to eco-friendly, renewable and sustainable fuels commonly termed as green fuels is gaining popularity in recent years to enhance the energy security (Shote, 2019; Hossain et al., 2020).

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The fuel obtained from biomass through biological and/or chemical process is termed as biofuel. The liquid and gaseous form of biofuels is being largely used in transportation sector which can be easily blended with the existing fossil fuels. The liquid biofuel included bioethanol, biodiesel, bio-oil and gaseous biofuels *viz*., biomethane, biohydrogen are anticipated to meet out at least one-fourth of the world's energy demand. Transesterification, anaerobic decomposition, fermentation and pyrolysis are the processes majorly involved in the production of three generations of alternative green fuels, they are i) first generation biofuels which are produced from edible oil seeds and crops, ii) second generation biofuels also termed as advanced fuels are produce from non-food agricultural biomass/feed stock and iii) third generation biofuels are produced from algae. Biofuel production greatly depends on saccharification, transesterification and fermentation by the action of microorganisms. The efficacy and economy of the process is enhanced by native species or improved by recombinant microorganisms. Bioprocessing methodology can be enhanced by manipulation of the starter culture involved and downstream processing with improved yield at reduced cost. Combination of different species of microorganisms with mixed potential paves a better option for increasing the efficiency of low-cost biofuel production rather than expensive and unpredictable genetic manipulation (Chakraborty and Mukhopadhyay, 2020).

Microbial consortia in green fuel production

Industrial biotechnology is regarded as a key technology to process natural resources by biological means to generate useful products such as chemical, material and fuels. The application of scientific and engineering knowledge to the bioprocesses provides future alternatives to the fading conventional resources. Fermentation of products using pure culture was considered as the prerequisite in late 1940s and 1950s when amino acids, vitamins, pharmaceutics were developed (Sabra et al., 2010). Presently, microbial consortia-based bioprocesses have emerged as a potential technology for the beverages production, treatment of wastewater and biogas generation.

The complex composition of the wastes and presence of inhibitory factors, the production of hydrogen is complicated by using a pure culture. Hence, a consortium of microbial cultures can solve this problem in a broader term. Microbial consortia targets not only on the production of several products by different pathways but also on a narrow product with a mixed substrate (Kleerebezem et al., 2007). The primary advantage of using microbial consortia is the efficient utilization of substrate with an increased product yield. Microbial consortia are able to perform complicated functions by compartmentalizing the pathway and intermediates of individual microorganism without potential interactions (Brenner et al., 2008). They are also robust to environmental fluctuations by means of resisting invasion by other species.Furthermore, a microbial population with active metabolic activity in a microbial consortium can withstand nutritional scarcity, which plays a major role in its community survival (Eiteman et al., 2008).

Microbial consortia provide potential advantages over pure cultures, such as diverse metabolic activity and high productivity. Generally, due to their practicability and easy handling in sterile conditions, they are preferred for industrial applications which extensively reduces processing costs (Johnson et al., 2009; Sivagurunathan et al., 2016). Microbial consortia technology can be broadly divided into three types: nature consortia, artificial consortia, and synthetic consortia (Wang et al., 2020).

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The multifunctional ability of the microbial consortia is based on the communication through exchange of signal molecules (Keller and Surette, 2006), biofilm formation (Greenberg, 2003) and trading metabolites (Wolfaardt et al., 1994). The bioprocesses are too complicated for the complex substrate using microbial consortia. The microbial consortia could be an alternative to pure culture for chemical and bioenergy production in industrial biotechnology. The new bioenergy economy uses biomass as energy resource substitute for fossil fuels creating an emission free environment. Biomass include agricultural residues, forest remaining, energy crop residues, municipal solid wastes, and other plant or algal based materials. One of the application of industrial bioprocesses involving microbial consortia is the development of biohydrogen (Sabra et al., 2010). Moreover, microbial consortia utilise potential interactions, to increase biofuel production efficiency and yield (Liu et al., 2019).

Microbial consortia for bioethanol production

Bioethanol is a popular biofuel that accounts for roughly 90% of all the biofuels in use. Various resources /substrates like sugarcane, sugarbeet, corn wheat, rice straw, wheat straw, wood chips, bagasse etc. are used. However, the simplicity of the bioprocess and recovery of bioethanol from food crops is always scientifically appreciable. Based on the substrates used for bioethanol production, it is categorised as 1st, 2nd and 3rdgeneration bioethanol. Initially bioethanol was produced by microbial fermentation of crop plants like sugarcane and corn and hence named as First-Generation bioethanol. But converting edible crops into fuel creates hike in food price and poses threat for food security. Hence the Second-Generation bioethanol came into picture by utilising feedstock materials like straw and other agricultural wastes which are rich in lignocellulosic materials. Again the 2nd Generation Bioethanol production also faced problems as the microbes with appropriate saccharolytic enzymes to digest thisnon-food biomass are very much limited in number. This paved way for the rise of 3rd Generation bioethanol wherein macroalgae (seaweeds) and microalgae are the producers. In algae 50% of their protein and lipids are utilised for bioethanol production.

They have the following advantages over 1st and 2nd Generation bioethanol producers.

1. Requirement for water resources and arable land is highly minimised.
2. There is no competition against food crops.
3. Capable of growing in diversified ecosystem.
4. Can produce 5-10 times more biomass than crop plants.
5. There is no need for presence of complex saccharolytic enzymes as required in second generation.
6. Absence of lignin in algae is helpful in pre-treatment and hydrolysis steps.
7. Cost of production is comparatively less.

The notable microbes involved in all these 3 generations' bioethanol production are tabulated below (*Table 1*).

