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REVIEW

Acetyl-CoA Pathway for Biosynthesis of Organics

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A great diversity of microorganisms have tendency to reduce numerous organic compounds and gases. Various acetogens have potential to produce valuable organic compounds by acquiring environmentally sustainable approaches. Acetogens like *Sporomusa ovata*, *Clostridium ljungdahlii*, *Clostridium aceticum*, *Moorella thermoacetica* and *Acetobacterium woodii* are attractive species for fixing waste greenhouse gases. Acetogens utilize acetyl Co-A pathway for acetate production with small amount of butanol and alcohols. Genetic mutations, metabolic engineering and bioelectrochemical synthesis can be adopted to divert the chemical reaction pathway apart from acetate production. In bioelectrochemical synthesis, electrodes material, electrodes surface areas, kind of biofilms, ion exchange membranes, internal resistances, *etc*. effect electron exchange between microorganisms and electron acceptors. Adapted strains provide an insight into the mechanisms of extracellular electron exchange. There's a requirement to modify the metabolic pathways of microorganisms by sequencing their genomes to obtain ethanol, isopropanol, *n*-butanol, *etc*. This review provides insight of natural and engineered methods for scavenging greenhouse gases using acetyl Co-A metabolic pathways adopted by acetogens. The unique approach is the critical discussion leading to the selection of acetyl Co-A pathway on the basis of its energy efficiency. The research on bioelectrochemical process, metabolic engineering and their applications are being focused to give a comprehensive review on the subject.

Keywords: Scavenging Greenhouse gases, Bio-electrode, Genetic mutation, Metabolic pathways, Biochemical synthesis.

INTRODUCTION

The global demand for energy has been increased due to its increased consumption rate. The increased energy demands can be met by utilizing existing renewable energy sources and exploring new ones. The energy rich substrates are currently being used as a source of bioenergy, biochemicals and biofuel. These substrates require agricultural land and food crops for the production of chemicals and biofuels instead of exploiting them for feeding extensively growing human population. It is also predicted that resources of fossil fuel are limited and are going to be eliminated in current century if are consumed at current rate. Therefore an innovative solution is required to device methods to obtain renewable chemicals or fuels.

Bio-electrochemical synthesis is a novel technique where anaerobes are able to convert greenhouse gases into valuable chemicals. Utilization of syngas addresses the challenges by converting atmospheric carbon dioxide to organics. The popular approach for biochemical production of chemicals is fermentation process which extract electrons from energy rich substrates *e.g*., sugar, CO_2, CO and H_2 to produce acetate, ethanol and other acids like butyric acid. The greenhouse gases are being fixed by using electrochemical or catalytic processes. This process requires high cost of electrodes, deactivation of electrodes in long run and inefficient synthesis of chemicals. Whereas in bioelectrochemical synthesis the bio-cathode development is cost effective. Microbial catalyzed electrochemical system a futuristic and promising technology for bioenergy generation as there is no additional supply of energy like other waste treatment processes. This system uses the potential of bacterial metabolism either for treatment processes or production of valuable bioproducts. Multiple and diverse applications of microbial active systems are microbial fuel cell (MFC for harnessing electricity), bioelectrochemical treatment (BET waste water treatment), microbial electrochemical synthesis (MES) and microbial electrolytic cell (MEC) for hydrogen production under low applied potential). The bio-catalyzed electrochemical system (BES) harnessing electrons by photovoltaic cells and electrode in the system are acting as an electron acceptor. Bio-catalyzed electrochemical system facilitates the direct conversion of chemical energy to valuable products in cathode chamber. Essential features of BES depend on electron transfer mechanism along with redox reactions in the absence of oxygen. H^+ ions migrate to cathode and get reduced to form

hydrogen and electrons from anode under the influence of applied voltage which is endothermic barrier to form hydrogen and other valuable bio products like ethanol, butanol, succinate and acetate[1]. Bio-electrosynthesis is a novel approach to develop new routes for the synthesis of fine chemicals. Electrochemically active microorganisms play a key role for the redox activity at electrodes. Microorganisms have ability to convert substrate to acetate are termed as acetogens. Acetogens exhibit tendency of CO_2 reduction to acetate through acetyl-CoA pathway. Acetate production is contributed by anaerobic bacteria like fermentative bacteria, syntrophic bacteria and homoacetogens. Fermentation process generates adenosine triphosphatehttp://en.wikipedia.org/wiki/Adenosine_triphosphate (ATP) by substrate-level phosphorylation in an anaerobic digestion. The trophic links between homoacetogens and syntrophic acetogens yields 29 % acetate and butyrate under suppressed methanogenesis conditions[2]. Autotrophic bacteria (Chemoautotroph and Photoautotroph) are able to utilize simple substances present in its surroundings, generally using energy from light (Photoautotroph) or inorganic chemical reactions (Chemoautotroph) and produces complex organic compounds.

This current review describes the potential of acetyl-CoA path way for the synthesis of various compounds utilizing simple inorganic or organic substances. The review of various studies for enhancing efficiency of acetyl-CoA path way in different bacterial strains by either metabolic engineering or bioelectrochemical process is presented. The applications of efficient acetyl-CoA path way for the synthesis of renewable chemicals have many advantages and are promising. This survey covers various aspects of acetogens, application of acetyl-CoA path way in fermentation and its improvement through bioelectrochemical process or genetic engineering.

