ISBN: 978-93-90847-18-1

6. Recent Approach for Butanol Production through Genetic Engineering Process to Overcome the Limitations of ABE Fermentation

Japleen Kaur

Department of Environmental Sciences,
Central University of Jammu,
Jammu and Kashmir, India.

Anita Singh

Department of Environmental Sciences,
Central University of Jammu,
Jammu and Kashmir, India.

Dr. Somvir Bajar

Department of Environmental Sciences,
Central University of Jammu,
Jammu and Kashmir, India.

Abstract:

Butanol is an important class of bio manufacturing. It has been widely used as an important feedstock for chemical production, renewable energy source and industrial solvent. For its superior properties it is used as a better substitute with gasoline. Butanol production through Acetone-Butanol-Ethanol fermentation tolerate only 2% of Butanol with other undesired products.

To overcome this limitation nowadays newly invent techniques is implemented, where Butanol is synthesis by Genetic and metabolically engineering pathways. In this technique, the organisms are genetically modified to produce desired product. This new era of technology not only solve the problem of biofuel production but also preserve the environment from destruction and for future generation.

Index Terms: Butanol, Fermentation, Genetic Engineering, Renewable energy

6.1 Introduction:

"Bio manufacturing is a type of manufacturing that utilizes biological systems which include living microbial cells, plant cells, tissues, enzymes or enzymatic system to synthesize commercially important macro and micro biomolecules for their commercial use in the various industrial and medical fields such as food, material, energy cosmetics, fuels and pharmaceutical industries [2, 7].

The products of bio-manufacturing may also extract from microbial cultures i.e. (primary and secondary metabolites). Bio manufacturing history classified into three main revolution on the basis of specific product, platform of production and research technologies [5]. The synthesis of primary metabolites such as Butanol, acetone, ethanol, citric acid through the process of mono-culture fermentation come under 1.0 Bio manufacturing, mainly focus on primary metabolites synthesis [2]. The 2.0 Bio manufacturing mainly focus on secondary metabolites production of antibiotics such as penicillin and streptomycin. The 3.0 bio manufacturing is for the synthesis of macro biomolecules such as enzymes and proteins mostly pharmaceutical compounds (human insulin, amylase, DNA polymerase, amylases, Follicle- stimulating hormone FSH, Bovine, proteases) through recombinant DNA technology. The 4.0 bio manufacturing target newly advanced products like artificial synthesis of human tissues and antibodies by hybridoma and stem cell technology, invitro through regenerative technology, production of artificial starch by invitro synthetic bio systems, production of iso-butanol for biofuels blending by metabolic engineering pathways as massive upgrade in the field of research.

The 4.0 bio manufacturing focus on the necessary and important challenges of mankind which mainly include food security, renewable energy and sustainability, water crisis, climate change and issues of health and also the conflict related to renewable energy i.e. Biofuels [6]. Nowadays more concern is given to bio manufacturing 1.0 and 1.4 [2]. Due to diminishing resources of oil and reinforcement of environmental concerns have shift our focus towards the production of alternative biofuels. To resolve this shortage of fuel production and supply, the concern towards eco-friendly renewable energy source is very necessary. This not only solve the problem of biofuel production but also preserve the environment from destruction and for future generation. At present, Butanol is interestingly potentially usable renewable energy source [1]. The energy content of Butanol is very high with low pollutant emanation make it feasible for easy liquid transportation biofuel and as a biofuel for existing cars [4].