First generation bioethanol (FGB)

Sugarcane and corn are the major substrates used for FGB production. In both the cases, *Saccharomyces cerevisiae* is the yeast used for fermentation. In sugarcane, the sugar extracted is directly fermented to ethanol but in corn, the starch has to be hydrolysed into sugar and then fermented by *S. cerevisiae* into ethanol and finally distilled to fuel grade. The performance efficiency of this yeast is reduced due to their low tolerance to

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ethanol and intolerance to accumulated toxic chemicals at the end of fermentation process. The substrate utilisation has been widened to sugarbeet, cassava, potato, sorghum etc.

Table 1. *Major contributory microbes in bioethanol production*

Microbial Group	Microorganisms Involved	Fermented Sugar Forms	Substrates Used
First Generation Bioethanol			
Yeast	*Saccharomyces cerevisiae*	Glucose, Fructose, Sucrose, Maltose	Sugarcane, Sugarbeet, Cassava, Potato, Sorghum
Bacteria	*Zymomonas mobilis*	Glucose, Fructose, Sucrose	
Second Generation Bioethanol			
Yeast	*Schizosaccharomyces pombe Pachysolen tannophilus, P. stipitis, Candida tropicalis, Candida shehatae*	Xylose, cellobiose, mannose, glucose, and galactose	Lignocellulosic biomass from coconut shells, wheat straw, rice straw, rice husks, maize cobs, cotton stalks, jute sticks, wood chips
	Kluyveromyces marxianus	Hexoses & Pentoses at 42-45^{0}C	
Third Generation Bioethanol			
Microalgae & Macroalgae (Chlorophyta, Phaeophyta, Rhodophyta)	*Chlorella vulgaris Ulva, Porphyra, Ascophyllum, Palmaria*	Photosynthetic	Lipid content of algal biomass

The industrially viable ethanol-generating bacteria are *Escherichia coli*, *Klebsiella oxytoca* and *Zymomonas mobilis*. Wild type strains of *Z. mobilis* prefer glucose-based raw materials for rapid and efficient production of bioethanol and give a 5-fold higher yield than other yeasts which corresponds to 97% yield and 12% (w/v) concentrations. Apart from glucose, *Z. mobilis* also utilises fructose and sucrose but not C5 sugars. This ethanol producing bacterium originated from African palm wine and Mexican pulque. It possessed higher sugar uptake, 2.5 times higher ethanol yield and increased tolerance to ethanol upto 16 % (v/v). These characters pose them to be superior to *S. cerevisiae*, albeit their commercial usage is limited due to the limited substrate range. They can't utilise xylose, arabinose present in lignocellulosic hydrolysates and hence restricted to FG bioethanol. Moreover, they cannot tolerate toxic inhibitors like acetic acid and phenolic compounds. When compared the efficiency of bioethanol production by *S. cerevisiae* and *Z. mobilis*, the bacteria under anaerobic condition produces 94% ethanol with lower biomass production (2.5 g/l) whereas the yeast recorded 88% ethanol yield with higher biomass (6.5 g/l) production. However, *Z. mobilis* found to be inferior to *S. cerevisiae* as it could bring down the pH from 6.3 to 3.3 which makes the sterilisation of the medium unnecessary. Li et al. (2019) reported that high cell density fermentation with cell recycling in *Zymomonas mobilis* 8b improved the ethanol productivity in lignocellulosic hydrolysate.

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Second generation bioethanol (SGB)

Second generation bioethanol is typically produced from lignocellulosic biomass and to some extent from industrial by-products which are relatively inexpensive and readily available (Achinas and Euverink, 2016). Lignocellulosic biomass obtained from agriculture industry consists of bagasse, coconut shells, wheat straw, rice straw, rice husks, maize cobs, cotton stalks, jute sticks etc. Municipal solid waste is also included in this category along with forestry waste like sawdust, bark and wood chips. In general, lignocellulosic biomass mainly has hemicellulose (25–40%), cellulose (45– 60%), and lignin (10–25%).

Lignocellulose consisting of lignin, cellulose, and hemicelluloses is found to be one of the promising and sufficient sources (Zaldivar et al., 2000). The conversion of lignocellulose into reducing sugars called enzymatic saccharification is more difficult than the conversion of starch into sugars. The hemicellulose and cellulose are converted to ethanol after undergoing mechanical or chemical or both pre-treatments. But the fermentation of the resulting diversified sugars like arabinose, galactose, rhamnose, mannose, xylose and galactose become a limiting factor.

Limitations in SG bioethanol production are

1. High cost invested for cellulose enzymes for conversion of cellulose in lignocellulosic biomass to sugars.
2. Cost input for removal of lignin covering the cellulose through pre-treatment so as to avoid adsorption of cellulose onto lignin.
3. Glucans and xylans make major part (50-75%) of hemicellulose and their 5C can't be fermented by FG yeast and genetic modification is needed.
4. Accumulation inhibitory substances which hinder the fermentation.
5. Not cost effective due to involvement of various pre-treatment methods.

The usage of *S. cerevisiae* for SGB from lignocellulosic biomass which is has D-xylose as major component is being limited due to the interference of furan released during hydrolysis with the activity of glycolytic enzymes and synthesis of macromolecules. But it could be replaced by *Pachysolen tannophilus, P. stipitis*, *Candida tropicalis*, and *Candida shehatae* that ferment xylose. One more alternate to overcome this problem is the co-culturing of *S. cerevisiae* with *Z. mobilis* to get synergistic effect on metabolic pathways (Singh et al., 2008).

Some strains of *Schizosaccharomyces pombe*, *S. cerevisiae* if provided with limited O_2 or oxygenic condition could convert D-Xylose to ethanol which could be improved by metabolic engineering (Kim et al., 2013). *Pachysolen stipitis*, *P. tannophilus*, *Candida tropicalis*, *C. shehatae* have also been found to convert xylose into ethanol. *P. stipitis* is having physiological characters suitable for acting upon lignocelluloses with a high affinity for xylose and also with an ability to ferment cellobiose, mannose, glucose, and galactose. The genetic mechanisms of *P. stipitis* and other yeasts have been utilised to create a genetically engineered *S. cerevisiae* strains with capability to ferment cellulose, rhamnose, xylose, xylan and arabinose though the assimilation is low (Koivistoinen et al., 2008).