Chemoautotroph: Chemoautotroph utilizes inorganic substances like carbon dioxide, carbon monoxide, water and hydrogen and converts them to carbohydrates. The bacteria which utilize inorganic compounds *e.g*., ammonia, nitrate, hydrogen sulfide, ferrous ions are known as chemolithoautotropic bacteria. Some of the chemolithoautotropic bacteria can grow not only autotrophically but also heterotrophically. These are known as facultative chemolithoautotropic bacteria.

Photoautotroph: Photoautotroph obtain energy from sunlight and turns light energy in the presence of inorganic substances to chemical energy Green plants and photosynthetic bacteria are Photoautotrophs[3].

Geologists suggest that life was emerged at hydrothermal vents in the absence of protein and metal sulphides catalyzed the bio chemical reactions. Biologists suggested that carbon dioxide fixation through acetyl-CoA pathway was very ancient. In early stages CO_2 from volcanoes and hydrothermal H_2 reaction catalyzed by metal sulphides provides hypothesis for primitive Acetyl-CoA where H_2 was electron donor and CO_2 was electron acceptor to synthesize the organic precursors to fuel. It proved to be first geochemical pathway[4]. The bioelectrochemical system is based on this primitive geochemical pathway where electron donors are electrodes instead of H_2 and greenhouse gas (CO or CO_2) as an electron acceptor.

Bio-electrosynthesis: Instead of deriving electrons by decomposing organic compounds bio-electrosynthesis is a process which can be called as an electro-synthesis where microorganisms drive electrons from electrodes for carbon dioxide reduction to extra cellular organics. Electrical energy is provided by solar energy cells or through potentiostat. In an artificial form of photosynthesis microbial electro synthetic cells are powered by solar energy. It eliminates the need of arable land and intensive agricultural activities[5]. The microorganism which exhibits tendency to derive electrons from an electrode is called electrotrophy where electrode is reducing the terminal electron acceptor. *Methanobacterium palustre* has ability to drive electrons from cathode and reduce carbon dioxide to methane, but low potential is required for methanogenesis which can produce significant hydrogen[6]. An autotrophic acetogenic microorganism *Sporomusa ovata* has the tendency to reduce carbon dioxide to acetate by capturing electron from graphite electrodes. Biofilm of *Sporomusa ovata* at cathode surface produces acetate along with small amount of 2-oxobutyrate. Other examples for autotrophic bacteria are *Clostridium ljungdahlii*, *Clostridium aceticum*, *Moorella thermoacetica* and *Acetobacterium woodii* which grow with H_2/CO_2 by using anaerobic conditions. In acetogens reduction of carbon dioxide to acetate utilize acetyl-CoA pathway which is acting as an intermediate for the production of a diversity of useful organic products. *Acetobacterium woodii* is unable to function at the cathode because of carbon dioxide reduction to organic acids which require protons and ATP produced is proton-dependent ATPase. *A. woodii* is unable to restore energy because it contains ATPase which is sodium-dependent[7,8]. Carbohydrates such as glucose, xylose, cellulose, fructose *etc.*, are acting as carbon and energy source by various acetogens. However, nonpathogenic bacteria like *Clostridium ragsdalei*, *Clostridium autoethanogenum*, *Clostridium ljungdahlii* can utilize waste green house gases as carbon and energy sources. The anaerobic acetogens have tendency to convert greenhouse gases to acetate, ethanol, acetone and butanol. Metabolic pathways and genetic mutations are helpful for the production of variety of organic solvents[9]. Examples of acetogenic bacteria are *Clostridium thermoautotrophicum*, *Clostridium ljungdahlii*, *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. Few acetogenic bacteria are listed in (Table-1) along with acetate, butyrate, ethanol and butanol production[10]. Current review is the study of Acetyl-CoA pathway along with the enzymes and co-enzymes for conversion of greenhouse gases to bio-organics.

Acetogens: Acetogens play a central role in carbon cycle and tons of acetate is produced in nature every year. Acetogens have tendency to grow on different substrates to yield valuable products. Apart from acetate synthesis different *Clostridium* species have potential for valuable biofuels like *Clostridium saccharoperbutylacetonicum* N1-4 produce butanol from rice bran hydrolyzates[21,22]. Butanol can also be produced from starch[23] corn and molasses and whey. A number of organisms are available including *Clostridium beijerinckii* P260, C. *beijerinckii* BA101, *Clostridium acetobutylicum* and *Clostridium saccharobutylicum* P262 for the bioconversion of maize, rye, molasses, potato, soya, dextrins, fructose, sucrose and lactose to acetone, butanol and ethanol[24]. The tendency to convert CO_2 and H_2 into acetic acid by acetogens is shown in (eqn. 1)[25] and conversion of glucose to acetate (eqn. 2)[26].

$$2CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3COO^- + 3H^+ \quad (2)$$

