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Chapter 5

Gas Fermentation for Commercial Biofuels Production

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1. Introduction

With diminishing global reserves of crude oil and increasing demand, especially from developing countries, the pressure on oil supply will grow. Although the 2007-2010 financial crisis brought down the price of crude oil (per barrel) from a record peak of US $145 in July 2008, factors such as recovering global economies and political instability in the Middle East have restored the price of crude oil to the US$100 mark. At current rate of consumption, the global reserves of petroleum are predicted to be exhausted within 50 years [1, 2]. This, coupled with the deleterious environmental impacts that result from accumulating atmospheric CO_2 from the burning of fossil fuels, the development of affordable, and environmentally sustainable fuels is urgently required. Many countries have responded to this challenge by legislating mandates and introducing policies to stimulate research and development (R&D) and commercialization of technologies that allow the production of low cost, low fossil carbon emitting fuels. For instance, the European Union (EU) has mandated member countries to a target of deriving 10% of all transportation fuel from renewable sources by 2020 [3]. Between 2005 and 2010, renewable energies such as solar, wind, and biofuels have been increasing at an average annual rate of 15-50% [4]. Renewable energy accounted for an estimated 16% of global final energy consumption in 2009 [4].

Biofuels have been defined as solid (bio-char), liquid (bioethanol, biobutanol, and biodiesel) and gaseous (biogas, biosyngas, and biohydrogen) fuels that are mainly derived from biomass [5]. Liquid biofuels provided a small but growing contribution towards worldwide fuel usage, accounting for 2.7% of global road transport fuels in 2010 [4]. The world's largest producer of biofuels is the United States (US), followed by Brazil and the EU [4]. In 2009, US and Brazil accounted for approximately 85% of global bioethanol

production while Europe generated about 85% of the world's biodiesel [6]. The global market for liquid biofuels (bioethanol and biodiesel) increased dramatically in recent years, reaching US$83 billion in 2011 and is projected to US$139 billion by 2021 [7].

The use and production of biofuels has a long history, starting with the inventors Nikolaus August Otto and Rudolph Diesel, who already envisioned the use of biofuels such as ethanol and natural oils when developing the first Otto cycle combustion and diesel engines [6]. While fermentative production of ethanol has been used for thousands of years, mainly for brewing beer starting in Mesopotamia 5000 B.C., fermentative production of another potential biofuel butanol, has only been discovered over the last century, but had significant impact. During the World War 1, Chaim Weizmann successfully applied a process called ABE (acetone-butanol-ethanol) fermentation using *Clostridium acetobutylicum* to generate industrial scale acetone (for cordites, the propellant of cartridges and shells) from starchy materials [6, 8]. His contribution was later recognised in the Balfour declaration in 1917 and he became the first President of the newly founded State of Israel [6, 8]. Intriguingly, the enormous potential of butanol produced at that time was not realized and the substance was simply stored in huge containers [6]. ABE fermentation became the second biggest ever biotechnological process (after the ethanol fermentation process) ever performed, but the low demand of acetone following the conclusion of the war led to closure of all the plants [8]. Although ABE fermentation briefly made a comeback during the Second World War, increasing substrate costs and increasing stable supply of low cost crude oil from the Middle East rendered the technology economically unviable. Recently, a resurgence of the technology is underway as some old plants are reopened and new plants are being built or planned in China, the US, the United Kingdom (UK), Brazil, France and Austria [6, 8].

Traditionally sugar substrates derived from food crops such as sugar cane, corn (maize) and sugar beet have been the preferred feedstocks for the production of biofuels. However, world raw sugar prices have witnessed significant volatility over the last decade or so, ranging from US$216/ton in year 2000 to a 30 year high of US$795/ton in February 2011 due to global sugar deficits and crop shortfall [9]. This has created uncertainty and raised sustainability issues about its use as a feedstock for large scale biofuel production. This review aims to shed light on the use of syngas and industrial waste gas as feedstocks, and the emerging field of gas fermentation to generate not only biofuels, but also other high-value added products. The advantages of gas fermentation over conventional sugar-based fermentation and thermochemical conversions, and their flexibility in utilizing a spectrum of feedstocks to generate syngas will be discussed. The biochemistry, genetic and energetic background of the microorganisms that perform this bioconversion process will be critically examined, together with recent advances in systems biology and synthetic biology that offer growing opportunities to improve biocatalysts in terms of both the potential products that can be produced and their process performance. The key processes such as gasification, bioreactor designs, media formulation, and product recovery will be analysed. Finally, the state of commercialization of gas fermentation will be highlighted and an outlook will be provided.