Utilisation of lignocellulose biomass is not only the limitation in classical yeast but also the intolerance to rising temperature (35-45°C) and ethanol concentration (over 20%) (Tofighi et al., 2014) fermentation through thermotolerant microbes would reduce the cooling cost and also the requirement of cellulases E.C.3.2.1.4 (Fonesca et al., 2008). *Kluyveromyces marxianus* survives at 42-45°C and ferments both hexose and pentose sugars which seems to solve both the limitations at a time (Yanase et al., 2010).

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Third generation bioethanol

The advantage of using algae is that it is phototrophic and can synthesises its own food. Moreover, they can grow in any kind of seasons and any kind of water ecosystems like marine water, fresh water, lakes, marshy to marginal lands. Bioethanol producing algae include both microalgae and macroalgae (seaweeds). Bioethanol producing Microalgae include both prokaryotic and eukaryotic photosynthetic microbes. They are capable of producing high amounts of lipids, proteins and carbohydrates in short time. These characters make them highly suitable for bioethanol production. Apart from biodiesel other high value products like biogas, biobutanol, acetone is also derived from algal biomass by changing the cultivation conditions. Normally the lipid content ranges between 70-90% of dry weight of microalgae (Mata et al., 2010). After several hydrolysis processes they are used for bioethanol production. In microalgae, by maintaining certain growth conditions carbohydrate content may be raised to 70% (Branyikova et al., 2011). Outer cell walls of microalgae have pectin, agar & alginate and cellulose, hemicellulose are present in inner walls (Wargacki et al., 2012).

Next major group is the macroalgae which are otherwise called as seaweeds, are very much present in marine environments. Alginate, mannitol, glucan and laminarin are the major polymers of macroalgae (Wargacki et al., 2012) unlike microalgae. Their cell walls are containing cellulose and hemicelluloses constituting only 2-10% dry weight and absence of lignin except in *Ulva* species with 3% of dry weight (Kraan, 2012). In case of macroalgae, absence of lignin or presence of little lignin simplifies the hydrolysis process. The carbohydrate content of macroalgae ranges from 25-50% in the green algae, 30-60% for red algae and 30-50% for brown algae. The maximum polysaccharide content is found to be in *Porphyra* (40-76%), *Ascophyllum* (42-70%), and *Palmaria* (38-74%). For efficient extraction of sugars and to limit the accumulation of toxic chemicals that inhibit the ethanol production pretreatment of algal biomass is essential. There are physical, chemical, physiochemical and biological pretreatments.

For commercial bioethanol production from algae, three methodologies are adopted namely open ponds, photo bioreactor systems (PBRs) and biofilm-based systems for algal growth. In open pond system, algae are grown in natural shallow ponds of one foot deep or lakes or in artificial ponds which are exposed to natural sunlight (Jorquera et al., 2010; Ashokkumar and Rengasamy, 2012). Artificial ponds have closed loop oval shaped recirculation channels (0.2 – 0.5 m depth) equipped with blenders and spreaders for stabilising algal growth. Even though this system is economical, it faces limitations like land use cost, high water usage, low production, ineffective agitating mechanisms and limited light. However, 10% of microalgal biomass, is derived from closed PBRs, while the major part of algal biomass is cultivated in open ponds (Moazami et al., 2012) even though it yields higher. It minimizes the chances of external pollution and contamination threats (Ugwu et al., 2008) and higher cell mass yield reduces the cutting costs considerably.

For outdoor algal mass cultures tubular PBRs are suitable due to large exposed surface area (Brennan and Owende, 2010) even though maintenance of the growth factors such as pH, dissolved O_2, CO_2 along the tubes is difficult (Ugwu et al., 2002). In Flat-plate PBRs maximum solar radiation can be captured and this facilitates growth of very thin but dense algal culture throughout the plate (Hu et al., 1998; Richmond et al., 2003). Algal biofilm system can provide better production, minimised dewatering operations, with decreased down processing costs and hence this system is used for wastewater treatment by certain industries (Wuertz et al., 2003). The major issue with biofuel production from

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algae is their high nutrient requirement in particular N and P. So their cultivation creates competition for these fertilizers (Peccia et al., 2013) and increased production cost. Since the effluents, wastewaters of various inductries and sewage water are rich sources of nutrients, they tend to be as good alternate growth medium for these algae.

Microbial consortia for biobutanol production

Butanol is having the advantage of replacing gasoline without engine modifications when compared to other conventional fuel alternatives which can be synthesised in both chemical process and also by microbial fermentation. The microbial fermentation has been indicated by Pasteur in 1861 itself. Normally it follows Acetone-Butanol-Ethanol (ABE) fermentation pathway wherein the yield of other two products namely acetone and ethanol would be of major part. Butanol has a final concentration of <20 g per L and low yield of 0.28 to 0.33 g kg-1. Biobutanol production is having limitations like high cost of substrate, high cost of downstream process, low tolerance of producer to the synthesised butanol.

Substrates for biobutanol production

The genus *Clostridium* is contributing much for the biobutanol production. It started since 1916, as Chaim Weizmann isolated *Clostridium acetobutylicum* for the synthesis of acetone which was used for production of cordite during WWII. This is capable of producing acetone, butanol and ethanol in the ratio of 7:2:1. The anaerobic nature of *Clostridium* always limits its applications. *C. acetobutylicum* is the most studied bacterial species for optimizing the yield of butanol. During log phase it undergoes acid formation wherein Acetic acid, butyric acid, CO_2 and H_2 liberated followed by second stage wherein these acids are converted into acetone, butanol and ethanol. They prefer starch and sugar based substrates. But engineered cellulolytic *C. cellulolyticum*, and *C. cellulovorans* are capable of converting cellulolytic biomass by the presence of cellulosomes. Glycerol is found to be a good substrate for *C. pastuerianum* which produces butanol, 1,3 propanediol, acetate, lactate, CO_2, and H_2 (Sarchami et al., 2016). However, the regular butanol producing *C. acetobutylicum*, *C. saccharobutylicum* and *C. beijerinckii* are unable to utilise glycerol. Recently *C. tetanomorphum* has been identified to utilise glycerol.