It has many uses in various industries of chemical and cosmetics [1]. Every year through petrochemical routes around 10-12 billion pounds of n-Butanol is produced [1]. Now-a-days, Butanol is achieving the global requirements of gasoline and diesel. The demand for Butanol exceeds every year and valued over 6$ billion as per according to 2016 data [4]. Biobutanol, particularly n-Butanol (C4H9OH) is a primary alcohol with four carbon chain. It is gaining more attention because it acts as substitute of gasoline and also called as next- generation fuel. The main advantage of n-Butanol (C4H9OH) includes less hazardous compound, less hygroscopicity, high octane number, high energy content, flexible for fuel blending, less volatile solvent. It can be used for blending in higher concentration in pure form with gasoline as compare to other solvents such as ethanol (CH3CH2OH) which shows 85% of blending with gasoline [4].It has superior fuel properties as compare to ethanol for the better replacement for gasoline. Butanol energy value is (29.2 MJ/L), which is probably 30% appreciable than (CH3CH2OH) ethanol and from gasoline it is 10% less [8]. It has closer ratio of air-to-fuel to gasoline, which indicates that they have similar fuel properties. Furthermore, low vapor pressure of Butanol allows its utilization in gasoline transport pipelines [4].Due to hike in the prices of crude oil generate our interest to produce Butanol as alternative fuel through biological process and metabolic engineering pathways [4].Metabolic engineering , which emerged in 20 years ago is directed modulation of metabolic pathways for over-production of desired metabolites or also the improvement of cellular properties, on the other hand newly develop approach of synthetic biology mainly focus on fundamental research facilitated by the utilization of synthetic DNA and genetic circuits.

Several common example of synthetic biology approach include antimalarial drug artemisinin by genetically engineered E.coli and yeast

In other words if the number of items constituting population is fixed, it is known as finite population. If the population consists of an infinite number of items, it is called infinite population.

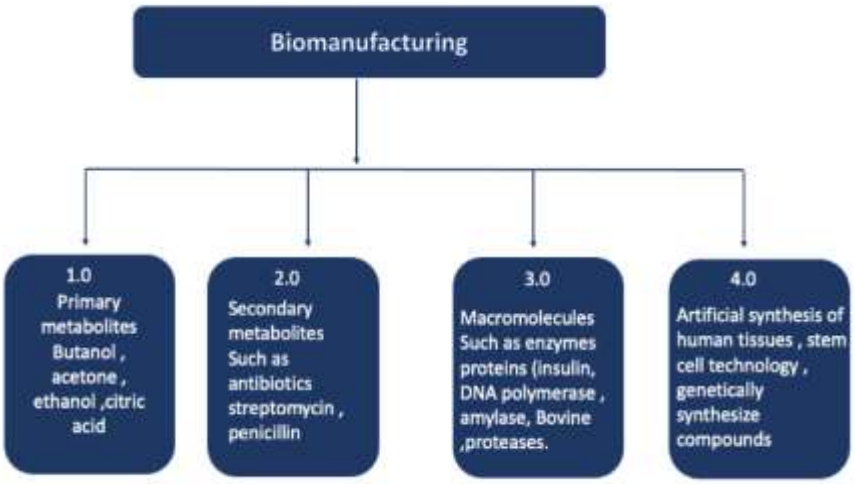

Fig. 6.1: Types of Bio manufacturing

6.2 Biological Production of Butanol through Abe Fermentation:

The acetone-butanol-ethanol fermentation or ABE fermentation is one of the prominent and ancient biological method of fermentation mainly accomplish through different Clostridium species which include C. acetobutylicum and C. beijerinckii in which glucose, xylose, cellobiose, mannose like sugars is use as soul carbon source to convert into primary metabolite's like butanol, ethanol and acetone. Corn, molasses, rice, potatoes, wheat are some conventional feedstocks used for the production of butanol. The only problem with these feedstocks they mainly depend on starch and restricts the Clostridium species amylolytic activity and not fulfil the requirement of upstream processing i.e. hydrolysis and pre-treatment activity [8]. The starch dependent feedstock merely not prove economical due to their high cost and it increases the concern of food verses fuel which lead to production of substrate like lignocellulosic biomass for butanol production. The lignocellulosic biomass for Acetone-butanol-Ethanol covers corn stover fiber, sunflower shells, timothy, and wheat straw [4]. The Acetone-butanol-Ethanol is batch culture type fermentation in which Glucose is used as carbon source i.e. substrate. All the constituents for fermentation prepared under sterile condition with anaerobic environment provide to the reactor through N2 or CO2 gases [4]. The inoculation is provided in the medium with overnight grown Clostridium with the incubation of 35°C.The total time of fermentation lasts for 36-72 hours with 20-25 g/l (butanol) of production. Among all the Clostridium family C. acetobutylicum studied in broad prospects due to high butanol producing ability.