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2. Advantages of gas fermentation

The production of first generation biofuels relies on food crops such as sugar beet, sugar cane, corn, wheat and cassava as substrates for bioethanol; and vegetable oils and animal fats for biodiesel. Although years of intense R&D have made methods of bioethanol production (typically using the yeast *Saccharomyces cerevisiae*) technologically mature, there remain some serious questions regarding its sustainability. The use of food crops as a source of carbohydrate feedstocks by these processes requires high-quality agricultural land. The inevitable conflict between the increasing diversion of crops or land for fuel rather than food production has been highlighted as one of the prime causes of rising global food prices. Furthermore, corn ethanol producers in the US, have historically enjoyed a 45-cent-a-gallon federal tax credit for years (which ended in early 2012), costing the government US$30.5 billion between 2005 to 2011, raising questions about its economic competitiveness with gasoline [10, 11].

These arguments have stimulated the search for so-called second generation biofuels, which utilize non-food lignocellulose biomass such as wood, dedicated energy crops, agricultural residues and municipal solid wastes as feedstocks. Biomass consists of cellulose, hemicellulose and lignin, and the latter of which is extremely resistant to degradation. One approach to unlocking the potential in this abundant feedstock is to separate the lignin from the carbohydrate fraction of the biomass via extensive pre-treatment of the lignocellulose involving, for example, steam-explosion and/or acid hydrolysis. These pre-treatments are designed to allow the carbohydrate portion of the biomass to be broken down into simple sugars, for example by enzymatic hydrolysis using exogenously added cellulases to release fermentable sugars [12]. Such approaches have been found to be expensive and rate limiting [6, 12, 13]. Alternatively, processes using cellulolytic microorganisms (such as *C. cellulolyticum*, *C. thermocellum*, and *C. phytofermentans*) to carry out both the hydrolysis of lignocelluloses and sugar fermentation in a single step, termed 'Consolidated Bioprocessing Process (CBP)' [12] have been proposed, however the development of these is still at an early stage, and again low conversion rates seem to be a major limitation that needs to be overcome.

Microorganisms such as acetogens, carboxytrophs and methanogens are able to utilize the CO_2 + H_2, and/or CO available in such syngas as their sole source of carbon and energy for growth as well as the production of biofuels and other valuable products. However, only acetogens are described to synthesize metabolic end products that have potentials as liquid transportation fuels. While biological processes are generally considered slower than chemical reactions, the use of these microbes to carry out syngas fermentation offers several key advantages over alternative thermo-chemical approaches such as the Fischer-Tropsch' process (FTP). First, microbial processes operate at ambient temperatures and low pressures which offer significant energy and cost savings. Second, the ambient conditions and irreversible nature of biological reactions also avoid thermodynamic equilibrium relationships and allow near complete conversion efficiencies [14, 15]. Third, biological conversions are commonly more specific due to high enzymatic specificities, resulting in higher product yield with the formation of fewer by-products. Fourth, unlike traditional chemical catalysts which require a set feed gas composition to yield desired product ratios or suite, microbial processes have freedom to operate for the production ofthe same suite of products across a wider range of CO:H_2 ratios in the feed gas [16]. Fifth, bio-

catalysts exhibit a much higher tolerance to poisoning by tars, sulphur and chlorine than inorganic catalysts [6, 16]. However, some challenges have been identified for syngas fermentation to be commercialized, including gas mass transfer limitations, long retention times due to slow cell growth, and lower alcohol production rates and broth concentrations. Recent progress and development to remedy these issues will be highlighted in this review.

3. Feedstock and gasification

Due to the flexibility of the microbes to ferment syngas with diverse composition, virtually any carbonaceous materials can be used as feedstock for gasification. Non-food biomass that can be employed as feedstock for gasification includes agricultural wastes, dedicated energy crops, forest residues, and municipal organic wastes, or even glycerol and feathers [16-20]. Biomass is available on a renewable basis, either through natural processes or anthropogenic activities (e.g. organic wastes). It has been estimated that out of a global energy potential from modern biomass of 250 EJ per year in 2005, only 9 EJ (3.6%) was used for energy generation [18]. The use of existing waste streams such as municipal organic waste also differentiate itself from other feedstocks such as dedicated energy crops because these wastes are available today at economically attractive prices, and they are often already aggregated and require less indirect land use. Alternatively, gasification of non-biomass sources such as coal, cokes, oil shale, tar sands, sewage sludge and heavy residues from oil refining, as well as reformed natural gas are commonly applied as feedstocks for the FTP and can also be used for syngas fermentation [15, 21]. Furthermore, some industries such as steel manufacturing, oil refining and chemical production generate large volume of CO and/or CO_2 rich gas streams as wastes. Tapping into these sources using microbial fermentation process essentially convert existing toxic waste gas streams into valuable commodities such as biofuels. The overall process of gas fermentation is outlined in Figure 1.