TABLE-1
PRODUCTION OF BIO-ORGANIC COMPOUNDS BY VARIOUS ACETOGENS

Organisms	Gas substrate (v/v) (%)	Fermentation time	Temperature (°C)	pH	Product
Clostridium aceticum	H_2/CO/Ar (4:78:18)	72 h	30	8.6	Acetate 2.2 g/L [Ref. 11]
Clostridium ljungdahlii	CO/CO_2/Ar /H_2 (55:10: 15:20)	560 h	36	4.50	Ethanol 48 g/L [Ref. 12]
Clostridium ljungdahlii	CO/CO_2/H_2/N_2 (20:20:10:50)	44 h	37	6.80	Acetate 3 g/L [Ref. 13]
Clostridium autoethanogenum	CO_2/CO (5:95)	60 h	37	4.7	Acetate 23 mmol ethano l259 mg/L [Ref. 14]
Butyribacterium methylotrophicum	100 % CO	NA	36	6.0	Acetate 7.9 mmol butyrate 5.9 g/L [Ref. 115]
Eubacterium limosum	/CO_2 /CO (20:80)	54 h	37	6.7	Acetate 0.75 mmol butyrate 0.001 mmol [Ref. 16]
Clostridium carboxidivorans	CO/N_2/CO_2 (25:60:15)	18 days	37	5.2	Ethanol 0.33 mol [Ref. 17]
Clostridium ragsdalei	CO/CO_2/N_2/H_2 (20:15:60:5)	15 days	32	6	Ethanol 1.8 g/L, Acetate 1.4 g/L [Ref. 18]
Butyribacterium methylotrophicum	CO_2/CO (30:70)	144 h	37	6.90	Acetic acid 1.3 g/L [Ref. 19]
Clostridium carboxidivorans	/N_2/CO/CO_2 (60:25:15)	30 days	37	5.8	Acetate 0.68 g/L, ethanol 0.56 g/L [Ref. 20]

Acetogens use variety of electron acceptors like nitrate, protons, CO_2, dimethylsulfoxide and fumarate as illustrated in Fig. 1[27]. Other Clostridium species like *Clostridium formicoaceticum* and *Clostridium magnum* are hydrogen-oxidizing autotrophic acetogens which can grow on H_2/CO_2. However, their growth rate is improved by medium containing yeast extract, trace elements and vitamins[28,29]. A list of vitamins and trace elements are essential for their growth[30]. Trace elements nickel, selenium, molybdenum, iron, tungsten and cobalt are all major constituent of enzymes. These enzymes can synthesize acetate from CO_2.

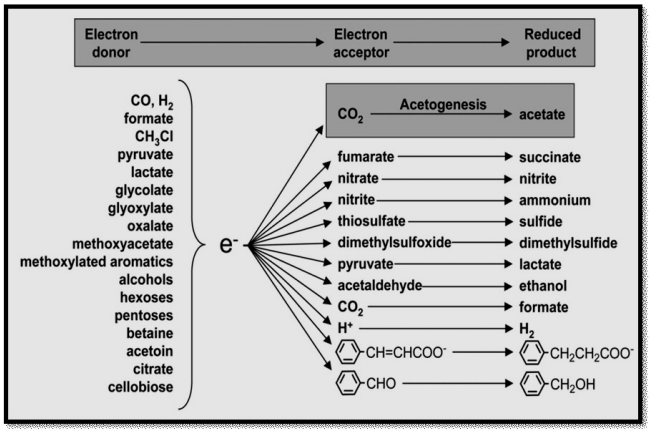

Fig. 1. Redox couples of acetogens

The fixation of CO_2 by prokaryotes and all plants is by reductive pentose phosphate (Calvin-Benson) cycle, but in prokaryotes five autotrophic pathways cannot be neglected[31]. The chemolithoautotrophic bacteria and archaea are considered among the first type of organisms on earth for carbon fixation. For the formation of organic compounds from CO_2 they use electron donors like (S^{2-}, H_2, NH_4) and electron acceptors (CO_2, O_2, SO_4). Energy yield depend on thermodynamics of these redox couples and bio-chemistry of the pathways used. In aerobic oxidized environment, thermodynamically more energy is required than anaerobic reducing habitats. So CO_2 fixation pathways are (reductive acetyl-CoA pathway, reductive tri-carboxylic acid cycle, rTCA and decarboxylate 4-hydroxy butyrate (DC/4-HB) cycle) which are more common in anaerobic microorganisms with oxygen sensitive enzymes. These cycles require quite less energy as compared to the pathways followed by aerobic microorganisms which are Calvin Benson Bassham (CBB) cycle, 3-hydroxy propionate (3-HP) bicycle and 3-hydroxy propionate/4-hydroxy butyrate (3-HP/4-HB) cycle[32]. Quantum requirement is another comparison for the efficiency among these cycles. It is the ratio of photons absorbed per CO_2 fixed. Anoxygenic photosynthesis requires 2 photons to reduce NAD^+ to NADH whereas oxygenic photosynthesis requires 4 photons to reduce $NADP^+$ to NADPH. Similarly oxygenic photosynthetic reactions absorb shorter wavelength of light so photon has high energy 176 KJ/mol as compared to anoxygenic photosynthetic reactions which absorb longer wavelength so energy is 162 kJ/mol. All these comparisons depict overall energy demands in aerobic and anaerobic microorganisms[33]. Acetyl-CoA path way is one carbon process and does not involve multi carbon intermediates for CO_2 fixation. In contrast the Calvin cycle, the reductive tricarboxylic acid cycle and hydroxypropionate cycle are dependent upon recycled intermediates ribulose bisphosphate, oxaloacetate and acetyl-CoA for CO_2 fixation. So acetyl-CoA path way is quite distinct from cyclic CO_2 fixing processes[34]. Current review is focused on acetogens following reductive acetyl-CoA pathway in anaerobes due to less energy demand for CO_2 and H_2 fixation. The potentials of bioelectrochemical processes and metabolic engineering for increasing the yield of desirable biochemicals are extensively described here:

Five alternative autotrophic pathways: Five alternative autotrophic pathways are;

- Reductive citric acid cycle
- Decarboxylate/4-hydroxybutyrate cycle
- 3-Hydroxypropionate/4-hydroxybutyrate cycle
- 3-Hydroxypropinate bicycle
- Reductive acetyl-coenzyme A pathway

Reductive citric acid cycle was discovered in anaerobes like *Chlorobium limicola*. This cycle is reverse of Krebs cycle

where hydrogen and carbon dioxide in the presence of numerous ATP molecules generate acetyl-CoA. ATP citrate lyase, 2-oxoglutarate ferredoxin oxidoreductase and fumarate reductase are enzymes catalyzing these reactions.

Decarboxylate/4-hydroxybutyrate cycle is common in autotrophic members like Thermoproteales and Desulfurococcales. This cycle comprises of two parts. Following conversion takes place in two parts, first acetyl-CoA, CO_2 and bicarbonate are converted to succinyl-CoA and second succinyl-CoA is converted to two molecules of acetyl-CoA. One molecule is for biosynthesis and other acts as a CO_2 acceptor. 4-hydroxybutyrate formation is through enzyme 4-hydroxybut-yryl-CoA dehydratase. This cycle was studied in Thermoproteus neutrophilus[35].

3-Hydroxypropionate/4 hydroxybutyrate cycle is common in aerobic autotrophic Sulfolobales and in anaerobic Stygiolobus azoricus. In this cycle two molecules of bicarbonate produce acetyl-Co-A. This cycle comprises of two parts, in the first part acetyl-CoA and two bicarbonate molecules transforms to succinyl-CoA *via* 3-hydroxypropionate. Secondly succinyl-CoA is changed to two acetyl-CoA *via* 4-hydroxybutyrate and this trait is common to autotrophic Crenarchaeota. By oxidative decarboxylation of malate in the presence of malic enzyme pyruvate is formed from succinyl-CoA[36].

3-Hydroxypropionate bi-cycle is used by bacterium *Chloroflexus aurantiacus* for autotrophic CO_2 fixation. The pathway initiate from acetyl-CoA, biotin-dependent acetyl-CoA, ATP and carboxylating enzyme propionyl-CoA carboxylase[37].

Reductive acetyl-coenzyme A (CoA) is in both reductive and oxidative, directions. For energy conservation reductive pathway is followed along with assimilation of carbon[38,39]. This pathway follows the CO and H_2 conversion to acetyl-CoA and then to acetate. In this pathway acetyl-CoA and carbon monoxide dehydrogenase (CODH) enzymes are key centre often called carbon monoxide dehydrogenase pathway. The organisms utilizing CO exhibit the tendency to use CO_2 and CO_2/H_2 and same set of enzymes and mechanisms is same as used in acetyl-coenzyme A pathway. Autotrophic organisms use acetyl-CoA pathway as electron acceptors for conservation of energy and CO_2 assimilation into biomass[40]. This is irreversible and non cyclic pathway under strict anaerobic conditions. It consists of two branches one is methyl and second is carbonyl branch as illustrated in (Fig. 2), where CO_2 is reduced to carbonyl and methyl level through several enzymatic reactions[41]. Methyl (Eastern branch) and carbonyl (Western branch) branches are important in Wood-Ljungdahl pathway. Eastern branch is for metabolism of carbon and Western branch is unique for anaerobic acetogens for fixing CO_2 or CO to acetate synthesis in Wood-Ljungdahl pathway[42]. Recently it is found that CO_2 is not only the electron sink but there are other electron acceptors too[43]. Fermenting organisms *via* substrate level phosphorylation can produce 1 to 4 mol of ATP per one mole of hexose (eqn 3).

$$C_6H_{12}O_6 + 4\ ADP + 4\ Pi \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ ATP + 8\ [H]^+ \quad (3)$$

Second case is the reduction of CO_2 to acetate through Wood-Ljungdahl pathway as expressed in eqn. 4.

$$2\ CO_2 + 8\ [H]^+ + nADP + nPi \rightarrow CH_3COOH + nATP \quad (4)$$

Wood-Ljungdahl pathway is common for both anabolism and catabolism in anaerobes[31,44]. In acetyl-CoA pathway methyl branch reduces CO_2 to formate by utilizing the enzyme formate dehydrogenase (FDH)

$$CO_2 + NADPH \rightarrow HCOO^- + NADP^+$$

and then to tetrahydrofolate (H_4F). The formation of 10-formyl-H_4folate is catalyzed by the enzyme 10-formyl-H_4folate synthetase[45].