The fermentation through Clostridium is biphasic, it involves both solvent genesis and acidogenesis. Moreover, acidogenesis is responsible for production of acids and solvent genesis is responsible for the production of solvents such as butanol, ethanol and acetone. The key enzyme involve for the production or biosynthesis of solvents with coded genes are acetoacetyl-CoA thiolase (thl), aldehyde/alcohol dehydrogenase, (adhE/adhE2), 3-hydroxybutyryl-CoA dehydrogenase (hbd), crotonase (CRT), butyryl-CoA dehydrogenase (BCD) [4].

These are the most essential genes that are required for the conversion of acetyl CoA to butanol. Further rate limiting steps are also present which decreases the rate of reaction. The Clostridium not only produce butanol as end desired product along with butanol other compounds are produced such as butyric acid, acetic acid, ethanol and acetone. The whole anaerobic fermentation process lasts for almost 72 hours. The complete Acetone-Butanol-Ethanol collected is approximately 20-25 g/L. The inhibition of synthesis of butanol identified at a 5-10 g/L of concentration. Clostridia tolerate more than 2 % Butanol [4].

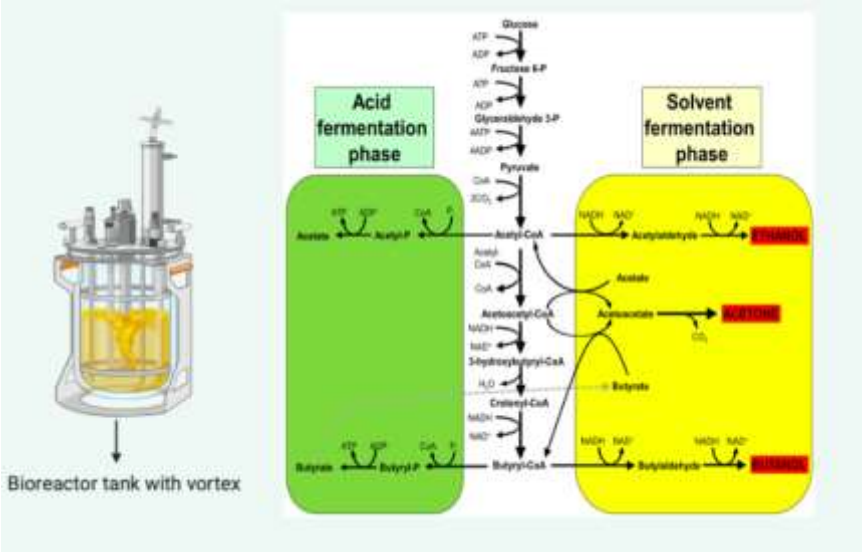

Fig. 6.2: ABE fermentation pathways

6.3 Butanol Production Challenges through Abe Fermentation:

6.3.1 Butanol Toxicity:

As we know that Clostridia tolerate only 2% of butanol. Butanol is more toxic that ethanol and acetone and a big drawback for the overall yield of butanol in the whole Acetone-Butanol -Ethanol fermentation process [4]. This declines the productivity of butanol in industrial level production. The butanol toxicity occurs due to hydrophobicity and related alcohols which may disturb the cell membrane of bacteria. In C. acetobutylicum high level of butanol cause changes in the structure of cell membrane. Ethanol, short chain aliphatic alcohol decrease fluidity of membrane whereas as long chain aliphatic alcohol increases the fluidity of membrane. Butanol cause fluidity in membrane by entering inside the membrane.

