



**EXAMINING THE METABOLIC PATHWAY OF MICROORGANISMS INCLUDING  
ENERGY PRODUCTION AND NUTRIENT UTILIZATION**

**Putta Rajesh Kumar<sup>1\*</sup>, Poojitha P.<sup>1</sup>, Sowjanya P.<sup>1</sup>, Shivaleela U.<sup>1</sup>, Meena N.<sup>1</sup> and JVC Sharma<sup>2</sup>**

<sup>1</sup>Department of Pharmaceutics, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.

<sup>2</sup>Department of Pharmacognosy, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.



\*Corresponding Author: Dr. Putta Rajesh Kumar

Department of Pharmaceutics, Joginpally BR Pharmacy College, Hyderabad - 500075, Telangana, India.

Article Received on 27/01/2024

Article Revised on 17/02/2024

Article Accepted on 07/03/2024

**ABSTRACT**

The use of fossil energy sources has a negative impact on the economic and socio-political stability of specific regions and countries, causing environmental changes due to the emission of greenhouse gases. Moreover, the stocks of mineral energy are limited, causing the demand for new types and forms of energy. Biomass is a renewable energy source and represents an alternative to fossil energy sources. Microorganisms produce energy from the substrate and biomass from substances in the microenvironment, to maintain their metabolism and life. However, specialized microorganisms also produce specific metabolites under almost abiotic circumstances that often do not have the immediate task of sustaining their own lives. This paper presents the action of biogenic and biogenic-thermogenic microorganisms, which produce methane, alcohols, lipids, triglycerides, and hydrogen, thus often creating renewable energy from waste biomass. Furthermore, some microorganisms acquire new or improved properties through genetic interventions for producing significant amounts of energy. In this way, they clean the environment and can consume greenhouse gases. Particularly suitable are blue-green algae or cyanobacteria but also some otherwise pathogenic microorganisms (*E. coli*, *Klebsiella*, and others), as well as many other specialized microorganisms that show an incredible ability to adapt. Microorganisms can change the current paradigm, energy-environment, and open up countless opportunities for producing new energy sources, especially hydrogen, which is an ideal energy source for all systems (biological, physical, technological). Developing such energy production technologies can significantly change the already achieved critical level of greenhouse gases that significantly affect the climate.

**KEYWORDS:** Microorganisms, Metabolic Pathway, Renewable energy, Clean environment.

**INTRODUCTION**

A metabolic pathway is any sequence of events or interactions between genes and the products they produce that leads to the creation or modification of a system component that is necessary for the proper operation of a biological system.<sup>[1]</sup> Thomas Carr believes there is much to be learned by considering our current energy crisis in that context. It is interesting to note that the word energy is synonymous with the Greek word for challenge. Global energy consumption is increasing faster than renewable energy sources can keep up with this demand. Oil and other natural resources will soon run out, so if we want to extend the life of the oil that we now have, we must make changes to our way of life. As a result, there is a growing global push for all kinds of innovations and ideas aimed at creating cleaner, more effective means of producing energy. The process of producing power through microorganisms. It was Potter who published the first paper nearly a century ago demonstrating that bacteria could produce electricity.<sup>[2]</sup>

In place of the conventional power sources like steam, wind, solar, or water, microorganisms can produce electricity. For almost a century, researchers have been examining the capacity of microbes, the tiniest living creatures on Earth, to generate energy for purposes other than their own survival. The term bioelectrochemical system refers to this conversion process. The ability of microbes, including bacteria, to generate electricity is demonstrated in this article, suggesting that they may one day serve as a renewable energy source. Microbial fuel cells, or MFCs, are a type of bio electrochemical system that produces electricity. Anode chambers, or negative electrodes, and cathode chambers, or positive electrodes, are typically seen in this setup. Batteries and MFC function similarly. The anode chamber's microorganisms break down organic and inorganic materials, or substrates, to produce electricity.

To create electricity, these electrons move from the anode to the cathode via an external circuit composed of

conductive materials, like copper wires. Additionally, nearly half of the photosynthesis that produces more oxygen and less carbon dioxide on our planet is carried out by microorganisms.<sup>[3]</sup> While preventing the spread of disease is crucial for maintaining the health of individuals and communities, students also need to learn about the advantages that microorganisms and microbial communities offer. Numerous websites concentrate on microbes and illness. When conducting this review, we looked for tools that instructors could utilize to enable biology majors and beginners both comprehend the advantages that bacteria offer.

## MATERIALS AND METHODS

**Major metabolic pathways of microorganism for biofuel productions:** Many chemicals and fuels are currently produced from fossil fuels, which will cause a worldwide decrease in fossil materials and many environmental problems. The emergence of metabolic engineering in the early 1990s provided ideas to solve the problems. Several studies have reported bioethanol, biodiesel, and other valuable petrochemicals, that is, butanol, isopentanol, terpenes, etc., production from lignocellulosic biomass (LCB). Its feasibility has been hampered by the Inherent recalcitrance of the biomass.<sup>[5-7]</sup>

**Pyruvate and derivatives:** Pyruvate and derivatives are widely utilized in food, pharmaceuticals, pesticides, feed additives, and bioenergy industries. A number of endogenous metabolic processes, including as amino acid, fatty acid, glycolysis, and the TCA cycle, are centered on pyruvate. The utilization of fossil fuels

results in environmental changes because of greenhouse gas emissions, which has an adverse effect on the socio-political and economic stability of particular nations and areas.<sup>[8]</sup>

As a renewable energy source, biomass can be used in place of fossil fuels. For the purpose of sustaining their metabolism and existence, microorganisms generate energy from biomass, or materials found in their surroundings. They can absorb greenhouse gasses and purify the atmosphere in this way. Blue-green algae or cyanobacteria, as well as many other specialized microorganisms with remarkable adaptability, are particularly suited. Some microorganisms, such as *E. Coli* and *Klebsiella*, are also acceptable.<sup>[8]</sup>

Producing Power with *Shewanella Oneidensis* MR-1 Lactate Utilization in Microbial Fuel Cells A promising technique for directly harnessing microbes to produce electricity from organic materials is the microbial fuel cell (MFC). The electrical performance of MFCs is influenced by the kind of electron donors supplied into them, and optimizing MFC performance requires a mechanistic knowledge of these effects. In this work, they examined the function of formate in the production of electricity and the associated microbial metabolism using a model organism in MFCs, *Shewanella oneidensis* MR-1, and <sup>13</sup>C pathway analysis. According to findings, formate and lactate work together to generate energy, and adding more formate to the initial lactate produced more power than utilizing either substance alone as an electron donor.