$$HCOOH + H_4folate + ATP \rightarrow HCO^- H_4folate + ADP + Pi$$

Acetyl-CoA is formed by uniting carbonyl group with methyl group. The enzyme carbon monoxide dehydrogenase (CODH) is important to reduce CO_2 to CO as illustrated in the equation

$$CO_2 + 2H^+ + 2e^- \rightarrow CO + H_2O$$

Enzyme nickel carbon monoxide dehydrogenase (Ni-CODH) catalyzes the oxidation of CO to CO_2 which reduces further to formate and then to methyl group. It is acting as monofunctional (CODH). The bifunctional CODH again reduces CO_2 to CO[41]. At final stage CO condenses with carbonyl-methyl group of coenzyme A and corrinoid protein to form acetyl-CoA. Methenyl group is reduced to methyl-H_4F with the splitting of water. Methyl group is then transferred to corrinoid iron sulfur protein (CFeSP) and then to CO dehydrogenase for acetyl-CoA synthesis which plays central role in this pathway[46,47]. Another mole of carbon dioxide is oxidized by carbon monoxide dehydrogenase (CODH) to acetyl-Co-A. Acetate is produced by acetyl-Co-A phosphotransacetylase and acetate kinase. One mole of ATP is produced by substrate level phosphorylation (SLP) is consumed in the formyl-H_4F synthetase reaction as represented in (Fig. 2)[48]. There are two groups of acetogens the Na^+-dependent acetogens like *Acetobacterium woodii*[48,49] and H^+-dependent acetogens like *Moorella thermoacetica*[42]. The *Moorella thermoacetica* contains cytochromes and electron transport chain. The Na^+-dependent acetogens does not possess cytochromes but with corrinoid proteins which can translocate Na^+ along with Wood-Ljungdahl pathway.

Autotrophic metabolism of acetogens: Several acetogens grow with H_2/CO_2 as H_2-oxidizing autotrophs. They use CO_2 to synthesize acetate and acetyl-CoA plays central part in this pathway. The first product of autotrophic fixation of CO_2 in acetogens is Acetyl-CoA. Enzymes isolated from *Clostridium thermoaceticum* are formate dehydrogenase[50], carbon monoxide dehydrogenase[51], a corrinoid protein and methyl transferase[52]. Formate dehydrogenase contains tungsten, selenium and iron, carbon monoxide dehydrogenase has iron, zinc and nickel whereas corrinoid enzyme has vitamin B_{12}. Acetyl-CoA is a source of cellular carbon depending on both catabolic and anabolic pathway. In anabolism acetyl-CoA is carboxylated to pyruvate *via* enzyme pyruvate synthase then to phosphoenolpyruvate and in the last to cell materials. In catabolism acetyl-CoA generates ATP and acetate in the presence of catalyst phosphotransacetylase followed by acetate kinase. This metabolic phase is called an acidogenic phase. But in solventogenic phase ethanol is produced and no ATP is evolved, the growth of bacteria is slow in this phase.

Applications of acetogens: Acetyl-CoA pathways play central role for the synthesis of organics like propane diol,