Other than butanol toxicity the ABE fermentation is also inhibit by other micro-organisms contamination and phage such as bacteriophage, concentration of nutrients and type of salts, types of substrate, oxygen diffusion, accumulation of macromolecules. High level of butanol alters the membrane of cell and may also disturb composition of cell membrane.

Aliphatic alcohol which are short chain reduces membrane fluidity on the other hand long chain aliphatic alcohol increases membrane fluidity. Other impact of butanol on the bacterial cells as a result of membrane fluidity are destabilization of cell membrane and disturbance of other physiochemical features such as permeability of membrane, glucose uptake, transport of solute, intracellular ATP level. Bacteriophage contamination is very common technical challenge. The ABE fermentation is also influenced by bacteriophage contamination. To overcome limitations of butanol, newly invent techniques implement nowadays where butanol is synthesis by Genetic and metabolically engineering pathways [4]

6.2.2 Butanol Recovery Limitations:

The partial miscibility of butanol in water is as progressive quality in preventing engine corrosion and use in higher concentration. This property various technical issues during the recovery of whole product. Biobutanol solubility in water is 7.7% (7g/L) at 20 °C [4]. While primary butanol is soluble in water, secondary butanol is largely water soluble, and tertiary is completely soluble.

The OH-group in alcohol increases their polarity and initiate aqueous solubility, due to increases in the length of carbon chain which tends to decrease its solubility in water. All the alcohol group such as Methanol, ethanol and propanol are completely soluble in water except butanol due to its long carbon chain. The recovery of butanol through fermentation includes some chemical technologies such as liquid-liquid extraction, prestraction, pervaporation, adsorption, distillation. During the butanol recovery by adsorption take place more chances of disturbance by other alcohol will also take place as ethanol could compete butanol at adsorption site.

The butanol recovery is also influenced by butyric acid and acetic acid as well as the nutrition in fermentation broth also compete and disturb overall recovery. The oldest method of butanol recovery is Distillation. It is one of the traditional methods which involve azeotrope to differentiate alcohol from solution which are diluted. The energy required for distillation is very high because boiling point of butanol(117.7°C) is very high as greater than water. The overall concentration of acetone, butanol and ethanol is 18-33 g/L in the fermentation broth and the concentration of butanol 13-18 g/L.

Gas stripping is another important technology that removes OH-group from vapor phase with the help of molecular sieve. In this process mixture of gases like, Nitrogen, carbon-dioxide and hydrogen expel from fermentation medium using different kind of shafts. The gases help to capture butanol fermenter. On the other hand, liquid-liquid extraction relies on coefficients of distribution and extraction solvent of fermentation medium. Acetone, butanol ad ethanol is recovered using an extracting solvent with the fermenter medium. Some important extracts studied for recovery of butanol include esters, alkaline, hexanol, vegetable oil. The one of the drawbacks of liquid-liquid extraction is it forms subsequent emulsion formation. Pevaporation is another technique other than liquid-liquid extraction to extract the alcohol [4].

6.3.3 Butanol Production through Metabolic Engineering Pathways:

Microorganisms that are metabolically engineered to switch renewable carbon sources into desired fuel products are considered as the best choice to obtain high volumetric productivity and yield. The new approach of butanol production through metabolic and genetic engineering pathways has deducted many limitations of butanol and increases butanol yield titer and productivity. This help to improve total productivity of Clostridium solvent genic pathways. The new era of bioinformatics, Recombinant DNA technology, mutagenesis metagenomics, proteomics like advanced tools genetically changed metabolic pathways of micro-organisms and target only on desired product. The mutant strain through metabolic pathways in Clostridium focus on high cell density, persistent cell viability, no spore formation, tolerance to aerobic condition and main focus on high tolerance to metabolites like butanol, ethanol and acetone. And also help to generate strain which directly utilize lignocellulosic feedstock. This synthetic biology approach works on carbon catabolite repression and mutates strains, glucose selective strain and xylose selective strain through glucose selective pathways for better productivity and also increases butanol tolerance [3]. Adding mutations in bacterial species help in boosting and reshaping overall industrial production of butanol refinery. Other than Clostridium, other genetically modified organisms like E.coli also used for advance butanol production.