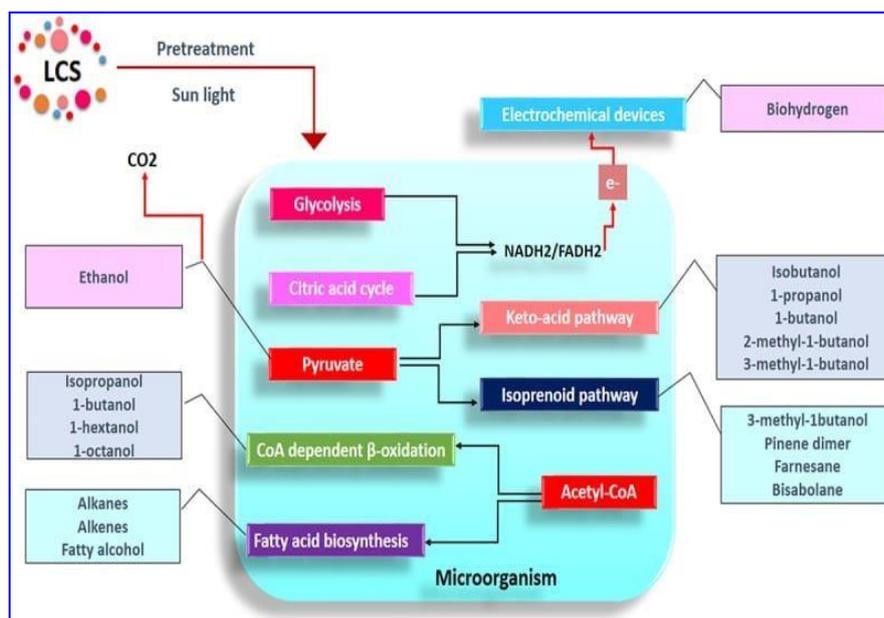


Fig. 1: Butanol as substitute for gasoline to alleviate pressure of petroleum resources.<sup>[4]</sup>

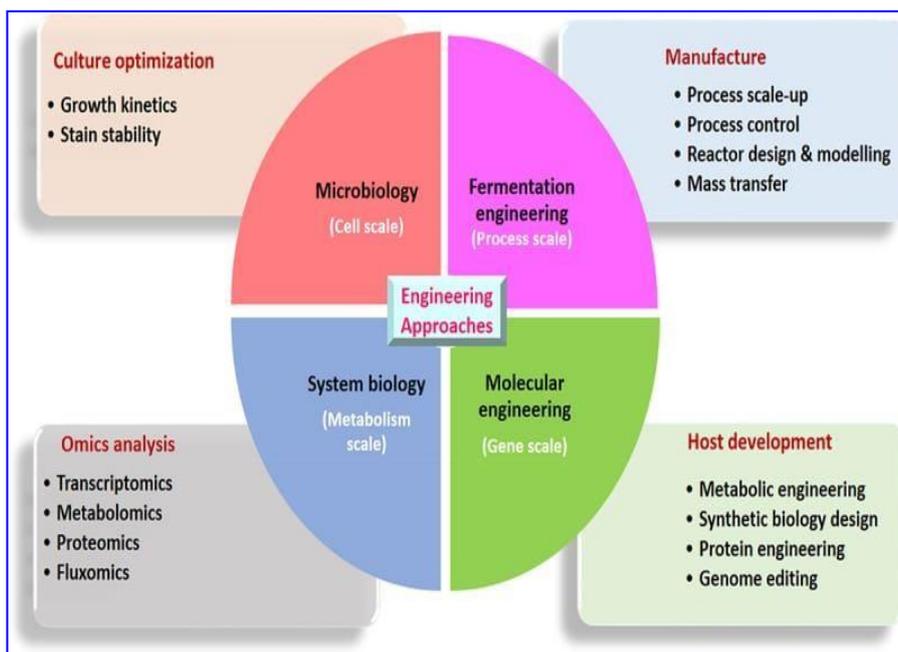


Fig. 2: Micro organisms engineering approaches for energy production.<sup>[4]</sup>

They observed decoupled cell growth and electricity generation during co-utilization of lactate and formate in *S. oneidensis* MR-1 based on the <sup>13</sup>C tracer analysis (i.e., while the lactate was mainly metabolized to support the cell growth, the formate was oxidized to release electrons for higher electricity generation). To the best of our knowledge, 13C tracer analysis has never been used to investigate microbial metabolism in MFCs, but it has proven to be an effective technique for comprehending how electron donors alter the metabolic pathways in the chosen electro chemically active bacteria. Potter published the first study on bacteria’s ability to produce electricity nearly a century ago, but at the time, his findings received little national attention.<sup>[9]</sup>

This power of bacteria has only been rediscovered in recent years. As previously noted, the demand for alternative energy sources, a deeper comprehension of the microbial system in connection to electron transport, and eventually the creation of microbial fuel cells are the driving forces behind this newfound interest. Using a microbe as a catalyst, a Microbial Fuel Cell (MFC) may produce power directly from a wide range of organic or inorganic chemicals. Traditionally, fuel cells use an oxidant at the cathode and a fuel at the anode to convert chemical energy to electrical energy.<sup>[10]</sup> Through the use of an external circuit, the released protons and electrons create electricity. An ion exchange membrane separates the anode and cathode in MFCs, and a mixture of microorganisms and organic materials is employed.<sup>[11]</sup>

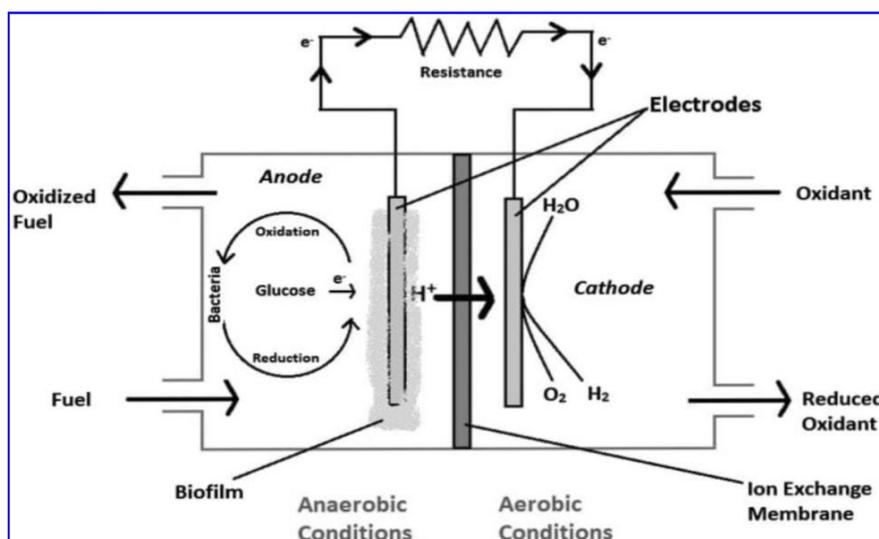


Fig. 3: Microbial fuel cell structure and working.<sup>[10]</sup>

A schematic representation of the MFC.<sup>[10]</sup> The cathode is exposed to air on one side, and the solution containing

the biodegradable substrate is present on the other side. The anode chamber containing the bacteria is sealed off

from oxygen. Biochemical treatment systems are being extensively studied for their potential to produce electricity directly. Microbial fuel cell (MFC) devices, on the other hand, use biodegradable raw materials, including wastewater, to convert chemical energy into electricity without the need for the Carnot cycle.<sup>[12,13]</sup> The possibilities of producing electricity directly through biochemical treatment systems are also being studied very closely. Moreover, the MFC device has been shown to contain possibly electrogenic bacteria.<sup>[14]</sup> These microorganisms consume organic substances and electrons onto the electrode, which causes electricity to

produced.<sup>[14]</sup>

**Synthesis and Metabolism of pyruvate:** The essential microbial metabolic pathways, enzymes, cofactors (e.g., NAD<sup>+</sup> and NADH) and energy transfer compounds (e.g., ATP and ADP) are involved in pyruvate formation and consumption. Pyruvate is synthesized from glucose through the glycolytic pathway. Subsequently, pyruvate enters the TCA cycle via acetyl-CoA and converts to various chemicals such as lactate, alanine,  $\alpha$ -acetolactate, and acetate.<sup>[15]</sup>