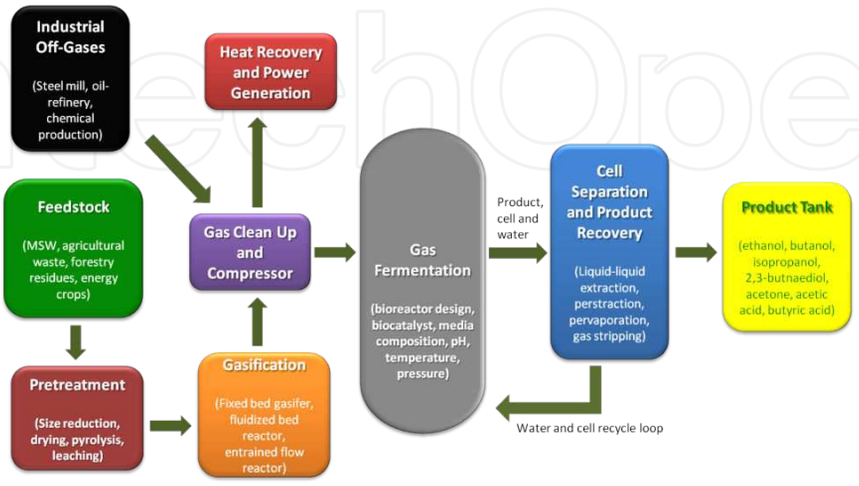

Figure 1. Overview of gas fermentation process

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Prior to gasification, biomass generally needs to go through a pre-treatment process encompassing drying, size reduction (e.g. chipping, grinding and chopping), pyrolysis, fractionation and leaching depending on the gasifier configuration [22, 23]. This upstream pre-treatment process can incur significant capital expense and add to the overall biomass feedstock cost, ranging from US$16-70 per dry ton [22]. Gasification is a thermo-chemical process that converts carbonaceous materials to gaseous intermediates at elevated temperature (600-1000^{o}C), in the presence of an oxidizing agent such as air, steam or oxygen [16, 22]. The resulting syngas contains mainly CO, CO_2, H_2 and N_2, with varying amounts of CH_4, water vapour and trace amount of impurities such as H_2S, COS, NH_3, HCl, HCN, NO_x, phenol, light hydrocarbons and tar [17, 22, 24]. The composition and amount of impurities of syngas depends on the feedstock properties (e.g. moisture, dust and particle size), gasifier type and operational conditions (e.g. temperature, pressure, and oxidant) [17, 22]. Table 1 summarizes typical composition of syngas and other potential gas streams derived from various sources.

4. Fixed bed gasifier

Depending on the direction of the flows of carbonaceous fuel and oxidant (air or steam), fixed bed gasifier can be further categorized into updraft or downdraft reactor. In the updraft (counter-current) version of the fixed bed gasifier, biomass enters from the top while gasifying agent from the bottom. The biomass moves down the reactor through zones of drying (100^{o}C), pyrolysis (300^{o}C), gasification (900^{o}C) and finally oxidation zone (1400^{o}C) [18]. Although this mode of gasifier is often associated with high tar content in the exit gas, recent advances in tar cracking demonstrated that very low tar level is achievable [31]. The direct heat exchange of the oxidizing agent with the entering fuel feed results in low gas exit temperature and hence high thermal efficiency [18, 23]. The downdraft (co-current) gasifier has very similar design as the updraft reactor, except the carbonaceous fuel and oxidizing agent flow in the same direction. In comparison to the updraft gasifier, the downdraft reactor has lower tar content in the exit gas but exhibit lower thermal efficiency [23]. Due to the size limitation in the constriction (where most of the gasification occurs) of the reactor, this mode of gasifier is considered unsuitable for large scale operation [18].

5. Fluidized bed reactor

In fluidized bed reactor, the carbonaceous fuel is mixed together with inert bed material (e.g. silica sand) by forcing fluidization medium (e.g. air and/or steam) through the reactor. The inert bed facilitates better heat exchange between the fuel materials, resulting in nearly isothermal operation conditions and high feedstock conversion efficiencies [18, 22]. The maximum operating temperature of the gasifier is typically around 800 - 900^{o}C, which is limited by the melting point of the bed material [18]. Furthermore, the geometry of the reactor and excellent mixing properties also means that fluidized bed reactors are suitable for

up-scaling [18, 22]. Due to these properties, fluidized bed reactor is currently the most commonly used gasifier for biomass feedstock [32]. However, this mode of gasifier is not suitable for feedstocks with high levels of ash and alkali metals because the melting of these components causes stickiness and formation of bigger lumps, which ultimately negatively affect the hydrodynamics of the reactor [18].