Biobutanol from lignocellulosic biomass

The production of biobutanol from lignocellulosic biomass includes 3 major steps. In the first step fractionation of feedstock into sugars by pretreatment. Common physical pretreatment methods include milling/grinding, extrusion, microwave and ultrasonication. Steam explosion, steam treatment, hydrothermolysis, hot water treatment and ammonium fibre expansion are some of physiochemical methods and chemical pretreatment includes alkali, acidic, ionic liquid, ozonolysis and organo solvent treatments.

The second step is detoxification wherein the chemical compounds like furfural, 5-hydroxymethyl furfural (HMF), acetic acid, formic acid, levulinic acid released during pretreatment step which may affect producer microbe and enzymes. *Saccharomyces cereviseae* are sensitive to furfural, HMF and acetic acid but *C. acetobutylicum* is not affected. Partial decomposition of lignin creates *p*-coumaric acid and syringe aldehyde (Maiti et al., 2016) which are toxic. Generally, electro dialysis, liming/overliming,

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activated carbon/charcoal, dilution, and resin treatments are used as detoxification techniques. In the third step fermentation is done by ABE pathway. Batch fermentation is the most adopted method for its simplicity and low risk of contamination (Lee et al., 2008). In Fed-batch mode substrate inhibition can be managed by gradually adding the substrate, so that the substrate concentration is kept below toxic levels and improved productivity is achieved in continuous fermentation by chemostat (Li et al., 2011).

Biobutanol from mixed sugars

The pre-processed lignocellulosic biomass feedstock contains both pentoses (xylose and arabinose) and hexoses (glucose and mannose). Many research works are being carried out for utilization of mixed sugars for the production of biofuels. Genes involved in xylose metabolism is present as a cluster in *C. beijerinckii* but, in *C. acetobutylicum* they are dispersed in different chromosomal locations (Gu et al., 2010). Moreover, in *C. beijerinckii* xylose metabolic pathway genes are in multiples than in *C. acetobutylicum* (Nölling et al., 2001). Simultaneous utilisation of pentose and hexose sugars is affected by Carbon Catabolite Repression (CCR) wherein the presence of preferred hexose inhibits the utilisation of pentoses (Ren et al., 2010). This leads to diauxic growth which results in inclusion of lag phase and hence residence time is increased. Metabolic engineering works are going on for utilisation of pentoses and hexoses simultaneously (Ren et al., 2010; Xiao et al., 2011, 2012). Besides, researchers have succeeded in setting different feeding and pre-growth strategies to achieve mixed sugar usage without any strain manipulation (Birgen et al., 2018; Magalhães et al., 2018).

Microbial consortia for biomethane production

Biomethane, one of the renewable gas among the emerging green fuels which is majorly used for internal combustion engines. Biomethane is produced by anaerobic fermentation of organic substrates. The octane rating of methane is 130 which is much higher than gasoline, and methane is also reported to produces less CO_2 when compared to conventional fossil fuels (Budzianowski and Brodacka, 2017). Polymeric materials are broken down by a complex process involving hydrolysis, acidogenesis, acetogenesis, and methanation to produce methane and carbon dioxide. Diverse group of microorganisms take care of each step of anaerobic digestion. During hydrolysis process, the complex lignocellulosic materials *viz*., cellulose, hemicellulose and other polymeric sugars are hydrolysed into simple sugar molecules by the action anaerobic microorganisms which secretes various hydrolytic enzymes. These free sugars are then converted into organic acids*viz*., acetic acid, propionic acids, butyric acid, hydrogen and carbon dioxide by acidogenic bacteria. Next to acidogenic phase is the acetogenic phase whereinthe organic acids produced during acidogenic phase are utilized by the anaerobic bacteria where hydrogen and CO_2 are reduced to acetic acid. Next and last step in methane production is methanogenesis that occurs under strict anaerobic conditions where methanogenic bacteria utilize hydrogen with CO_2, acetate, formate, and alcohols to generate methanegas (Chandra et al., 2012).

Pre-treatment methods *viz*., acid, alkali, thermal and ultrasonic treatments of several lignocellulosic materials have been investigated by many researchers and they reported that 53.6% increased methane production in thermal biomass treatment and referred it as the optimum pre-treatment method. In addition to pre-treatment method, the type of feedstock also plays major role in methane production by anaerobic digestion. Similar

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finding was reported by Bhatia et al. (2017) who suggested that banana stem with low lignin content can be a suitable substrate for methane production as compared to corn stover and wheat straw by anaerobic digestion. As algae also lacks lignin and possess hemicellulose in lesser proportion, it is largely studied in the production of biogas and biomethane by simpler milder pre-treatments (Barbot et al., 2016; Saratale et al., 2018). Gruduls et al. (2018) investigated the combinatorial effect of carbon dioxide pretreatment with three different thermal pre-treatments on algae species *viz*., *Fucus vesiculosus*, *Furcellaria lumbricalis*, *Cladophora* sp. and *Ulva intestinalis* from Baltic sea for the production of biomethane. The results showed that CO_2 at low pressure along with autoclaving pre-treatment increased the biomethane production by 12-14%. Lee et al. (2020) used bioaugmentation in anaerobic digestion for the biomethane production from Cowgrass (*Axonopus compressus*). Six different anaerobic microorganisms *viz*., *Clostridium cellulolyticum* strain ATCC35319 and *Clostridium cellulovorans* strain ATCC35296 with cellulose-hydrolyzing ability, *Clostridium aceticum* strain ATCC35044 and *Mesotoga infera* strain DSM25546 acetogenic cultures for acetogenic phase and methanogenic archeae, *Methanosarcina barkeri* strain ATCC43569 and *Methanosaeta concilii* strain DSM3671 for methanogenic phase were used for bioaugmentation study. In the work, researchers reported that double culture augmentation does not show any significant increase in biomethane production whereas the optimal mixture involving C. *cellulolyticum*, *M.infera* and *M. concilii* for triple bioaugmentation exhibited 20.7% increase in methane production when compared to the control. The reason suggested behind the failure of biomethane yield increase was the incompatibility of certain microbes with each other.