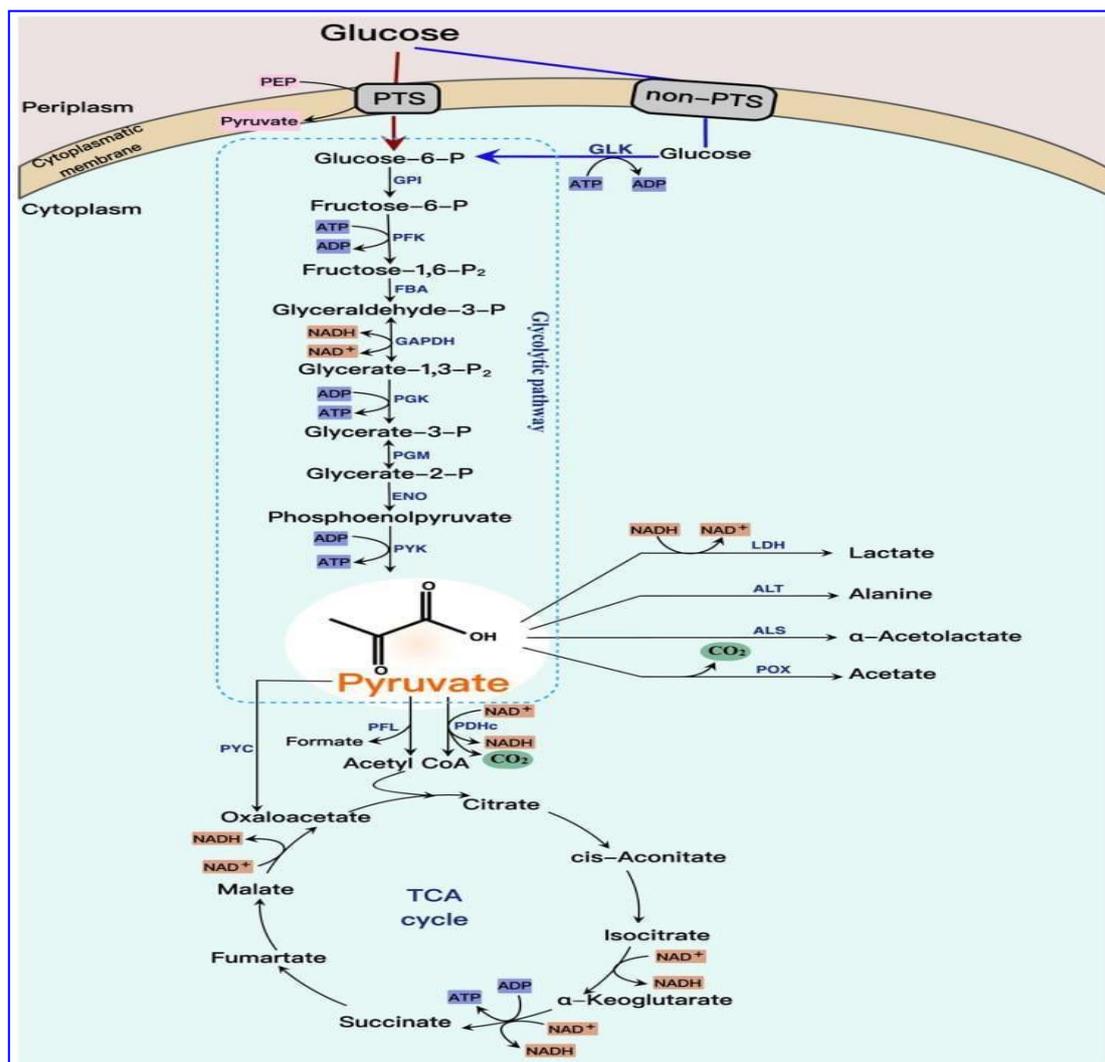


Fig. 4: Synthesis and Metabolism of pyruvate.

The maximal theoretical yield of pyruvate from glucose is 0.966 g/g, as indicated by the following equation, which describes the biochemical process of pyruvate generation from glucose through the glycolytic pathway:  $\text{glucose} + 2 \text{NAD}^+ + 2 \text{Pi} + 2 \text{ADP} \rightarrow 2 \text{pyruvate} + 2 \text{NADH} + 2 \text{ATP}$ .

Through the cyclic regeneration of cofactors, NADH/NAD<sup>+</sup> and ATP/ADP influence the cell's

metabolic network while taking part in enzymatic reactions as products or substrates. Pyruvate is created by the glycolytic process, which yields two mol ATP and two mol NADH. Moreover, under aerobic conditions, pyruvate enters the TCA cycle through acetyl-CoA to create 1 mol ATP and 3 mol NADH. Through NADH-dependent dehydrogenase, NADH enters the lactate and ethanol pathways for NAD<sup>+</sup> regeneration or generates ATP through oxidative phosphorylation (Figure 5)

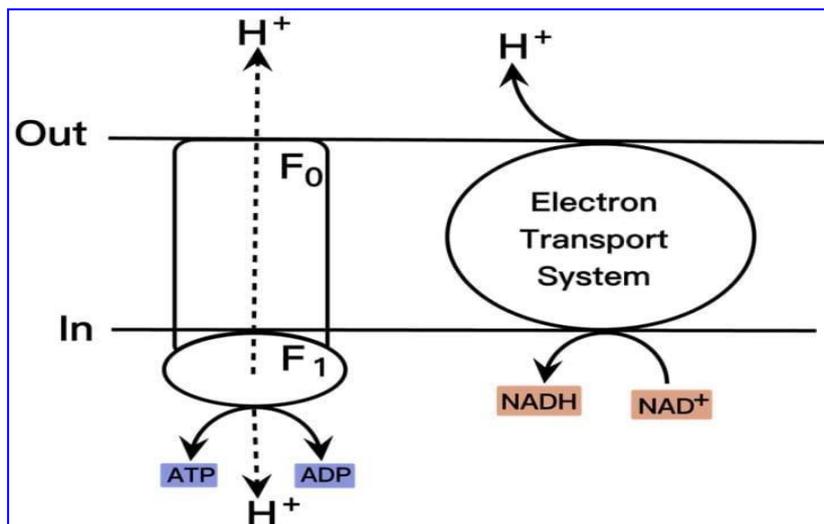


Fig. 5: Oxidative phosphorylation of NADH in microorganisms.