	Composition vol%, dry basis						Ref.
	CO	CO_2	H_2	N_2	CH_4	Other	
Non-biomass source							
Coal gasification	59.4	10	29.4	0.6	0	0.6	[25]
Coke oven gas	5.6	1.4	55.4	4.3	28.4	4.9	[25]
Partial oxidation of heavy fuel oil	47	4.3	46	1.4	0.3	1	[26]
Hardwood chips + 20 wt%liquid crude glycerol	19.73	11.67	19.38	NR*	3.82	NR*	[19]
Steam reforming of natural gas	15.5	8.1	75.7	0.2	0.5	0	[25]
Steam reforming of Naphtha	6.7	15.8	65.9	2.6	6.3	2.7	[25]
Water gas	30	3.4	31.7	13.1	12.2	9.6	[25]
Steel Mill	44	22	2	32	0	0	[27]
Biomass and organic waste source							
Demolition wood + sewage sludge	10.53	15.02	8.02	60.46	3.19	2.78	[28]
Cacao shell	8	16.02	9.02	61.45	2.34	3.17	[28]
Dairy biomass	8.7	15.7	18.6	56	0.6	0.4	[29]
Switchgrass	14.7	16.5	4.4	56.8	4.2	3.4	[13]
Kentucky bluegrass straw	12.9	17.4	2.6	64.2	2.1	0.8	[30]
Willow	9.4	17.1	7.2	60.42	3.3	2.58	[28]

Note: NR, not reported

The factors that determine which type of gasifier to employ are scale of operation, feedstock size and composition, tar yield and sensitivity towards ash [18]. Currently, three main types of gasifier are commercially employed: fixed bed, fluidized bed and entrained flow reactors [18].

Table 1. Typical composition of syngas and other potential gas streams from various sources

6. Entrained flow reactor

Entrained flow reactor is the preferred route for large scale gasification of coal, petcoke and refinery residues because of high carbon conversion efficiencies and low tar production [22]. This mode of gasifier does not require inert bed material but relies on feeding the feedstocks co-currently with oxidizing agent at high velocity to achieve a pneumatic transport regime

[18]. At operating temperature of 1200-1500°C, this method is able to convert tars and methane, resulting in better syngas quality [18]. Importantly this technology requires the feedstocks to be pulverised into fine particles of ~50 μm before feeding, which is not a major issue for coal but very difficult and costly for biomass sources [18, 22].

7. Microbes and biochemistry of gas fermentation

Acetogens are defined as obligate anaerobes that utilize the reductive acetyl-CoA pathway for the reduction of CO_2 to the acetyl moiety of acetyl-coenzyme A (CoA), for the conservation of energy, and for the assimilation of CO_2 into cell carbon [33]. In addition to the reductive acetyl-CoA pathway, four other biological pathways are known for complete autotrophic CO_2 fixation: the Calvin cycle, the reductive tricarboxylic acid (TCA) cycle, the 3-hydroxypropionate/malyl-CoA cycle and the 3-hydroxypropionate/4-hydroxybutyrate cycle [34]. Since the earlier atmosphere of earth was anoxic and the acetyl-CoA pathway is biochemically the simplest among the autotrophic pathways (the only linear pathway, whereas the other four pathways are cyclic), it has been postulated to be the first autotrophic process on earth [35, 36]. The reductive acetyl-CoA pathway is also known as the 'Wood-Ljungdahl' pathway, in recognition of the two pioneers, Lars G. Ljungdahl and Harland G. Wood, who elucidated the chemical and enzymology of the pathway using *Moorella thermoacetica* (formerly: *Clostridium thermoaceticum*) [35] or CODH/ACS pathway after the key enzyme of the pathway Carbon Monoxide dehydrogenase/Acetyl-CoA synthase. This ancient pathway is diversely distributed among at least 23 different bacterial genera: *Acetitomaculum*, *Acetoanaerobium*, *Acetobacterium*, *Acetohalobium*, *Acetonema*, *Alkalibaculum*, "*Bryantella*", "*Butyribacterium*",*Caloramator*, *Clostridium*, *Eubacterium*, *Holophaga*, *Moorella*, *Natroniella*, *Natronincola*, *Oxobacter*, *Ruminococcus*, *Sporomusa*, *Syntrophococcus*, *Tindallia*, *Thermoacetogenium*, *Thermoanaerobacter*, and *Treponema* [33]. A selection of mesophilic and thermophilic acetogens are presented in Table 2. Acetogens are able to utilize gases CO_2 + H_2, and/or CO to produce acetic acid and ethanol according to the following stoichiometries:

$$2\,CO_2 + 4\,H_2 \Rightarrow CH_3COOH + 2\,H_2O \qquad \Delta H = -75.3\ kJ/mol \tag{1}$$

$$2\,CO_2 + 6\,H_2 \Rightarrow C_2H_5OH + 3\,H_2O \qquad \Delta H = -97.3\ kJ/mol \tag{2}$$

$$4\,CO + 2\,H_2O \Rightarrow CH_3COOH + 2\,CO_2 \qquad \Delta H = -154.9\ kJ/mol \tag{3}$$

$$6CO + 3\,H_2O \Rightarrow C_2H_5OH + 4\,CO_2 \qquad \Delta H = -217.9\ kJ/mol \tag{4}$$

The Acetyl-CoA pathway is essentially a terminal electron-accepting process that assimilates CO_2 into biomass [35]. It constitutes an *Eastern* (or Carbonyl) branch and a *Western* (or Meth-

Species	Substrate	Product(s)	T_{opt} (°C)	pH_{opt}	Genome Status	Ref.
Mesophilic Microorganisms						
Acetobacterium woodii	H_2/CO_2, CO	Acetate	30	6.8	Available	[41, 42]
Acetonema longum	H_2/CO_2	Acetate, butyrate	30-33	7.8		[43]
Alkalibaculum bacchi	H_2/CO_2, CO	Acetate, ethanol	37	8.0-8.5		[44, 45]
Blautia producta	H_2/CO_2, CO	Acetate	37	7		[46]
Butyribacterium methylotrophicum	H_2/CO_2, CO	Acetate, ethanol, butyrate, butanol	37	6		[47-49]
Clostridium aceticum	H_2/CO_2, CO	Acetate	30	8.3	Under construction	[50-52]
Clostridium autoethanogenum	H_2/CO_2, CO	Acetate, ethanol, 2,3-butanediol, lactate	37	5.8-6.0		[27, 53]
Clostridium carboxidivorans or "P7"	H_2/CO_2, CO	Acetate, ethanol, butyrate, butanol, lactate	38	6.2	Draft	[54, 55]
Clostridium drakei	H_2/CO_2, CO	Acetate, ethanol, butyrate	25-30	5.8- 6.9		[55-57]
Clostridium formicoaceticum	CO	Acetate, formate	37	NR		[50, 58, 59]
Clostridium glycolicum	H_2/CO_2	Acetate	37-40	7.0-7.5		[60, 61]
Clostridium ljungdahlii	H_2/CO_2, CO	Acetate, ethanol, 2,3-butanediol, lactate	37	6	Available	[27, 62, 63]
Clostridium magnum	H_2/CO_2	Acetate	30-32	7.0		[64, 65]
Clostridium mayombei	H_2/CO_2	Acetate	33	7.3		[66]
Clostridium methoxybenzovorans	H_2/CO_2	Acetate, formate	37	7.4		[67]
"Clostridium ragsdalei" or "P11"	H_2/CO_2, CO	Acetate, ethanol, 2,3-butanediol, lactate	37	6.3		[68]
Clostridium scatologenes	H_2/CO_2, CO	Acetate, ethanol, butyrate	37-40	5.4-7.5		[55, 56]
Eubacterium limosum	H_2/CO_2, CO	Acetate	38-39	7.0-7.2	Available	[41, 69]
Oxobacter pfennigii	H_2/CO_2, CO	Acetate, butyrate	36-38	7.3		[70]
Thermophilic Microorganisms						
Moorella thermoacetica	H_2/CO_2, CO	Acetate	55	6.5-6.8	Available	[71-73]
Moorella thermoautotrophica	H_2/CO_2, CO	Acetate	58	6.1		[74]
Thermoanaerobacter kiuvi	H_2/CO_2	Acetate	66	6.4		[72]