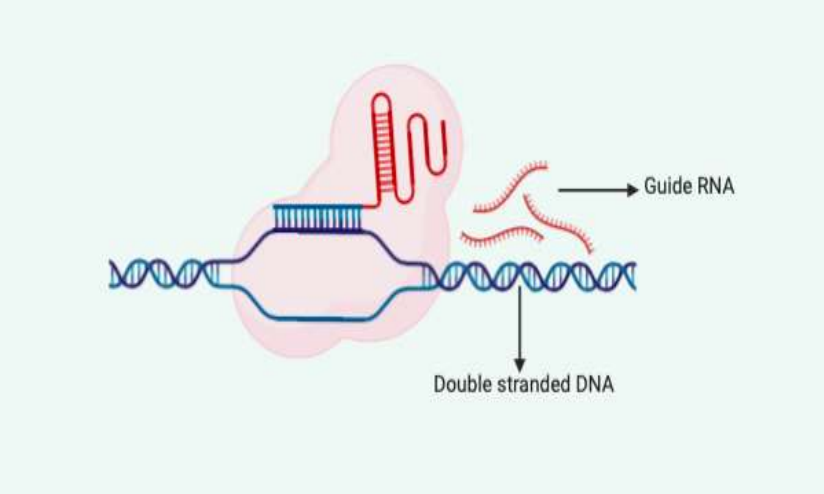

Fig. 6.3: Genome editing from Biotechnological tools

6.4 Recombinant Butanol Production in Escherichia Coli:

E.coli is gram-negative, facultative rod-shaped bacteria with known rod-shaped. E.coli has the unique advantage of being the best-studied model organism in terms of gene-regulation and expression. The vast knowledge of genetic, metabolic and physiological properties of E.coli allow its reconstruction for production of different target compounds. The important relevant characteristics of industrially important butanol include induce genetic modifications to increase the capacity to grow on mineral media, easy and quick growth, capacity to grow on both anerobic and aerobic conditions. It also successfully engineered to produce hormones and different proteins [9].

E.coli proven to be as potential host for expressing important genes and for the process of cloning to produce bioethanol. E.coli has the unique property to utilize two sugars through Carbon Catabolite repression. The genes responsible for glucose uptake ptsGcrr then become dephosphorylated and prevents activation of adenylate cyclase.

The overall reaction gives limited amount of catabolite activator protein (CAP) and cAMP complex. To overcome the problem of CCR two strains genetically engineered and utilization of glucose and xylose simultaneously. Two strains metabolically engineered to develop glucose selective strain and Xylose selective strain [9]. Both the strains sequentially consume sugar and only constructed for the production of butanol as desired product.

The bioinformatically tools plays an important role for the construction of strain. As we know that the whole data of E.coli is available and coded bioinformatically. Nowadays synthetic biology approach evolves for butanol production of butanol. Antisense RNA technology also been applied for the production of improved ABE fermentation. The antisense RNA is single stranded RNA complementary to as transcribed messenger RNA strand. Addition of antisense RNA strand inside the cell with the help of stoichiometric effects inhibits the translation of complementary mRNA.

Synthetic biology approach applied to develop microbial strains tolerant to high butanol concentration and other solvent stress. An increase in the concentration of butanol in the whole process of fermentation induces as response which is almost similar to heat shock.

The important role of heat shock in this process in not understood properly. So, the synthetic biology approach plays an important role for metabolically production of butanol. It makes an easier way for the production of butanol. Metabolic engineering of microorganism has enabled the incorporation of non-native pathways to generate high value drop in alcohol biofuels. [4]