Wei et al. (2020) experimented the effect of combining corn stover biochar on biomethane potential by anaerobic digestion of primary sludge. In addition to biomethane potential, the researchers also analysed the role of biochar on the anaerobic microbial communities in the control and experimental continuous digesters. Bacterial populations in control and experimental digesters were majorly dominated by Acidobacteria, Bacteroidetes, Firmicutes and Proteobacteria and these microbial communities are reported to have the ability of degradation of organic substrates by hydrolysis, acidogenesis and methanogenesis.

Wang et al. (2017) also reported the significant impact of biochar addition on anaerobic fermentation. *Rhodobacter* sp. was identified as the hydrolytic microorganism abundant in the biochar-dosed reactor. Organic matter degrading bacterial strains *viz*., *Paludibacter* sp. and *Proteinclasticum* sp. have been documented forthe production of Volatile Fatty Acid (VFA) and hydrogen generation. The population of these strains increased by $39.4 \pm 0.1\%$ and $46.2 \pm 0.1\%$ by incorporation of biochar (Wang et al., 2017). In addition, *Methanosaeta* sp. and *Methanolinea* sp. known as acetoclastic and hydrogenotrophic methanogens were also found in theanaerobic digesters. Among the methanogens, dominant strain reported was *Methanosaeta* sp. which indicates that the utilization of acetate is the main pathway of methanogenesis. The results concluded that addition of biochar increased the populations of methanogens which in turn can improve methane production. Overall, these variations in different digesters with and without biochar addition suggested that biochar changed the population in microbial community in an anticipated path with increased methane production by anaerobic digestion.

Similar study was conducted by Zheng et al. (2020) who studied the change in microbial community after addition of different ratios of 3:1, 1:1, 1:3 corn stalk and pig manure with or without recirculation of liquid digestate. There is an increased methane

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production by recirculation when added with 3:1 ratio of corn stalk and pig manure, while 1:1, 1:3 ratio of corn stalk and pig manure inhibited the methane production. Among the diversified microbial community, the dominant methanogen in digester were *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, *Methanocorpusculum*, *Methanosaeta*, *Methanosphaera* and *Methanosarcina*. Out of three different ratios, 3:1 with recirculation and 1:1 ratio of corn stalk/pig manure without recirculation has maximum biomethane production and *Methanosaeta* sp. was reported as dominant among the above-mentioned methanogens (Zheng et al., 2020).

Microbial consortia for biohydrogen production

Biohydrogen technology is one of the promising bioprocessing technologies which can be biologically synthesized through photofermentation or dark fermentation. Dark fermentation is commonly employed for conversion of most of the lignocellulosic waste and wastewater into biofuels. Biohydrogen production from biomass feedstock is a cost effective and energy saving strategy. Hydrogen is the final product of dark fermentation process by group of anaerobic microorganisms. It can be converted to electrical energy using microbial fuel cells. Usually, bacterial oxidation of the organic substrate takes place in the anodic chamber where the bacteria act as catalysts. The produced electrons are transferred to the cathode externally and protons moves internally through a permeable membrane which resulted in the generation of power or metabolites such as methane or hydrogen gas. The thermodynamic barrier was overcome by the application of potential in a Microbial Fuel Cell (MFC) externally, to form hydrogen at the cathode by the combination of electrons and protons and this possibility directed it towards biohydrogen production (Rabaey et al., 2004).

Cultures of *Clostridia a*nd *Enterobacter* species were employed for biohydrogen production through dark fermentation process. Thermophilic bacteria such as *Caldicellulosiruptor saccharolyticus* or *Thermotoga elfii* (Herbel et al., 2010; van Niel et al., 2002; de Vrije et al., 2009) are able to produce some by-products beside hydrogen production. Recently, thermophilic consortium has found to be effective for hydrogen production. Moreover, large-scale biohydrogen production shows low fermentation rate and hydrogen yield. During fermentation process, hydrogen scavengers such as methanogens or homoacetogens lower the hydrogen yield by converting hydrogen into acetate or methane, thus they are eliminated through pretreatment of biomass using physical (freezing, thawing, heat, etc.) and chemical methods (acid, alkali, chloroform, etc.) (Saady, 2013; Yasin et al., 2013). Among which, heat treatment is found to be effective against methanogens with promising hydrogen yield. The aim of pretreatment is to inhibit the endospores of hydrogen producers such as *Clostridium* sp. (Wang et al., 2020).

Mixed culture of anaerobic sludge and fermentative bacteria *Rhodobacter sphaeroides* (Ozmihci and Kargi 2010), *Rhodobacter capsulatus* (Uyar et al., 2009) and *Rhodopseudomonas palustris* (Adessi et al., 2012) have shown highest hydrogen yield. A percentage of energy is retained in the byproducts during substrate fermentation, which makes the use of second fermentation phase possible for complete substrate conversion (Sabra et al., 2010). Photosynthesis microorganisms are able to produce oxygen and electron from water, thereby producing energy and biomass through anabolic reactions. These electrons can be converted to hydrogen by the action of hydrogenase or nitrogenase enzymes E.C. 1.18.6.1 which are active only under anoxic environment. The

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microorganisms are first allowed with oxygenic photosynthesis followed by anoxic photosynthesis to enhance hydrogenase activity and produce biohydrogen (Claassen et al., 2004). Purple non sulphur bacteria is able to produce hydrogen from organic substrates (Lazaro et al., 2012).