Notes: NR, not reported

Table 2. Acetogens

yl) branch (Figure 2.). The *Western* branch employs a series of enzymes to carry out a six-electron reduction of CO_2 to the methyl group of acetyl-CoA, starting from the conversion of CO_2 to formate by formate dehydrogenase. Formyl-H_4folate synthase then condenses formate with H_4folate to form 10-formyl-H_4folate, which is then converted to 5,10-methenyl-H_4folate by a cyclohydrolase. This is followed by a dehydrogenase that reducesmethenyl- to 5,10-methylene-H_4hydrofolate, before (6S)-5-CH_3-H_4folate is formed by methylene-H_4folate reductase [37]. A B12-depedent methyltransferase (MeTr) then transfer the methyl group of (6S)-5-CH_3-H_4folate to corrinoid iron-sulphur protein (CoFeSP) of the bi-functional carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex [37]. The bi-functional CODH/ACS enzyme complex is formed by two autonomous proteins, an $\alpha_2\beta_2$ tetramer (CODH/ACS) and a $\gamma\delta$ heterodimer (CoFeSP), and the genes are often arranged in an operon, together with MeTr [37, 38]. In the *Eastern* branch, the CODH component catalyzes the reduction of CO_2 to CO. The central molecule, acetyl-CoA, is finally generated when CO, methyl group (bound to CoFeSP) and CoASH are condensed by ACS. Given the pivotal role of CODH/ACS, it is unsurprising that this complex was found to be the most highly expressed transcripts under autotrophic conditions in *C. autoethanogenum* [27], and can represent up to 2% of the soluble cell protein of an acetogen [39]. CODH/ACS is not unique to acetogenic bacteria, as it is also present in sulphate-reducing bacteria, desulfitobacteria, and Archaea (methanogens and Archaeoglobus) [38, 40].

The reducing equivalents required for fixation of CO_2 carbon into acetyl-CoA come from the oxidation of molecular hydrogen under chemolithoautotrophic growth, or NADH and reduced ferredoxin under heterotrophic growth [75]. An extensive review by Calusinska *et al.* (2010) highlighted the diversity of ubiquitous hydrogenases that Clostridia possess although only one acetogen *C. carboxidivorans* was included in this study [76], which catalyze the reversible oxidation of hydrogen:

$$H_2 \Leftrightarrow 2\,H^+ + 2\,e^- \tag{5}$$

The direction of the hydrogenase reaction is directed by the redox potential of the components able to interact with the enzyme. Hydrogen evolution occurs when electron donor is available, whereas the presence of electron acceptor results in hydrogen oxidation [77]. Hydrogenases can be classified into three phylogenetically distinct classes of metalloenzymes: [NiFe]-, [FeFe]-, and [Fe]-hydrogenases [76]. In *Methanosarcina barkeri*, the Ech hydrogenase, a [NiFe]-hydrogenase, was demonstrated to oxidize H_2 to reduce ferredoxin [78]. During acetoclastic methanogenesis, Ech hydrogenase oxidize ferredoxin to generate H_2 [78]. Although genome analysis revealed the presence of Ech-like hydrogenase in *C. thermocellum, C. phytofermentans*, *C. papyrosolvens*, and *C. cellulolyticum*, their physiological roles remained unknown [76]. Clostridia harbour multiple distinct [FeFe]-hydrogenases, perhaps reflecting their ability to respond swiftly to changing environmental conditions [76]. The monomeric, soluble [FeFe]-hydrogenase of *C. pasteurianum* is one of the best studied. It transfer electrons from reduced ferredoxins or flavodoxins to protons, forming H_2 [79]. A trimeric [FeFe]-hydrogenase found in *C. difficile*, *C. beijerinckii*, and *C. carboxidivorans* were hypothesized to

couple formate oxidation to reduce protons into H_2 [76]. In *Thermotoga maritima*, an electron bifurcating, trimeric [FeFe]-hydrogenase was identified, that was shown to simultaneously oxidize reduced ferredoxin and NADH to evolve hydrogen under low H_2 partial pressure [80]. Under high H_2 partial pressure, the authors hypothesized that the NADH is oxidized to produce ethanol. *In silico* analysis revealed homologs of this bifurcating hydrogenase in a few Clostridia including *C. beijerinckii* and *C. thermocellum* [80]. In addition to classical hydrogenases, CODH/ACS and pyruvate:ferredoxin oxidoreductase (PFOR) from *M. thermoacetica* were shown to have hydrogen evolving capability, possibly as a mean of disposing excess reducing equivalents when electron carriers are limited and/or CO concentration is sufficient to inhibit conventional hydrogenases [81].