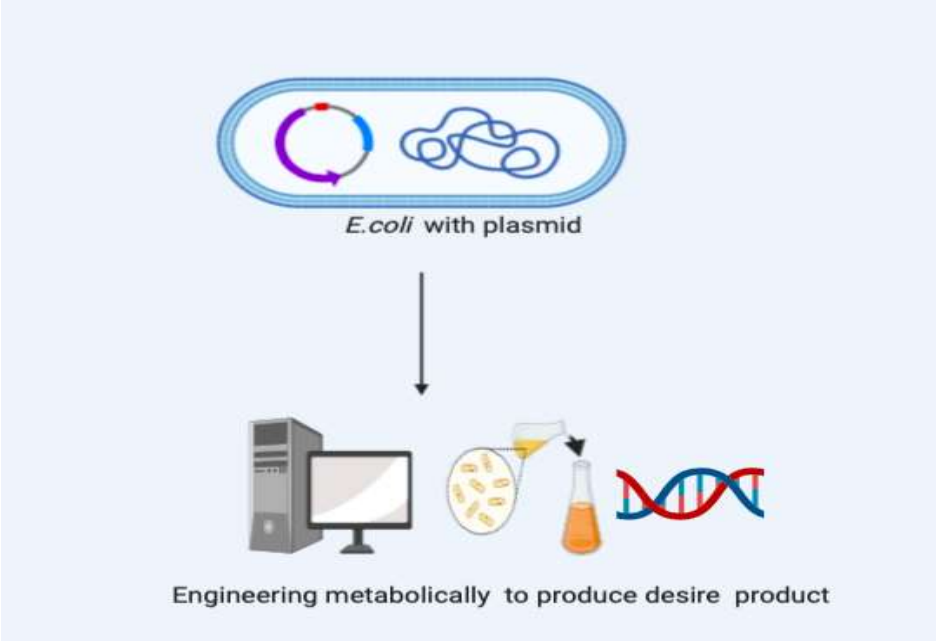

Fig.6.4: E. Coli Genome Editing

6.5 Conclusion:

Bio manufacturing mainly focus on human welfare and development. It addresses several important issues of mankind such as water crisis, renewable energy and sustainability, issues of health and major concern on biofuels (ethanol, butanol). Butanol serves as advanced biofuel having all properties similar to gasoline and produced through ABE fermentation in which only 2% butanol is produced (2% tolerance) with other solventogenic compounds (ethanol, citric acid, acetone) a big drawback to overall industrial progress. Newly develop synthetic biology approach is applied nowadays in which metabolic pathways are genetically engineered to increases the butanol tolerance and selectivity. This newly developed technique improves the whole recovery process of butanol and could make it as drop-in fuel replacement for gasoline.

6.6 References:

1. Abo, B. O., GAO, M., Wang, Y., Wu, C., Wang, Q., & Ma, H. (2019). Production of butanol from biomass: recent advances and future prospects. Environmental Science and Pollution Research, 26(20), 20164–20182.
2. Lee, J. Y., Geraldi, A., Rahman, Z., Lee, J. H., & Kim, S. C. (2015). Improved n-butanol tolerance in Escherichia coli by controlling membrane related functions. Journal of Biotechnology, 204, 33-44.
3. Maleki, N., Safari, M., &Eiteman, M. A. (2018). Conversion of glucose-xylose mixtures to pyruvate using a consortium of metabolically engineered Escherichia coli. Engineering in Life Sciences, 18(1), 40–47.
4. Nanda, S., Golemi-Kotra, D., McDermott, J. C., Dalai, A. K., Gökalp, I., & Kozinski, J. A. (2017). Fermentative production of butanol: Perspectives on synthetic biology. New Biotechnology, 37, 210–221.
5. Saini, M., Li, S. Y., Wang, Z. W., Chiang, C. J., & Chao, Y. P. (2016). Systematic engineering of the central metabolism in Escherichia coli for effective production of n-butanol. Biotechnology for biofuels, 9(1), 69.
6. Saini, M., Lin, L. J., Chiang, C. J., & Chao, Y. P. (2017). Synthetic consortium of Escherichia coli for n-butanol production by fermentation of the glucose–xylose mixture. Journal of agricultural and food chemistry, 65(46), 10040-10047.
7. Zhang, Y. H. P., Sun, J., & Ma, Y. (2017). Bio manufacturing: history and perspective. Journal of Industrial Microbiology and Biotechnology, 44(4–5), 773–784.
8. Zheng, Y. N., Li, L. Z., Xian, M., Ma, Y. J., Yang, J. M., Xu, X., & He, D. Z. (2009). Problems with the microbial production of butanol. Journal of industrial microbiology & biotechnology, 36(9), 1127-1138.
9. Ferreira, S., Pereira, R., Wahl, S. A., & Rocha, I. (2020). Metabolic engineering strategies for butanol production in Escherichia coli. Biotechnology and bioengineering, 117(8), 2571-2587.
10. Vogt, T. (2010). Phenylpropanoid biosynthesis. Molecular plant, 3(1), 2-20.
11. Lim, J. H., Seo, S. W., Kim, S. Y., & Jung, G. Y. (2013). Model-driven rebalancing of the intracellular redox state for optimization of a heterologous n-butanol pathway in Escherichia coli. Metabolic engineering, 20, 56-62.
12. Chae, T. U., Choi, S. Y., Kim, J. W., Ko, Y. S., & Lee, S. Y. (2017). Recent advances in systems metabolic engineering tools and strategies. Current opinion in biotechnology, 47, 67-82.