Microalgal cultures have shown extended use in hydrogen production which include *Chlamydomonas reinhardtii*, *Chlorella fusca* and *Scenedesmus obliquus* (Chader et al., 2009; Touloupakis et al., 2021). Microbial consortia used for hydrogen production is summarized in *Table 2*.

Table 2. *Microbial consortia for hydrogen production*

Consortia	Substrate	Hydrogen yield	Reference
Activated sludge	Glucose	1.93 mol H_2 mol^{-1} glucose	(Guo et al. 2013)
H_2-producing sludge	Food waste hydrolysate	85.6 mL g^{-1} food waste	(Han et al. 2015)
Anaerobic digester sludge	Coffee drink wastewater	1.78 mol H_2 mol^{-1} glucose	(Jung et al., 2011)
Enterobacter aerogenes and *Clostridium butyricum*	Crude glycerol	1.5 m mol H_2 mol^{-1}glycerol	(Pachapur et al. 2015)
Escherichia coli and *Enterobacter aerogenes*	municipal solid waste	2.12 L H_2 L^{-1} substrate	(Sharma and Melkania 2018)
Caldicellulosiruptor saccharolyticus and *C. kristjanssonii*	Glucose	3.7 mol H_2 mol^{-1}glucose	(Zeidan et al.2010)
Bacillus cereus A1 and *Brevumdimonas naejangsanensis* B1	Corn starch	1.94 mol H_2 mol^{-1} glucose	(Bao et al. 2013)
Bacillus cereus A1 and *Brevundimonas naejangsanensis* B1	Cassava starch	1.72 mol H_2 mol^{-1} glucose	(Wang et al. 2017)

Hydrogen producing bacteria are widely found in activated sludge, municipal solid wastes etc. (Wang et al., 2010; Yasin et al., 2013). Anaerobic sludge was used as a natural consortia, it shows a significant improvement in hydrogen production by fermentation of the ozonated palm oil mill effluent (POME). Ozonated POME showed the maximum hydrogen yield of 182.3 mL g^{-1} COD, which was 49% more than raw POME (Pisutpaisal et al., 2014). Similarly, hydrogen yield was enhanced from 1.6±0.1 to 2.24±0.03 mol H_2 mol^{-1} hexose in an anaerobic sequencing batch reactor fed with nutrient supplemented POME in addition to thermophilic microflora as the seed. The nutrient supplementation increased the hydrogen production (Thong et al., 2013).

In contrast, the availability of non-hydrogen producing bacteria hinders the activity of hydrogen producers such as nitrate, sulphate and iron reducing bacteria. In natural environment, the hydrogen producers are inhibited by the hydrogen utilising bacteria. An artificial optimised condition for the hydrogen production by the microbial consortia has been needed for generating maximum yield. Dark fermentation of microbial consortia of two isolated strains, *Bacillus cereus* A1 and *Brevumdimonas naejangsanensis* B1 from activated sludge were effectively used for hydrogen production from cassava starch and corn starch with a yield of 94.1% (Bao et al., 2012; Wang et al., 2017). On the other way, some of the artificial consortia such as *Enterobacter* spH1, *Enterobacter* spH2, and *Citrobacter freundii* H3 yield lower hydrogen production (Li and Liu, 2012).

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Hydrogen production using genetic engineered strains have also been reported. One of the study stated hydrogen production using a genetically engineered dual-organism system. Formate consumption was eliminated by deleting two formate dehydrogenases E.C.1.17.1.10 of *S.cerevisiae*, and pyruvate-formate lyase pathway from *E. coli* was integrated to develop formate-overproducing strain which yielded formate 4.5 times higher (Waks and Silver, 2009). A detailed cell interaction study of *Bacillus cereus* A1 and *Brevundimonas naejangsanensis* B1, hydrogen producing consortia showed enhancement in hydrogen yield. Lactate was metabolized from starch by strain A1 and used by the strain B1 for growth and hydrogen generation. In response to strain A1, formate was produced by strain B1 for electron shuttle and thereby producing hydrogen (Wang et al., 2019).

Sarcocarp harvested from coconuts was utilized as feedstock for bioenergy production using a consortium of *E. coli* and *Shewanella oneidensis* in microbial fuel cells (MFCs) (Wang et al., 2014). Wastewater treatment coupled with hydrogen production is achieved in MFCs. The Romanian water sample used for hydrogen production showed 57% of hydrogen yield through electrolysis in MFC without aid of mediators. Also the nitrate removal was noted (Cucu et al., 2013).

Engineered consortium of *Klebsiella pneumoniae–Shewanella oneidensis* was designed to harness electricity from corn stalk hydrolysate by the degradation of xylose and glucose. *Klebsiella pneumoniae* was able to convert glucose and xylose to lactate. Meanwhile electrons generated were accepted by *Shewanella oneidensis* mediated through a biosynthetic flavins pathway from *Bacillus subtilis.* More lactate synthesis was possible by eliminating the ethanol and acetate pathway governed by *pta* (phosphotransacetylase gene) and *adhE* (alcohol dehydrogenase gene). Also by constructing synthesis system by the expression of *ldhD* (lactate dehydrogenase gene) and *lldP* (lactate transporter gene). By these mechanisms, favin synthesis was enhanced, and electron transfer was easily facilitated by the adhesion of *Shewanella oneidensis* on the carbon electrode (Li et al., 2019).

Implementing immobilization technology for hydrogen production using microbial consortia is in current trend. The technology shows much higher proficiency in hydrogen yield and provided recycling of carrier material for several batches of experiment. It shows superiority over suspended cell culture systems by increasing biomass concentration and reusability of carrier. Immobilized consortia of *Bacillus cereus* and *Brevundimonas naejangsanensis* on three different carriers such as polyester fiber, activated carbon, and corn stalk was studied for hydrogen production. Among which corn stalk exhibited good performance yielding 1.50 mol H_2 mol^{-1} glucose and repeatedly used for the next ten batches (Ma et al., 2017).