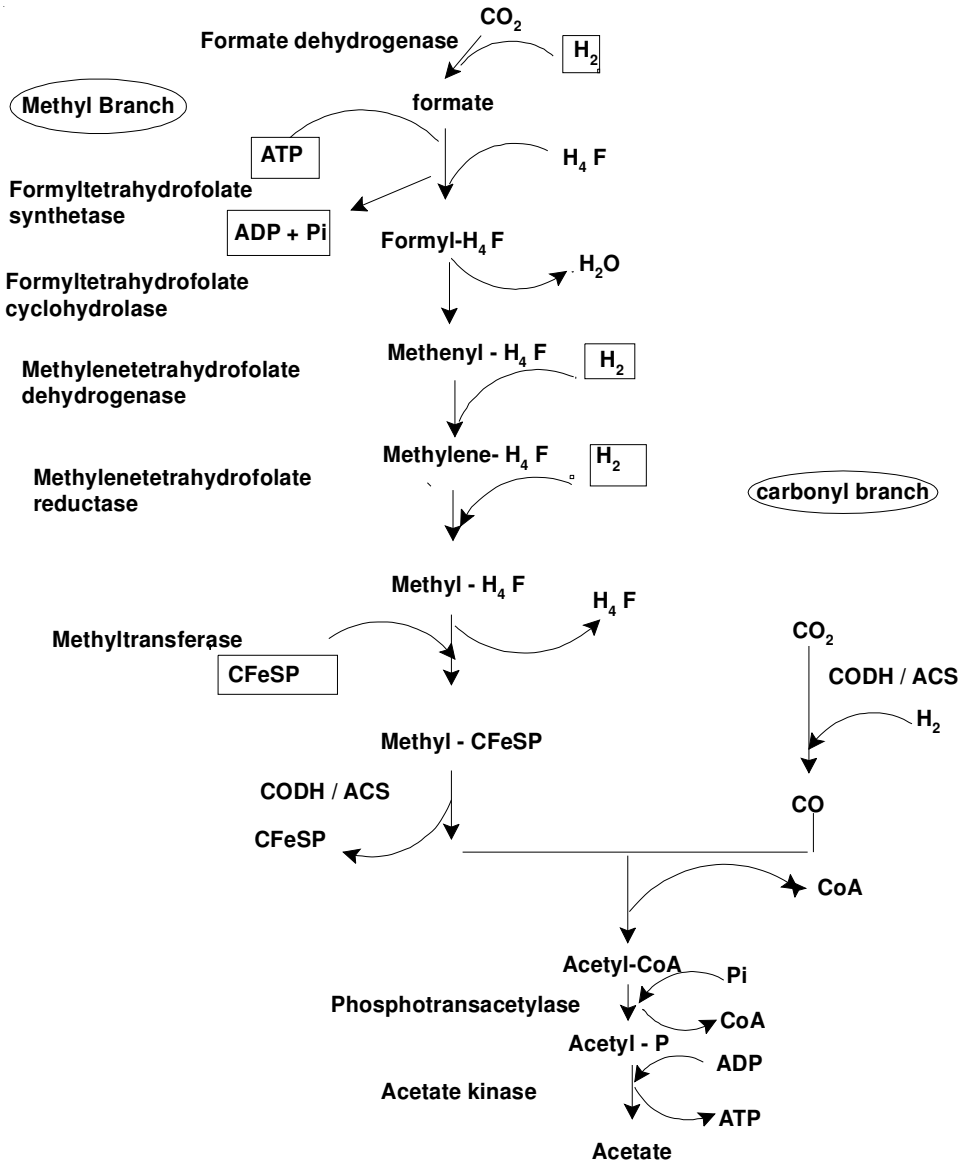

Fig. 2. Wood-Ljungdahl pathway; Electrons are derived from organic substrate by oxidation

2,3-butane diol and isopropanol by various *Clostridium* species. Nature of substrate, experimental conditions and genetic engineering are basic keys for the amendments in the pathways to get desirable results. Various examples are discussed below.

Production of carboxylic acid: The carbohydrates conversion to acids and solvents by *Clostridium acetobutylicum* involve acetyl-CoA and butyryl-CoA for butyrate and acetate production. Butyryl and acetyl phosphate are produced from Coenzyme-A derivatives in the presence of a catalyst phosphotransacetylase (PTA) and phosphortransbutyrylase (PTB). In the next step, the acyl phosphates are converted to acetate or butyrate. ATP is generated in the presence of catalyst acetate and butyrate kinase. In solvent production, the reduction of acetyl-CoA and butyryl-CoA to acetaldehyde and butyraldehyde takes place, after that ethanol and butanol are formed respectively. For the production of acetoacetate from acetoacetyl-CoA, acetate and butyrate are assimilated in the presence of enzymes acetoacetyl-CoA acetate/butyrate transferase. Acetone and carbon dioxide are formed by decarboxylation of acetoacetate *via* enzymes acetoacetate decarboxylase[53].

Production of alcohols

Propane diol: Clostridium species are well known for the production of propane diol. The main product product of the strain was *n*-butanol but other products like ethanol and acetic acid were also observed[54,55]. The metabolic pathway of glycerol fermentation by *Clostridium pasteurianum* is represented in (Fig. 3)[56]. Number of other *Clostridium* species like *Clostridium acetobutylicum, Clostridium butylicum, Clostridium beijerinckii, Clostridium kainantoi* and *Clostridium butyricum* has the potential to ferment glycerol for the production of ethanol, acetic acid, butyric acid, butanol, acetone and 1,3-propane diol[57,58].

2,3-Butane diol: Three nonpathogenic acetogen, *Clostridium autoethanogenum*[59], *Clostridium ljungdahlii*[60] and *Clostridium ragsdalei*[61] produce 2,3-butane diol (2,3BD) by using carbon monoxide and waste green house gases. Genes involved were confirmed and study demonstrated the relation between mRNA accumulation from 2,3-butane diol biosynthetic genes and 2,3-butane diol production[62]. Acetogens produce acetate, ethanol and butyrate by utilizing acetyl-CoA