13. Chae, T. U., Choi, S. Y., Kim, J. W., Ko, Y. S., & Lee, S. Y. (2017). Recent advances in systems metabolic engineering tools and strategies. Current opinion in biotechnology, 47, 67-82.
14. Chen, C. T., & Liao, J. C. (2016). Frontiers in microbial 1-butanol and iso-butanol production. FEMS microbiology letters, 363(5), fnw020.
15. Chin, W. C., Lin, K. H., Chang, J. J., & Huang, C. C. (2013). Improvement of n-butanol tolerance in Escherichia coli by membrane-targeted tilapia metallothionein. Biotechnology for biofuels, 6(1), 1-9.
16. Choi, K. R., Jang, W. D., Yang, D., Cho, J. S., Park, D., & Lee, S. Y. (2019). Systems metabolic engineering strategies: integrating systems and synthetic biology with metabolic engineering. Trends in biotechnology, 37(8), 817-837.
17. Clomburg, J. M., & Gonzalez, R. (2010). Biofuel production in Escherichia coli: the role of metabolic engineering and synthetic biology. Applied microbiology and biotechnology, 86(2), 419-434.
18. Conrad, T. M., Lewis, N. E., & Palsson, B. Ø. (2011). Microbial laboratory evolution in the era of genome-scale science. Molecular systems biology, 7(1), 509.
19. Ferreira, S., Pereira, R., Wahl, S. A., & Rocha, I. (2020). Metabolic engineering strategies for butanol production in Escherichia coli. Biotechnology and bioengineering, 117(8), 2571-2587.
20. De Graef, M. R., Alexeeva, S., Snoep, J. L., & de Mattos, M. J. T. (1999). The steady-state internal redox state (NADH/NAD) reflects the external redox state and is correlated with catabolic adaptation in Escherichia coli. Journal of bacteriology, 181(8), 2351-2357.
21. Nanda, S., Mohammad, J., Reddy, S. N., Kozinski, J. A., & Dalai, A. K. (2014). Pathways of lignocellulosic biomass conversion to renewable fuels. Biomass Conversion and Bio refinery, 4(2), 157-191.
22. Nanda, S., Dalai, A. K., Gökalp, I., & Kozinski, J. A. (2016). Valorization of horse manure through catalytic supercritical water gasification. Waste management, 52, 147-158.
23. Gong, M., Nanda, S., Romero, M. J., Zhu, W., & Kozinski, J. A. (2017). Subcritical and supercritical water gasification of humic acid as a model compound of humic substances in sewage sludge. The Journal of Supercritical Fluids, 119, 130-138.
24. Dürre, P. (2007). Biobutanol: an attractive biofuel. Biotechnology Journal: Healthcare Nutrition Technology, 2(12), 1525-1534.
25. Green, E. M. (2011). Fermentative production of butanol—the industrial perspective. Current opinion in biotechnology, 22(3), 337-343.
26. Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2007). Bio production of butanol from biomass: from genes to bioreactors. Current opinion in biotechnology, 18(3), 220-227.
27. Atsumi, S., Hanai, T., & Liao, J. C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature, 451(7174), 86-89.
28. Benner, S. A., &Sismour, A. M. (2005). Synthetic biology. Nature Reviews Genetics, 6(7), 533-543.
29. Bornscheuer, U. T., Huisman, G. W., Kazlauskas, R. J., Lutz, S., Moore, J. C., & Robins, K. (2012). Engineering the third wave of biocatalysis. Nature, 485(7397), 185-194.
30. Chen, H. G., & Zhang, Y. H. P. (2015). New biorefineries and sustainable agriculture: Increased food, biofuels, and ecosystem security. Renewable and Sustainable Energy Reviews, 47, 117-132.
31. Davies, H. M. (2010). Commercialization of whole-plant systems for bio manufacturing of protein products: evolution and prospects. Plant biotechnology journal, 8(8), 845-861.
32. Demain, A. L. (2004). Pickles, pectin, and penicillin. Annu. Rev. Microbiol., 58, 1-42.
33. Demain, A. L. (2007). The business of biotechnology. Ind Biotechnol 3: 269–283.