Utilizing Metabolic Engineering to Produce Compounds Derived from Pyruvate. 2,3-BD with Acetoin The production of 2,3-BD and acetoin follows two main routes (Figure 3). One approach is that ALS condenses two pyruvate molecules to  $\alpha$ -acetolactate, which  $\alpha$ -acetolactate decarboxylase (ALDC) then breaks down into acetoin.

The essential microbial metabolic pathways, enzymes, and cofactors (e.g., NAD<sup>+</sup> and NADH) are involved in acetoin and 2,3-BD formation and consumption. The reversible transformation between acetoin and 2,3-BD is associated with the cellular NADH/NAD<sup>+</sup> ratio balance.<sup>[16]</sup> Cofactors are closely related to the 2,3-BD pathway. The production of acetoin and 2,3-BD could be improved by regulating the ratio of NADH/NAD<sup>+</sup>.<sup>[17,18]</sup>

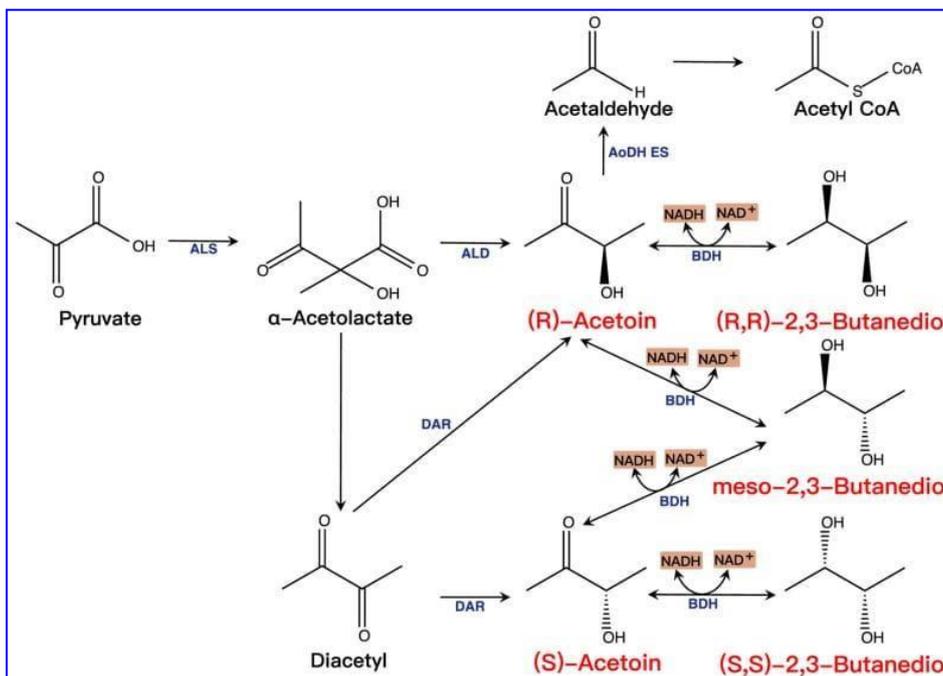


Fig. 6: Synthesis and Metabolism of acetoin and 2,3-BD.

## DISCUSSION

**Synthesis of Butanol and Butyrate:** The essential microbial metabolic pathways, enzymes, cofactors (e.g., NADH), and energy transfer compounds (e.g., ATP) are involved in butanol and butyrate formation. Green is the butanol synthesis pathway in the solvent-producing phases. Yellow is the butyrate synthesis pathway in the acid-producing phase. Butanol is generated in the solvent-

producing phases. Enhancing the synthesis pathway and reducing by-products are fundamental to increasing butanol yield. On the one hand, overexpression of crucial enzymes of the synthesis pathway could increase butanol yield.<sup>[19]</sup>

**L-Alanine:** Most microbes use ALT to convert L-alanine, which is primarily produced for cellular

biosynthesis (Figure 6). However, due to the low amounts of L-alanine accumulation and the presence of the DL-alanine form, there is no practical purpose for a strain to manufacture L-alanine utilizing ALT. Certain organisms, such as *Bacillus sphaericus*, *Lysinibacillus*

*sphaericus*, *Arthrobacter oxydans*, and *Clostridium sp.*, can accumulate L-alanine in response to L-alanine dehydrogenase (ALD), which is then converted to D-alanine by alanine racemase (ALR).<sup>[20,21,22,23,24]</sup>

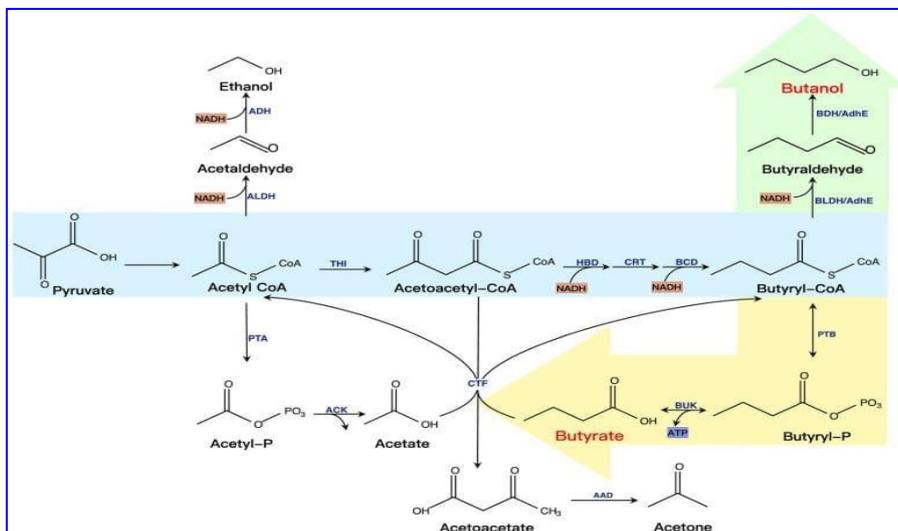


Fig. 7: Synthesis of butanol and butyrate.

**L-alanine synthesis by (A) alanine transaminase (ALT) and (B) L-alanine dehydrogenase (ALD):** The creation of byproducts during the fermentative synthesis of L-alanine presents another difficulty. Genes involved in competing for metabolic pathways were deleted to develop strains that could fulfill the challenge.<sup>[25]</sup> A two-stage batch fermentation procedure yielded 32 g/L of L-alanine in the *E. coli* mutant strain following the deletion of the *aceF* and *ldhA* genes. In addition, the *E. coli*

mutants lacked the pyruvate-formate lyase, phosphoenolpyruvate synthase, POX, LDH, and parts of the PDHc. The strain that was obtained yielded 88 g/L L-alanine, which is about the theoretical maximum.<sup>[26]</sup> *C. glutamicum* was also treated using a similar approach. In *C. glutamicum*, the genes for organic acid production and ALR were eliminated, leading to a carbon flow from organic acid to L-alanine and the production of 98 g/L L-alanine.<sup>[27]</sup>