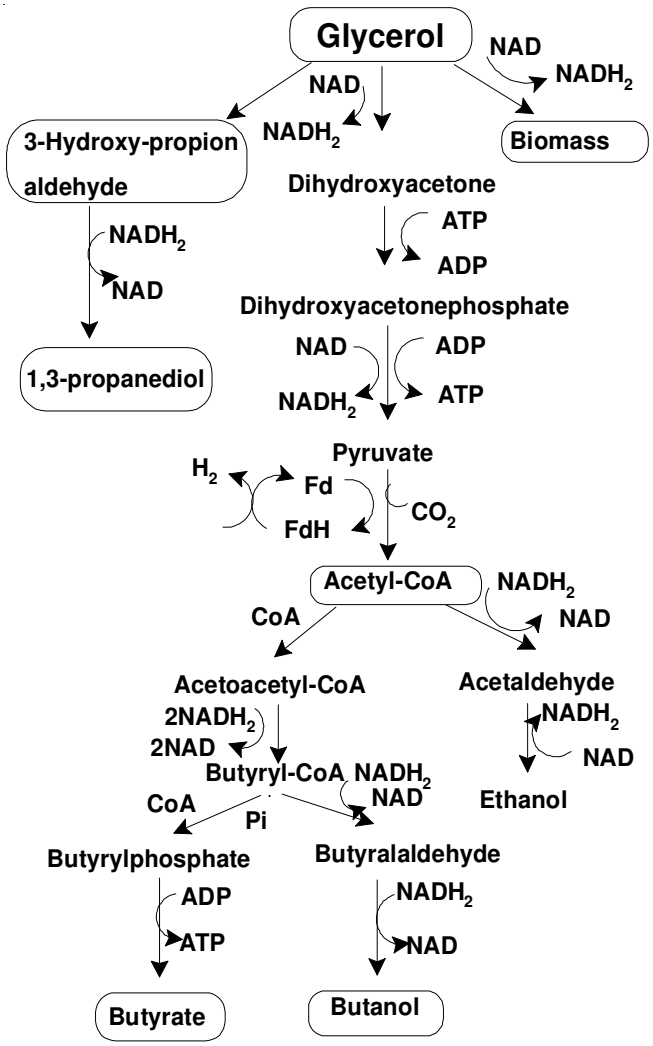

Fig. 3. Metabolic pathway in glycerol fermentation by *C. pasteurianum*

has been reported. The biochemistry related to this pathway is described in numerous articles[63,64]. But the metabolites which are synthesized from precursor like pyruvate can be produced by microorganisms by utilizing waste green house gases. the metabolites, 2,3 butane diol and lactate by acetogenic bacteria are illustrated in (Fig. 4). Responsible gene was also found in all three strains. Finally acetoin is reduced to 2,3-butane diol *via* enzyme 2,3-butane diol dehydrogenase. This enzyme is NADH and zinc dependent. Homologus enzymes in *Clostridium beijerinckii* was also expressed in acetoin-producing organisms.

Isopropanol: Metabolic engineering is an attractive approach for the synthetic pathways to fuel production. Like *n*-butanol is produced by *Clostridium* species[65,66], but genome information and molecular biology techniques are helpful for the production of organic compounds like fatty acid esters and isoprenoids in non-native organisms[67,68]. *Escherichia coli* or *Saccharomyces cerevisiae* can be engineered for fuel production.

Isopropanol is produced by *Clostridium* species by using acetone pathway[69] as represented in (Fig. 5). Three genes from *Clostridium acetobutylicum* ATCC 824, ctfAB, thl, adc were introduced in *E. coli* coding acetoacetyl-CoA transferase, acetyl-CoA acetyltransferase and acetoacetate decarboxylase[70] which were helpful for acetone production. This proved that

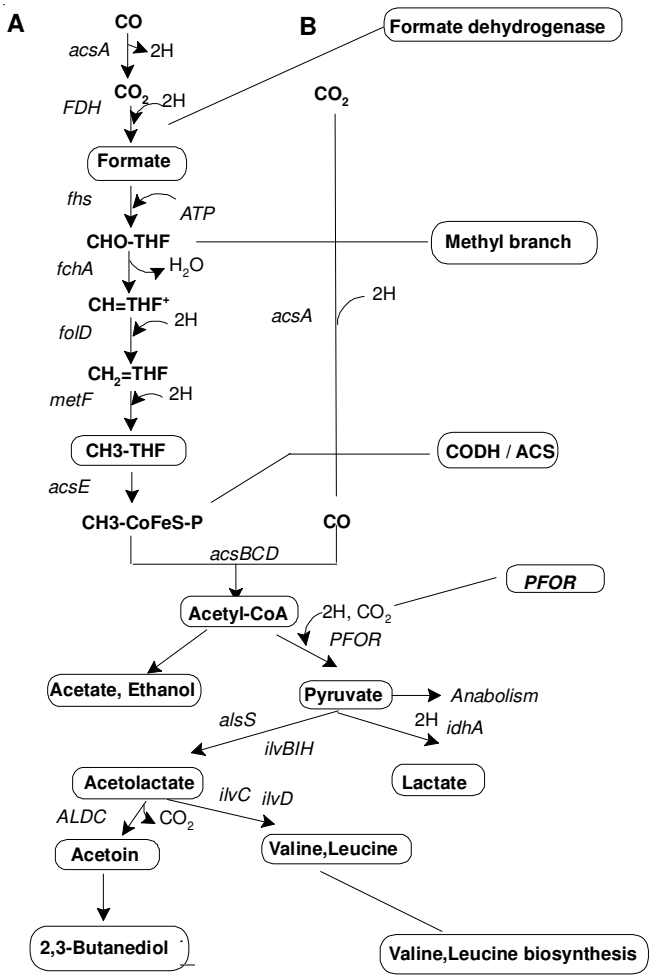

Fig. 4. 2,3-Butanediol production by gas fermentation

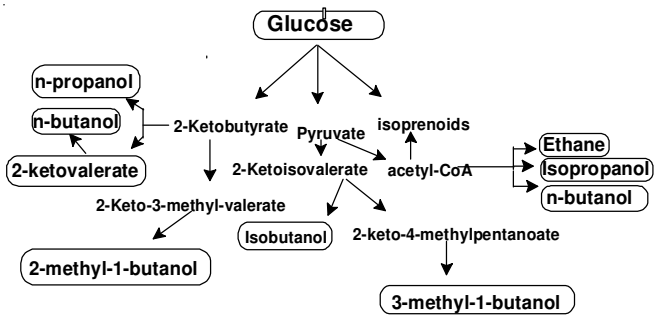

Fig. 5. Engineered metabolic pathways to produce biofuels

E. col was best for acetone and Isopropanol production. Another enzyme alcohol dehydrogenase (ADH) is required for the conversion of acetone to isopropanol[71]. This secondary (ADH) was over expressed in *E. coli* from *Clostridium acetobutylicum* following acetone biosynthetic pathway. Acetoacetyl-CoA transferase (*ato*AD) and acetyl-CoA acetyltransferase (*ato*B) were used by *E. coli* for the optimization of the pathway[72,73]. The comparison between *Clostridium beijerinckii* and *Clostridium brockii* was observed with activity of secondary enzyme (ADH) encoded by adh and found that these combined genes gave high yield than native Clostridium species[74,75].