34. Demain, A. L., & Vaishnav, P. (2009). Production of recombinant proteins by microbes and higher organisms. Biotechnology advances, 27(3), 297-306.
35. United Nations. Department of Economic and Social Affairs. (2013). World economic and social survey 2013: Sustainable development challenges. UN.
36. Surisetty, V. R., Dalai, A. K., & Kozinski, J. (2011). Alcohols as alternative fuels: An overview. Applied Catalysis A: General, 404(1-2), 1-11.
37. MacLean, H. L., & Lave, L. B. (2003). Evaluating automobile fuel/propulsion system technologies. Progress in energy and combustion science, 29(1), 1-69.
38. Szulczyk, K. R. (2010). Which is a better transportation fuel--butanol or ethanol? International Journal of Energy & Environment, (3).
39. Donaldson, G., Eliot, A., Nagarajan, V., Nakamura, C., & Huang, L. (2007). U.S. Patent Application No. 11/741,892.
40. Donaldson, Gail, Andrew Eliot, Vasantha Nagarajan, Charles Nakamura, and Jean-francois Tomb. "Fermentive production of four carbon alcohols." U.S. Patent Application 11/741,916, filed December 20, 2007.
41. Lane, J. G. (2013). Rhodia ink pact for bio based n-butanol. Brazil, from bagasse. Biofuels Digest: http://www. Biofuels digest. com.
42. Sillers, R., Chow, A., Tracy, B., & Papoutsakis, E. T. (2008). Metabolic engineering of the non-sporulating, non-solventogenic Clostridium acetobutylicum strain M5 to produce butanol without acetone demonstrate the robustness of the acid-formation pathways and the importance of the electron balance. Metabolic engineering, 10(6), 321-332.
43. Sillers, R., Al-Hinai, M. A., & Papoutsakis, E. T. (2009). Aldehyde–alcohol dehydrogenase and/or thiolase overexpression coupled with CoA transferase down regulation lead to higher alcohol titers and selectivity in Clostridium acetobutylicum fermentations. Biotechnology and bioengineering, 102(1), 38-49.
44. Papoutsakis, E. T. (2008). Engineering solventogenic clostridia. Current opinion in biotechnology, 19(5), 420-429.
45. Harris, L. M., Desai, R. P., Welker, N. E., & Papoutsakis, E. T. (2000).
46. Characterization of recombinant strains of the Clostridium acetobutylicum butyrate kinase inactivation mutant: need for new phenomenological models for solventogenesis and butanol inhibition? Biotechnology and bioengineering, 67(1), 1-11.