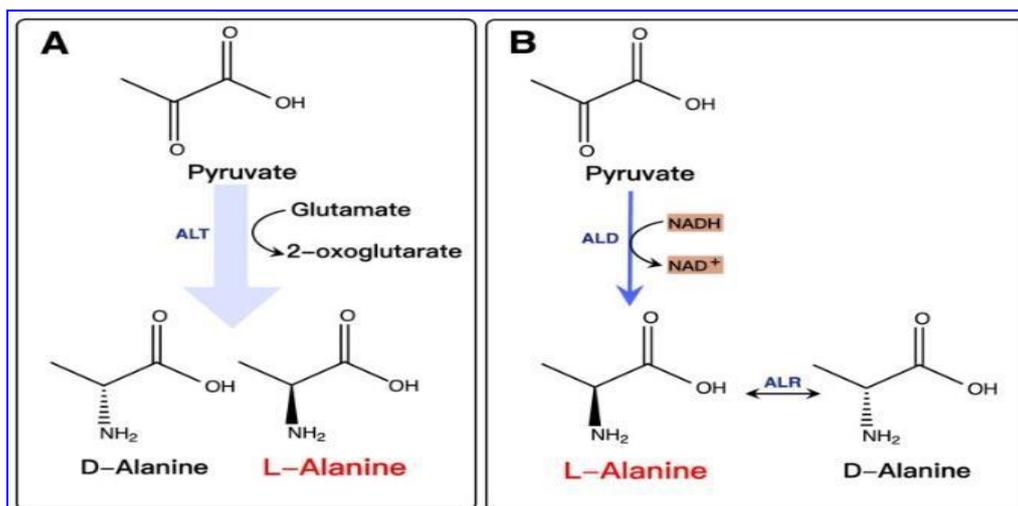


Fig. 8: L-alanine synthesis by alanine transaminase (ALT) and alanine dehydrogenase (ALD).

**Biofuel production:** The basic feature of life is oxidoreduction, which creates energy from matter.<sup>[28]</sup> Thus, microorganisms are undoubtedly crucial in developing waste purification and use strategies.<sup>[29]</sup> Bioenergy research is the center of scientific and technological research in the strategy of finding cost-

effective biorefineries.<sup>[30]</sup> The main reason for increased interest in biomass as an energy source is application to sustainable development paradigm. In addition, biomass sources are often present at local level, and conversion into biofuel is possible with low initial costs.<sup>[31]</sup>

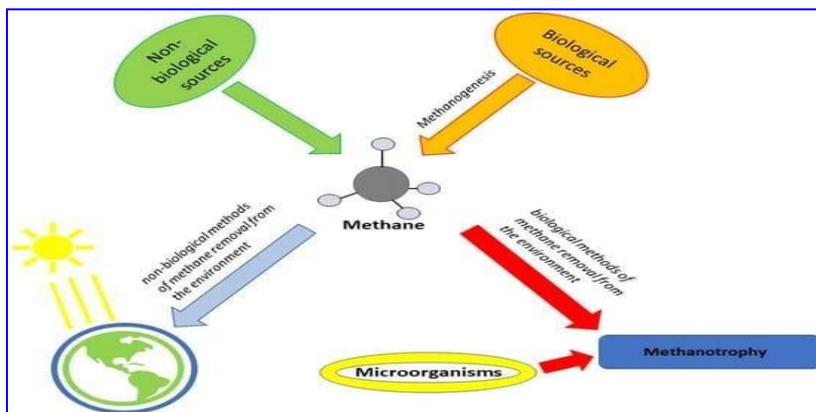


Fig. 9: Methane Formation and Decomposition via biological and non-biological means.

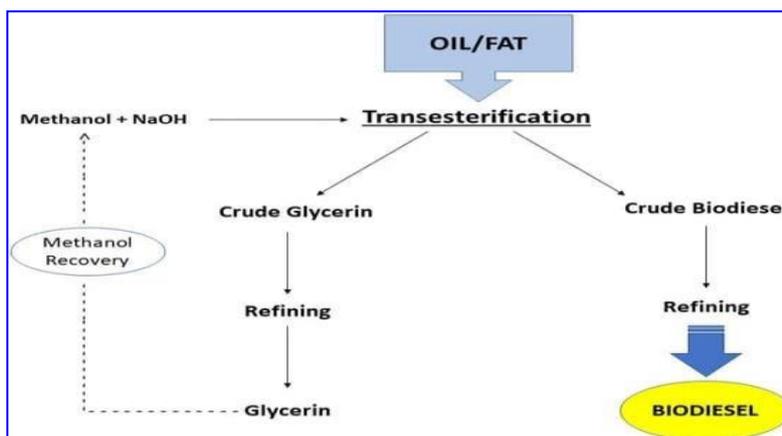


Fig. 10: Biodiesel production pathway.

The possibilities of direct electricity production by means of biochemical treatment systems are also being studied very intensively, and microbial fuel cell (MFC) devices

convert chemical energy into electricity (without the Carnot cycle) from biodegradable raw materials and even from wastewater.<sup>[32,33]</sup>

**Bioenergy carrying Molecules and Metabolic pathways**

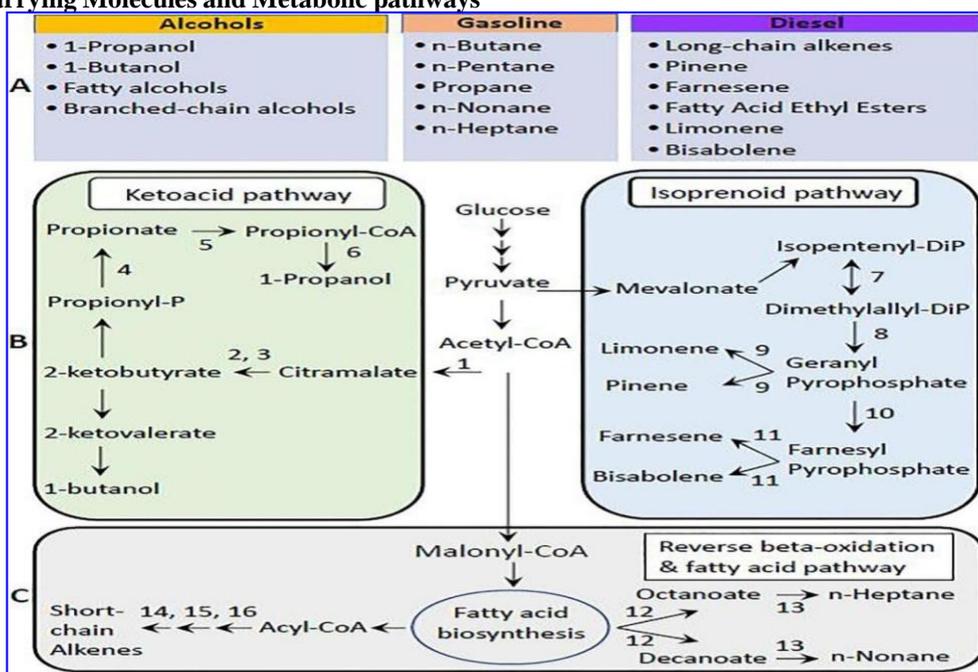


Fig. 11: Main bioenergy carrying molecules and metabolic pathways generated in biosynthetic fuels.