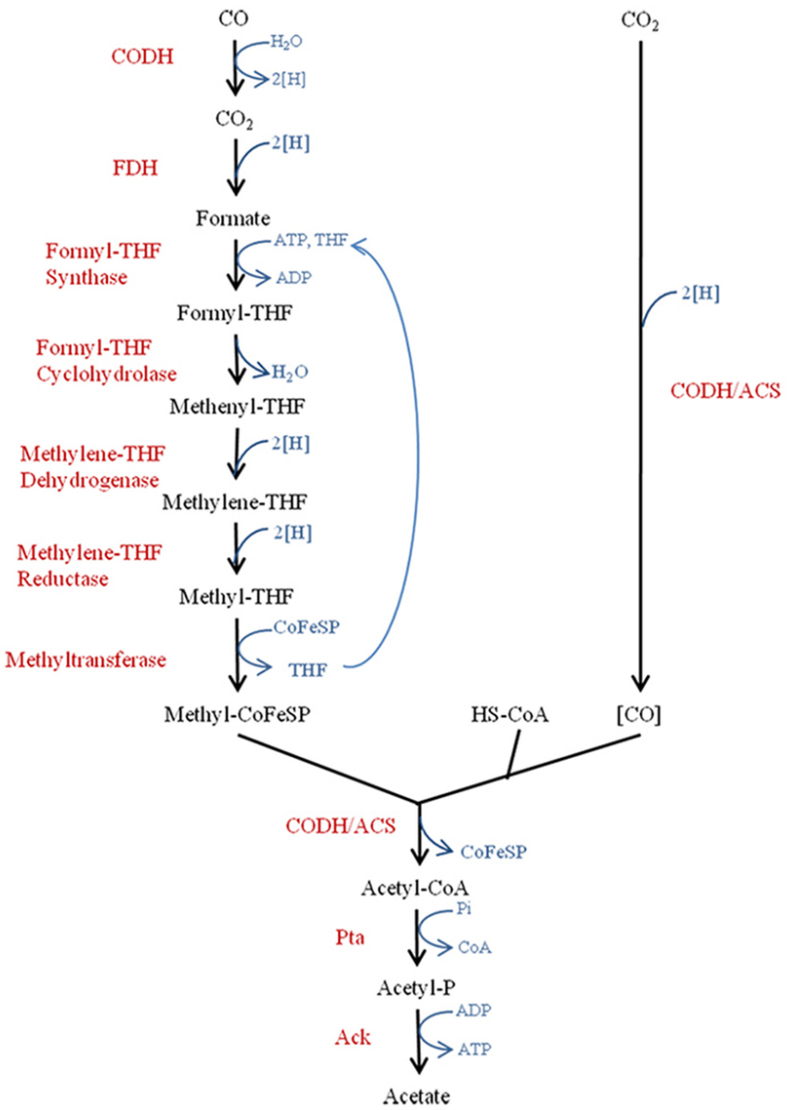

Figure 2. Wood-Ljungdahl Pathway. Ack, acetate kinase; ACS, acetyl-CoA synthase; CODH, carbon monoxide dehydrogenase; CoFeSP, corrinoid iron sulfur protein; FDH, formate dehydrogenase; Pta, phosphotransacetylase; THF, tetrahydrofolate.