Microbial consortia for biodiesel production

Biodiesel is mainly obtained by the transesterification of fat and vegetable oils in the presence of a catalyst by an alcohol leading to a fatty acid methyl esters (FAMEs) or a fatty acid ethylester (FAEEs) (Parawira, 2009). The transesterification may be acid catalysed, alkali catalysed or enzyme catalysed. The microbial production has the potential to overcome these challenges due to some advantages such high yield in short time, low labour requirement, easy to scale up regardless of venue, season, climate change and other factors (Zhao et al., 2015).

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Fatty acid supplying microbes

Microbes for biodiesel production should accumulate at least 50% of lipid in cell, should be fast grower with simple industrial requirements and with high recovery percent. Grease microorganisms also called as oleaginous microorganisms are engaged in biodiesel production to supply fatty acids source for transesterification. Grease strains are found to be in the species of bacteria, yeasts, molds and algae (Kosa and Ragauskas, 2011). They can utilize or convert various agro-industrial materials (e.g., plantbiomass) to cellular lipids (Peralta-Yahya and Keasling, 2010).

Many autotrophic microalgae capable of biodiesel synthesis have been found, such as *Chlorella vulgaris*, *Botryococcus braunii*, *Navicula pelliculosa*, *Scenedesmus acutus*, *Crypthecodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochlorisoleo abundans*, *Phaeodactylum tricornutum* and *Tetraselmiss ueica* (Li et al., 2008). Algae need very low requirements to grow including carbon dioxide, sun light, water even in non arable land or in some kind of waste water. They have short generation time, i.e., they can double their mass every few hours and produce at least 30 times more oil per acre than seed plants. There is scope for utilising arid coastal lands which are unsuitable for conventional agriculture, using wastewater or sea water. Many screening studies reported that green algae represent the largest taxonomic groups from which oleaginous candidates have been identified not because of the higher lipid content of them than other algal taxa, but rather because many of green algae can easily be isolated from diverse habitats and grow faster than species from other taxonomic groups (Lotero et al., 2005). However, some limitations such as low growth rate, strict breeding condition and large upfront investment, need to be overcome for effective utilisation of microalgae as an economically viable biofuel feedstock (Radakovits et al., 2010).

Ethanol supplying microbes

Methanol or ethanol is the most frequently used acyl acceptor for biodiesel production. The sourcing of ethanol is having high chances as ethanol can be derived from renewable sources through microbial fermentation (Yusuf et al., 2011). The use of *S. cerevisiae*, *Z. mobilis* and genetically engineered *E. coli* has already been well established for ethanol production. The ethanol producing gene from *Z. mobilis* and the wax ester synthase/acyl-CoA-diacylglycerol acyl transferase (WS/DGAT) gene from *Acinetobacter baylyi* have been overexpressed in *E. coli* for obtaining biosynthetic pathway of biodiesel (Kalscheuer et al., 2006). A newly engineered *E. coli* strain utilizing hemicellulose as raw materials is developed. Likewise in *S. cerevisiae*, ethanol accumulation in high concentration is achieved and hence ethanol supply won't be a limitation factor. Recent understanding on the genetic manipulation technologies in oleaginous bacteria, such as *Rhodococcus opacus* (Holder et al., 2011) and *Yarrowia lipolytica* (Loira et al., 2012) pave way for their potential applications in biodiesel production. Establishing the plasmid for FAEEs production in cellulosic strains, may help to decrease the cost from both raw materials and production process (Lin et al., 2013).

Emerging field of synthetic microbial consortia for green fuels

Biological engineering is a newly emerging field where in the feasibility of microorganisms is being explored in the application of bioprocessing. Mostly, single culture otherwise referred as monoculture system and recombinant microbes such as

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E. coli or yeast is being adopted in various industrial bioprocesses including biofuel production (Shong et al., 2012). Genetic modification is the most common strategy adopted to improve the yield efficiency gives metabolic stress on a single organism leading to unsatisfactory and unpredictable performance. There are many more disadvantages in using monoculture system which lacks competency and genetic diversity which can be surmounted by mixed population of microorganisms with diverse capabilities. Such mixed population referred as microbial consortia is made to coexist which can be tapped for its catalytic specialities, decrease the undesired toxic byproducts and utilize the substrate entirely (Jiang et al., 2020). Ding et al. (2016) reported that construction of synthetic microbial consortia would be an alternative for programming novel complex behaviours and optimal features for bioprocessing providing new frontier for synthetic biology. Understanding of the multicellular systems and engineering novel cell-cell interaction capabilities was highly needed field of synthetic biology (Widder et al., 2016). But the major limitation in establishing microbial consortia for bioprocessing is the control and compatibility of the microbial communities as each organism involves complex biochemical pathways and is difficult for optimization.

Consolidated bioprocessing (CBP) is one such recent cost-effective biofuel production technology that involves microorganisms capable of performing enzymatic saccharification of lignocellulosic substrates and fermentation of the converted sugars into biofuel. This all in one process can be made feasible by synthetic microbial consortia and could efficiently produce biofuels from lignocelluloses through labor division and metabolic speciation between different groups of organisms which can be used for multitasking and tolerate much more variable environments (Qian et al., 2020). The realization of carbon distribution flow within microbial consortia is the key in designing synthetic CBP microbial consortia using lignocellulose. As lignocelluloses degradation and biofuel production are completed by different microbial species, the sugar-production rate by lignocellulose degraders and the sugar consumption rate by biofuel producers should be compatible. However, the incompatible growth conditions between lignocellulose degraders and biofuel producers might limit the overall conversion efficiency (Jiang et al., 2020). The construction of microbial consortia for CBP needs to harmonize the growth condition for establishing synchrony among different species for comparable sugar-release and consumption rates. This approach leads to longer fermentation durations and affects the final product titer. The designing of more specific bioreactors and biomaterials promote stable microbial consortia. In addition to gas permeable dense membranes, a bioreactor equipped with other inlets, such as light intensity fibers or temperature-controlled fluid, could form a gradient of cultivation conditions, which would also meet the requirements of different microbial consortia members and development of microcapsule and microfluidic laminar flow techniques to create a relatively optimal microenvironment for each cell, and the mechanical separation of each microbial species would not affect microbial growth in a complex microbial consortium system.