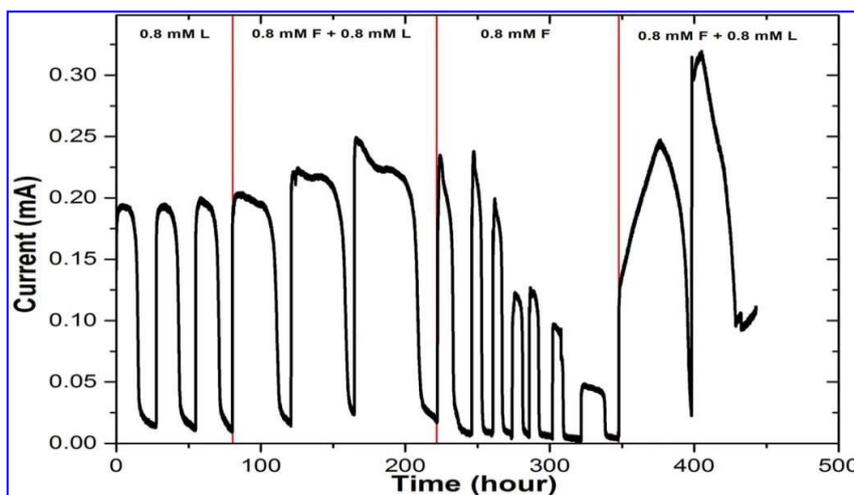
A. Molecules according to the fuel category; orange-alcohols; pink-gasoline, and purple-diesel. B. Metabolic pathways involved in the generation of the above molecules. C. Reversed  $\beta$ -oxidation and fatty acid synthesis for the production of branched-chain alcohols, long-chain alkanes, and fatty acid methyl esters.<sup>[34,35,36]</sup>

Abbreviations for enzymes : 1, citramalate synthase; 2, 3-isopropylmalate isomerase; 3, 3-isopropylmalate dehydrogenase; 4, acetate kinase; 5, acetyl-CoA:acetoacetyl-CoA transferase; 6, alcohol dehydrogenase; 7, isopentenyl pyrophosphate isomerase; 8, geranyl pyrophosphate synthase; 9, monoterpene synthases; 10, farnesyl pyrophosphate synthase; 11, sesquiterpene synthases; 12, carboxylic acid reductase; 13, a fatty aldehyde decarboxylase; 14, acyl-ACP thioesterase I; 15, acyl-CoA synthetase and 16, aldehyde deformylating oxygenase (or aldehyde decarboxylase).

As an example, certain strains of *Clostridium*, such as *Clostridium acetobutylicum*, have been shown to naturally create 1-butanol through the acetone-butanol-ethanol fermentation route.<sup>[37]</sup> The three primary metabolic pathways utilized in the design and comprehension of: A) The kind of biofuel generated; B) The primary pathway for producing branched-chain alcohols is 2- ketoacid; C) Reversed  $\beta$ -oxidation and fatty acid synthesis for producing branched-chain

alcohols, short- and long-chain alkanes, and fatty acid methyl esters; D) Isoprenoid pathways for producing branched and cyclic hydrocarbons.<sup>[38,39,40]</sup>

**Electrochemically active bacteria:** Under anaerobic circumstances, electrochemically active bacteria (EAB) function as efficient microbial catalysts to move electrons from substrates to solid electron acceptors (such as an anode electrode).<sup>[41,42,43,44]</sup> One of the most crucial phases in the production of electricity by MFCs is the transfer of electrons from the EAB to the electrode. This process is greatly impacted by the kind of electron donors used, which can alter the anode electrode's microbial composition, structure, and metabolism<sup>[45,46,47]</sup> To increase the effectiveness of energy recovery, it is crucial to comprehend how electron donors affect the generation of electricity in MFCs.<sup>[48,49,50]</sup> Coulombic efficiency (CE) and Coulombic recovery (CR) are quantitative indicators that display the fraction of collected electrons through EET vs total ideal electron production, which is used to evaluate the efficiency of electricity generation.<sup>[51,52,53]</sup> The purpose of this work is to look into the function of formate in *S. oneidensis* MR-1's catabolic metabolism and power production in MFCs. We specifically used the <sup>13</sup>C tracer experiment, a technique that is frequently employed to help metabolic rewiring in a variety of non-model environmental microbes.<sup>[54,55,56]</sup>



**Fig. 12:** From: <sup>13</sup>C Pathway Analysis for the Role of Formate in Electricity Generation by *Shewanella Oneidensis* MR-1 Using Lactate in Microbial Fuel Cells. Current generation in the MFC supplied with various electron donors.

Note: "L" means lactate; "F" means formate; "(F + L)" means addition of both substrate together; "0.8 mM" means 0.8 mM of each substrate added each cycle. Current generation in the MFC supplied with various electron donors. Note: L means lactate; F means formate; (F + L) means addition of both substrate together; 0.8 mM means 0.8 mM of each substrate added each cycle.

Electricity generation in the MFC clearly competed for electron donors and the comparable CR (Coulombic recovery) with other MFC studies of *S. oneidensis* MR-1

indicates that the anode electrode has strong ability to compete with oxygen for the electron donors to collect electrons.<sup>[57,58]</sup>

#### CONCLUSION AND FUTURE PROSPECTIVE

The hardest part of creating biofuels with "microbial factories" is producing a lot of fuel with a lot less money and a lot more efficiency than with traditional fossil fuels. To put it another way, bioethanol should be less expensive than gasoline when it comes to replacing it, albeit this could be a very difficult undertaking in terms

of reaching the daily requirement (quantity). For instance, almost 19 million barrels of gasoline are consumed daily in the United States; producing this quantity on an industrial scale could be difficult. Thus, future output of microbial biofuel should be given priority in order to raise its acceptability.

The goal of this review was to summarize the developments in the field of pyruvate and its related molecules through metabolic engineering. Provides outlines production techniques for pyruvate and its derivatives. The microbial synthesis of these chemicals has a lengthy history, as we discovered. Usually, these microbes create artificial metabolic pathways that result in the production of particular products. In the past, the primary method used by microbes to create synthetic metabolic pathways to increase output was gene overexpression or deletion. On the other hand, issues like cell growth inhibition and metabolic flow may arise due to incorrect gene expression or specific metabolic abnormalities. In order to better understand their effects on sustainability, exhaust gas emissions, and compatibility with both current and future modes of transportation and energy production, we have covered the most recent developments in microbial fuel development and fuel design in this study. Numerous investigations have looked into the possibility of using microbes to transform trash and biogenic residue into fuels and chemicals that convey energy.

Microorganisms cultured on various available organic substrates can produce advanced microbial biofuels, which are renewable energy sources. These substrates come from organic wastes and affordable, sustainable feedstock. In order to manufacture new fuels or achieve high product yields, the capacity to manipulate or modify the native biofuel producing metabolic pathways is a fundamental challenge in converting feedstocks into advanced biofuels using native hosts using biocatalysts and genetically tractable microorganisms that can be induced to manufacture desired fuels from a range of feedstocks is essential. Advanced biofuel production and use have been improved by fuel design and microbial metabolic engineering. Although advancements are still in their infancy, new metabolic pathways have been found and employed for synthesis of metabolites.