The pathway to produce Isobutanol and butanol by *Saccharomyces cerevisiae* where glycine was used as a substrate. During the kinetic growth with glycine the isobutanol and

butanol concentrations were 58, 90 mg/L, respectively. The formation of α-isoketovalerate and α-ketovalerate are intermediate in the pathway which converts to the isobutanol and butanol, respectively with one possible encoding gene for enzymes responsible for the production of butanol.

The metabolic engineering in *Saccharomyces cerevisiae* for synthesizing *n*-butanol. *S. cerevisiae* was engineered with biosynthetic pathway for *n*-butanol from organisms like *Clostridium beijerinckii*, Ralstonia eutropha and *Escherichia coli*. *S. cerevisiae* was selected as a host because it is well characterized organism, genetically tractable and has tolerance for *n*-butanol.

***n*-Butanol:** *Clostridium acetobutylicum* utilize the acetone and butyrate pathways for the production of *n*-butanol along with byproducts like butyrate, acetone and ethanol. CO_2 is released when glucose is used for *n*-butanol production. Genes from *Clostridium* species were expressed in *E. coli* by using expression plasmids[76]. The genes activity was detected by enzymes assays. The two genes from *Clostridium acetobutylicum* thiolase and acetyl-CoA acetyltransferase and from *E. coli* (thl), (atoB), respectively were over expressed. The host pathway were deleted which was competing with *n*-butanol pathway for acetyl-coenzyme A and NADH[77-79]. This improved the production of *n*-butanol nearly threefold in wild-type *E. coli* along with the deletion of frdBC, pta, ldhA, adhE and fnr. The production of acetate, ethanol, succinate and lactate was less than the original reaction[80-84] (Fig. 6).

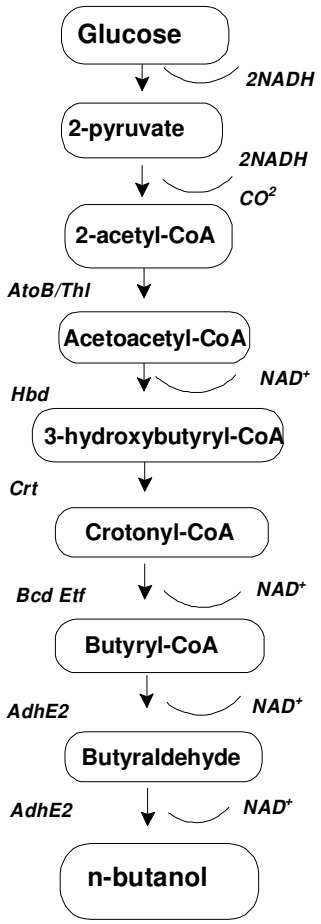

Fig. 6. *n*-Butanol productions in engineered *E. coli*

Challenges and future targets: There are number of promising research avenues in microbiology. Fermentation and electrosynthesis are costly and time consuming techniques. The recent innovative methodology is the bio-electrochemical synthesis of organics by utilizing waste greenhouse gases. The positive aspects of this technique are to utilize waste gases instead of food substrates and to eliminate the pollution and global warming. There is a need of intensive study for environmental friendly microbes having the potential to accept electrons from electrodes. The investigation of proton exchange membranes, more conductive electrode materials, nature of microbial strains and in-depth investigation of electron transfer are future challenges. Bio-electrochemical synthesis is in infancy stage so electrodes and microbes interactions should be envisioned. Intensive study of genetic engineering can prove to be a strong pillar to bring genetic mutations in metabolic pathways for alternate products. Still through investigations for genetic mutations in organisms are warranted.

Conclusion

The energy demands at world level are increasing due to the growing population rate. Renewable energy sources like food crops for the production of chemicals and biofuels are money and time consuming. Cost effective energy renewable sources are basic demand to meet the future challenges of exhausting fossil fuels.

Utilization of syngas addresses the challenges by converting atmospheric carbon dioxide to organics. The popular approach for biochemical production is fermentation process which extract electrons substrates *e.g*., sugar, CO_2, CO and H_2 to produce acetate, ethanol and other acids like butyric acid. Other electrochemical synthesis require high cost of electrodes, substrates and expensive chemicals which result in long run inefficient synthesis, electrodes deactivation and hazards to environment. Bio-electrochemical synthesis is novel and emerging technology where anaerobes are able to convert substrates and greenhouse gases into valuable chemicals. In bio-electrochemical synthesis the bio-cathode development is cost effective results in production of valuable organics and reduction of the pollution threats to environment. Acetogens focused are working in anoxic conditions by utilizing acetyl-CoA pathway for the biofuels. Genetic engineering is another tool for the mutational changes in the metabolic pathways for value added products.

The five metabolic pathways are compared in terms of terms of energy efficiency and found that Acetyl CoA pathway requires less energy for fixing CO_2. These acetogens ultimately utilize CO_2, H_2 to produce acetate or ethanol in the presence of enzymes and co-enzymes.The bioelectrochemical process and metabolic engineering are explored to increase process efficiency. The review of literature showed that there are some *Clostridium* and *Sporomusa* species which have tendency to accept electrons and gases from environment for biochemical synthesis. The genomes sequencing in adapted strains provides insight into the metabolic routes to get desirable compounds. Genome analysis enhances the understanding of microorganisms and is key factor to optimize microbial electrosynthesis. Significant efforts should continue for more suitable and reliable processes in this field which could prove to be cost effective.

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