The understanding of synthetic multicellular communication in the synthetic consortia, integration of metabolic engineering, ways and means to optimize the synthetic consortia are the known advancements in synthetic biology that have enabled the population-level coordination and control of ecosystem stability and dynamics (Choi et al., 2011). Furthermore, several computational tools have been developed by researchers for screening and predicting community behaviour (Choi et al., 2012). Usually, microbial cells in communities communicate and coordinate among the population by cell

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signalling, which is the major challenge in coordinating the signals among species (Hooshangi and Bentley, 2008). The most popular tools for engineering communication are based on quorum-sensing systems used by bacteria to sense and respond to changes in their local population density. Research and understanding are limited in the area of cell–cell communication in terms of independent communication modules, crosstalk between signals, and interspecies communication (Weber et al., 2007). The field of biofuel production depends on usage of cocultures, where a bioprocessing approach that converts biomass to biofuel in a single reactor has significant potential for producing low-cost biofuel. Shin et al. (2010) constructed two strains of *E. coli*, one secreted hemicellulases E.C.3.2.1.4 and other ferments sugars into ethanol synergistically to transform xylan into ethanol. The main challenge in such synergistic biofuel production was the balancing the populations of the two strains considering both function and ecology. Similarly, Tsai et al. (2010) developed synthetic yeast consortia recorded to produce high yield of ethanol from cellulose demonstrates the potential of a division of labor approach. The consortia population was modulated by adjusting the inoculation ratio of each of the four strains of *S. cerevisiae* as 7:2:4:2 and this optimized ratio produced 87% of the theoretical ethanol production. The biosynthetic potential of synthetic microbial consortia represents both exciting opportunities and challenges that require system-level approaches. Microbial consortia can enable complex behaviours through the combined strengths of the individual organisms.

Hu et al. (2017) developed a series of microbial consortia to improve lignocellulolytic enzyme activity using fungal strains *viz*., *Trichoderma reesei*, *Penicillium decumbens*, *Aspergillus tubingensis*, and *Aspergillus niger* with 16 bacterial species for assessing the synergistic effect. They findings of the study are that the cellulolytic activity of bacteria is more important for lignocellulolytic enzyme activity than the fungi in the consortia. And they found that *Trichoderma reesei* alone have synergistic interaction with 16 bacterial species in lignocellulose degradation assessed by increased carboxy methylcellulase E.C.3.2.1.203 and beta glucosidase E.C. 3.2.1.21 activity. Such devised microbial consortia may potentially be applied to effectively and economically degrade lignocellulose. Brethauer and Studer (2014) demonstrated a polyculture of *Trichoderma reesei*, *S. cerevisiae* and *Scheffersomyces stipites* could achieve cellulolytic enzyme production, hexose conversion and pentose sugar utilization in one bioreactor, realizing ethanol production from acid pre-treated wheat straw without detoxification. Many researchers have designed more such microbial consortia for consolidated bioprocessing for biofuel production using bacteria-bacteria-yeast, fungi-bacteria-yeast, fungi-fungi-yeast combinations (Minty et al., 2013; Sgobba et al., 2018).

In developing synthetic microbial consortia, mainly commensalistic and mutualistic interactions based grouping occurs. Metabolic engineering plays a major role in ensuring proper carbon and energy distribution among the different microbial species i.e., substrate converter and production organism. Engineered microbial strains will be able to cooperatively convert the complex polysaccharides into biofuel without dominating each other which can be achieved by pre-growing the strains before community assembly and creation of interdependency. Removal of toxic metabolite by one of the strains would be another method of achieving interdependency. Bayer et al. (2009) has experimented the toxic removal in a developing synthetic community between cellulolytic bacteria and yeast. The cellulolytic bacterium *Actinotalea fermentans* converts cellulose to ethanol and acetate, by growing with acetate producing organism, the production organism *S. cerevisiae* was engineered to express methyl halide transferase that relieves

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A. fermentans of its toxic metabolic end product (Bayer et al., 2009). Micro bead encapsulation of microorganisms, microfluidic chip technology, sequential layering of microbes onto a synthetic biofilm can be used as novel approaches for the construction of synthetic communities.

Conclusion

The transition from fossil to green biofuelsis mainly to lower the emission of greenhouse gases, incredible price hike of fossil fuels, climate deterioration and for the preservation of natural resources in the environment. But this shift towards use of green fuels in place of fossil fuels faces lot many challenges and obstacles in terms process standardization, yield, efficacy, compatibility and commercial applicability. In order to avoid exhaustion of sugar and starchy food/feedstocks, suitable alternates have been derived by introducing microbes that convert lignocellulosic biomass into liquid and gaseous biofuels. But the use of lignocellulosic substrates needs pretreatment and cost-effective methods, also further explored to minimize the process cost and further curtail the accumulation of fermentation inhibitors. Identification of novel microbial consortia either by natural selection or by applying molecular techniques for the conversion of substrates skipping pretreatments would be a milestone in the biofuel industries. A super strain of yeast with ability to convert pentoses, hexoses of lignocellulosic biomass and mixed sugars is a dream of this industry. Algae are gaining importance due to their higher growth rate, high productivity, high lipid content, less land requirement with little attention. Usage of industrial effluent, wastewater and sewage water as alternate for growth medium for these algae is a promising arena and further research in this line would give an effective solution for overcoming present limitations. A plausible technological breakthrough in the field of biochemical and genetic engineering is much needed to have a successful usage of biofuels and in near future green fuels will take over as alternate for fossil fuels.

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