The amounts of fuels and energy molecules produced are still quite minimal for the majority of metabolic processes. However, the synthesis of microbial fuel molecules can be improved, with the potential to surpass fossil fuels in terms of clean and efficient combustion, with the advent of CRISPR genome editing technologies and advances in synthetic and systems biology. The increasing need to discover new renewable energy sources that could displace gas and oil and lessen adverse effects on the ecology has prompted a plethora of studies. Scientists are therefore increasingly looking to microorganism-based biofuels. By employing a sufficient number of microorganisms that are present in

our immediate surroundings, civilization can be preserved and even thrive. Microorganisms are prepared to guide us into new human-environment partnerships in this way, acting as our companions in a future that is safer and more assured.

## REFERENCES

1. Encyclopedia of Bioinformatics and Computational Biology, 2019.
2. Potter MC. Electrical effects accompanying the decomposition of organic compounds. *Proc R Soc Lond B*, 1911; 84: 260–276. Doi: 10.1098/rspb.1911.0073.
3. Research publication by Professor Bruce Ernest Logan et al from Pennsylvania State University in, 2006. <https://theconversation.com/this-is-how-microorganisms-can-produce-renewable-energy-for-us-149933#:~:text=We%20can%20generate,University%20in%202006>
4. Veza I., Said M.F.M., Latiff Z.A. Recent advances in butanol production by acetone-butanol- ethanol (ABE) fermentation. *Biomass Bioenergy*, 2021; 144: 105919.
5. Sharma V, Joshi A, Ramawat KG, et al. Bioethanol production from halophytes of thar desert: a “green gold. In: Basu SK, Zandi P, Chalaras SK, editors. *Environment at crossroads: Challenges, dynamics and Solutions*. Guilan Prov., Iran: Haghshenass Publishing, 2017; 219–235.
6. Vu HP, Nguyen LN, Vu MT, et al. A comprehensive review on the framework to valorise lignocellulosic biomass as biorefinery feedstocks. *Sci Total Environ*, 2020; 743: 140630.
7. Joshi A, Kanthaliya B, Arora J. Halophytes: The nonconventional crops as source of biofuel production. In: Grigore MN, editor. *Handbook of halophytes: From molecules to ecosystems towards biosaline agriculture*, 2020; 1–28.
8. <sup>13</sup>C Pathway Analysis for the Role of Formate in Electricity Generation by *Shewanella Oneidensis* MR-1 Using Lactate in Microbial Fuel Cells, 2016; 6: 56-69.
9. Ghangrekar MM, Shinde VB. Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. *Bioresour Technol*, 2007; 98(15): 2879–2885.
10. Pham TH, Rabaey K, Aelsterman P, Clauwaert P, De Schampelaire L, Boon N, Vestraete W. Microbial fuel cells in relation to conventional anaerobic digestion technology. *Eng Life Sci*, 2006; 6: 285–292.
11. Logan BE, Regan JM. Feature article: microbial fuel cells—challenges and applications. *Environ Sci Technol*, 2006; 40(17): 5172–5180.
12. Moradian, J.M.; Fang, Z.; Yong, Y.-C. Recent advances on biomass-fueled microbial fuel cell. *Bioresour. Bioprocess*, 2021; 8: 1–13.
13. Pandit, S.; Savla, N.; Sonawane, J.M.; Sani, A.M.; Gupta, P.K.; Mathuriya, A.S.; Rai, A.K.; Jadhav,

- D.A.; Jung, S.P.; Prasad, R. Agricultural Waste and Wastewater as Feedstock for Bioelectricity Generation Using Microbial Fuel Cells: Recent Advances. *Fermentation*, 2021; 7: 169.
14. Taylor-Cornejo, E. Empowering Undergraduates to Fight Climate Change with Soil Microbes. *DNA Cell Biol*, 2022; 41: 58–63.
  15. Maleki N., Eiteman M.A. Recent progress in the microbial production of pyruvic acid. *Fermentation*, 2017; 3: 8-14.
  16. Magee R.J., Kosaric N. *Advances in Applied Microbiology*. Elsevier; Amsterdam, The Netherlands: The microbial production of 2, 3-butanediol, 1987; 32: 89–161.
  17. Lu P., Gao T., Bai R., Yang J., Xu Y., Chu W., Jiang K., Zhang J., Xu F., Zhao H. Regulation of carbon flux and NADH/NAD<sup>+</sup> supply to enhance 2, 3-butanediol production in *Enterobacter aerogenes*. *J. Biotechnol*, 2022; 358: 67–75.
  18. Ji X.-J., Huang H., Zhu J.-G., Ren L.-J., Nie Z.-K., Du J., Li S. Engineering *Klebsiella oxytoca* for efficient 2, 3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. *Appl. Microbiol. Biotechnol*, 2010; 85: 1751–1758.
  19. Bao T., Hou W., Wu X., Lu L., Zhang X., Yang S.T. Engineering *Clostridium cellulovorans* for highly selective n-butanol production from cellulose in consolidated bioprocessing. *Biotechnol. Bioeng*, 2021; 118: 2703–2718.
  20. Jojima T., Fujii M., Mori E., Inui M., Yukawa H. Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid L-alanine under oxygen deprivation. *Appl. Microbiol. Biotechnol*, 2010; 87: 159–165.
  21. Hashimoto S.-I., Katsumata R. L-alanine fermentation by an alanine racemase-deficient mutant of the DL-alanine hyperproducing bacterium *Arthrobacter oxydans* HAP-1. *J. Ferment. Bioeng*, 1998; 86: 385–390.
  22. Zhang X., Jantama K., Moore J.C., Shanmugam K.T., Ingram L.O. Production of L-alanine by metabolically engineered *Escherichia coli*. *Appl. Microbiol. Biotechnol*, 2007; 77: 355–366.
  23. Ohashima T., Soda K. Purification and properties of alanine dehydrogenase from *Bacillus sphaericus*. *Eur. J. Biochem*, 1979; 100: 29–30.
  24. Orlygsson J., Anderson R., Svensson B.H. Alanine as an end product during fermentation of monosaccharides by *Clostridium* strain P2. *Antonie Van Leeuwenhoek*, 1995; 68: 273–280.
  25. Lee M., Smith G., Eiteman M., Altman E. Aerobic production of alanine by *Escherichia coli* aceF ldhA mutants expressing the *Bacillus sphaericus* alaD gene. *Appl. Microbiol. Biotechnol*, 2004; 65: 56–60.
  26. Smith G.M., Lee S.A., Reilly K.C., Eiteman M.A., Altman E. Fed-batch two-phase production of alanine by a metabolically engineered *Escherichia coli*. *Biotechnol. Lett*, 2006; 28: 1695–1700.
  27. Jojima T., Fujii M., Mori E., Inui M., Yukawa H. Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid L-alanine under oxygen deprivation. *Appl. Microbiol. Biotechnol*, 2010; 87: 159–165.
  28. Gupta, A.; Gupta, R.; Singh, R.L. *Microbes and Environment. Principles and Applications of Biotechnology for a Sustainable Future*, 2017; 43–84.
  29. Waldrop, M.M. News Feature: Microbes for better sewage treatment. *Proc. Natl. Acad. Sci. USA*, 2021; 118: e2112863118.
  30. Zetterholm, J.; Bryngemark, E.; Ahlström, J.; Söderholm, P.; Harvey, S.; Wetterlund, E. Economic Evaluation of Large-Scale Biorefinery Deployment: A Framework Integrating Dynamic Biomass Market and Techno-Economic Models. *Sustainability*, 2020; 12: 7126.
  31. Rather, R.A.; Wani, A.W.; Mumtaz, S.; Padder, S.A.; Khan, A.H.; Almohana, A.I.; Almojil, S.F.; Alam, S.S.; Baba, T.R. Bioenergy: A foundation to environmental sustainability in a changing global climate scenario. *J. King Saud. Univ. Sci*, 2022; 34: 101734.
  32. Moradian, J.M.; Fang, Z.; Yong, Y.-C. Recent advances on biomass-fueled microbial fuel cell. *Bioresour. Bioprocess*, 2021; 8: 1–13.
  33. Pandit, S.; Savla, N.; Sonawane, J.M.; Sani, A.M.; Gupta, P.K.; Mathuriya, A.S.; Rai, A.K.; Jadhav, D.A.; Jung, S.P.; Prasad, R. Agricultural Waste and Wastewater as Feedstock for Bioelectricity Generation Using Microbial Fuel Cells: Recent Advances. *Fermentation*, 2021; 7: 169.
  34. P.P. Peralta-Yahya, J.D. Keasling Advanced biofuel production in microbes *Biotechnol J*, 2010; 5: 147-162.
  35. E.J. Steen, R. Chan, N. Prasad, S. Myers, C.J. Petzold, A. Redding, et al. Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol *Microb Cell Fact*, 2008; 7: 36.
  36. F. Zhang, S. Rodriguez, J.D. Keasling Metabolic engineering of microbial pathways for advanced biofuels production *Curr Opin Biotechnol*, 2011; 22: 775-783.
  37. S.A. Angermayr, A.D. Van der Woude, D. Correddu, A. Vreugdenhil, V. Verrone, K.J. Hellingwerf. Exploring metabolic engineering design principles for the photosynthetic production of lactic acid by *Synechocystis* sp. PCC6803. *Biotechnology for biofuels*, 2014; 7: 99.
  38. H. Liu, J. Zhang, J. Yuan, X. Jiang, L. Jiang, G. Zhao, et al. Omics-based analyses revealed metabolic responses of *Clostridium acetobutylicum* to lignocellulose-derived inhibitors furfural, formic acid and phenol stress for butanol fermentation *Biotechnol Biofuels*, 2019; 12: 1-20.
  39. Cheon, H.M. Kim, M. Gustavsson, S.Y. Lee Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels *Curr Opin Chem Biol*, 2016; 35: 10-21.
  40. E.J. Steen, R. Chan, N. Prasad, S. Myers, C.J.

- Petzold, A. Redding, et al. Metabolic engineering of *Saccharomyces cerevisiae* for the production of n-butanol *Microb Cell Fact*, 2008; 7: 36-42.
41. Phuc Thi Ha, Beomseok Tae & Chang, I. S. Performance and Bacterial Consortium of Microbial Fuel Cell Fed with Formate. *Energy Fuels*, 2008; 22: 164–168.
  42. Li, W., Yu, H. & He, Z. Towards sustainable wastewater treatment by using microbial fuel cell-centered technologies. *Energy Environ. Sci*, 2013; 7: 911–924.
  43. Rosenbaum, M. A. & Henrich, A. W. Engineering microbial electrocatalysis for chemical and fuel production. *Curr. Opin. Biotechnol*, 2014; 29: 93–98.
  44. Pant, D. et al. Bioelectrochemical systems (BES) for sustainable energy production and product recovery from organic wastes and industrial wastewaters. *RSC Adv*, 2012; 2: 1248–1263.
  45. He, Z. Microbial fuel cells: now let us talk about energy. *Environ Sci Technol*, 2013; 47: 332–333.
  46. Schroder, U. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. *Phys. Chem. Chem. Phys*, 2007; 9: 2619–2629.
  47. Dhere, N. G. et al. Application of acetate, lactate and fumarate as electron donors in microbial fuel cell, 2013; 8825: 1–7.
  48. Lovley, D. R. The microbe electric: conversion of organic matter to electricity. *Curr. Opin. Biotechnol*, 2008; 19: 564–57.
  49. Clauwaert, P. et al. Minimizing losses in bioelectrochemical systems: the road to applications. *Appl. Microbiol. Biotechnol*, 2008; 79: 901–913.
  50. Kim, B. H., Chang, I. S. & Gadd, G. M. Challenges in microbial fuel cell development and operation. *Appl. Microbiol. Biotechnol*. 2007; 76: 485–494.
  51. Qin, M., Molitor, H., Brazil, B., Novak, J. T. & He, Z. Recovery of nitrogen and water from landfill leachate by a microbial electrolysis cell-forward osmosis system. *Bioresour Technol*, 2015; 200: 485–492.
  52. Ge, Z., Ping, Q., Xiao, L. & He, Z. Reducing effluent discharge and recovering bioenergy in an osmotic microbial fuel cell treating domestic wastewater. *Desalination*, 2015; 312: 52–59.
  53. Ge, Z., Ping, Q. & He, Z. Hollow-fiber membrane bioelectrochemical reactor for waste water treatment. *J. Chem. Tech. Biotechnol*, 2013; 88: 1584–1590.
  54. Feng, X., Zhuang, W. Q., Colletti, P. & Tang, Y. J. Metabolic pathway determination and flux analysis in nonmodel microorganisms through <sup>13</sup>C-isotope labeling. *Methods Mol Biol*, 2012; 881: 309–330.
  55. Zhuang, L., Guo, W., Yoshida, M., Feng, X. Goodell, B. Investigating Oxalate Biosynthesis in Wood-decaying Fungus *Gloeophyllum trabeum* using <sup>13</sup>C Metabolic Flux Analysis. *RSC Advances*. In press, 2015.
  56. Y. J. et al. Invariability of central metabolic flux distribution in *Shewanella oneidensis* MR-1 under environmental or genetic perturbations. *Biotechnol. Progr*, 2009; 25: 1254–1259.
  57. Newton, G. J., Mori, S., Nakamura, R., Hashimoto, K. & Watanabe, K. Analyses of current-generating mechanisms of *Shewanella loihica* PV-4 and *Shewanella oneidensis* MR-1 in microbial fuel cells. *Appl Environ Microbiol*, 2009; 75: 7674–7681.
  58. Watson, V. J. & Logan, B. E. Power production in MFCs inoculated with *Shewanella oneidensis* MR-1 or mixed cultures. *Biotechnol. Bioeng*, 2010; 105: 489–498.