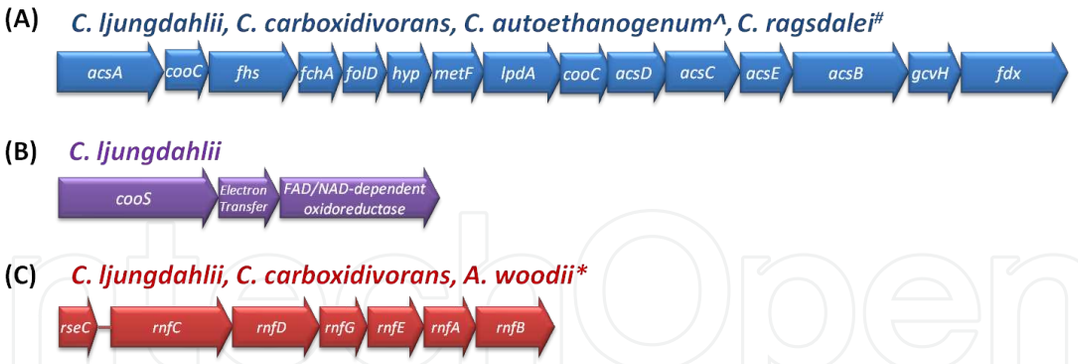

Figure 3. The organization of genes involved in acetogenesis and energy conservation from sequenced key acetogens. (A) Wood-Ljungdahl cluster; (B) carbon monoxide dehydrogenase (CODH) cluster; (C) Rnf complex cluster. *acsA*, CODH subunit; *acsB*, ACS subunit; *acsC*, corrinoid iron-sulfur protein large subunit; *acsD*, corrinoid iron-sulfur protein small subunit; *acsE*, methyltransferase subunit; *cooC*, gene for CODH accessory protein; *cooS*, CODH; *fchA*, formimido-tetrahydrofolate cyclodeaminase; *fdx*, ferredoxin; *fhs*, formyl-tetrahydrofolate synthase; *folD*, bifunctional methylene-tetrahydrofolate dehydrogenase/formyl-tetrahydrofolate cyclohydrolase; *gcvH*, gene for glycine cleavage system H protein; *hyp*, hypothetical protein; *lpdA*, dihydrolipoamide dehydrogenase; *metF*, methylene-tetrahydrofolate reductase; *rnfA, rnfB, rnfC, rnfD, rnfE, rnfG,* electron transport complex protein subunits; *rseC,* sigma E positive regulator. ^, truncated *acsA*. #, truncated fdx. *, lack *rseC*.

Most acetogens are also able to utilize another gas carbon monoxide (CO). In contrast to CO_2, CO can serve as both a source of carbon btut also as source of electrons such that hydrogen is not necessarily required. With a CO_2/CO reduction potential of -524 to -558mV, CO is approximately 1000-fold more capable of generating extremely low potential electrons than NADH, capable of reducing cellular electron carriers such as ferredoxin and flavodoxin [38, 82]. The reducing equivalents generated from CO oxidation can be coupled to reduction of CO_2 into acetate, butyrate and/or methane, evolution of molecular hydrogen from protons, reduction of nitrate/nitrite, reduction of sulfur species and reduction of aldehydes into alcohols [35, 83]. However, relatively few microorganisms are able to utilize CO as sole carbon and energy source, probably due to growth inhibition from sensitivity of their metalloproteins and hydrogenases towards CO [38, 83]. During exponential growth of *Pseudomonas carboxydovorans* (an aerobic carboxydotroph), it was demonstrated via immunological localization studies that 87% of the key enzyme CODH is associated with the inner cytoplasmic membrane, but this association was lost at the end of the exponential growth phase and a reduction in CO-dependent respiration rate was observed [84, 85]. It should be mentioned that aerobic and anaerobic CODH enzymes are structurally very different. CODH has been reported to be a very rapid and efficient CO oxidizer at rates between 4,000 and 40,000 s^{-1}, and reduces CO_2 at $11s^{-1}$ [86, 87]. Other electron donors commonly used by acetogens include formate, CH_3Cl, lactate, pyruvate, alcohols, betaine, carbohydrate, acetoin, oxalate and citrate [88]. CODH is able to split water in a biological water-gas shift reaction into hydrogen and electron according to the stoichiometry:

$$CO + H_2O \Rightarrow CO_2 + 2H^+ + 2e^- \quad (6)$$

The operation of this water gas shift reaction is the biochemical basis for the tremendous flexibility that acetogens have in terms of input gas composition. Via this reaction these organisms can flexibly use CO or H_2 as a source of electrons.Recently, some acetogens such as *C. ljungdahlii*, *C. aceticum*, *M. thermoacetica*, *Sporomusa ovata*, and *S. sphaeroides* have additionally been shown to utilize electrons derived from electrodes to reduce CO_2 into organic compounds such as acetate, formate, fumarate, caffeine, and 2-oxo-butyrate [89]. Termed microbial electrosynthesis, this nascent concept offers another route for acetogens to harvest the electrons generated from sustainable sources (e.g. solar and wind) to reduce CO_2 into useful multi-carbon products such as biofuels [90].

Under chemolithoautotrophic conditions, acetogenesis must not only fix carbon but also conserve energy. Approximately 0.1 mol of ATP is required for generation of 1g of dry biomass in anaerobes [82]. Acetyl-CoA is an energy rich molecule that through the combined actions of Pta (phosphotransacetylase) and Ack (acetate kinase), one ATP can be generated via substrate level phosphorylation (SLP). However, the activation of formate to 10-formyl-H4folate in the methyl-branch of Acetyl-CoA pathway consumes one ATP so no net gain in ATP is achieved via this mechanism [35, 75]. Furthermore, the reduction of CO_2 to the carbonyl group also requires energy, estimated at one third of ATP equivalent [35]. Recent advances indicated that other modes of energy conservation such as electron transport phosphorylation (ETP) or chemiosmotic processes that are coupled to the translocation of protons or sodium ions are implicated in acetogens. Acetogens such as *M. thermoacetica* harbour membrane-associated electron transport system containing cytochrome, menaquinones, and oxidoreductases that translocate H^+ out of the cell [33]. For acetogens that lack such membranous electron transport system, such as *Acetobacterium woodii* and *C. ljungdahlii*, a membrane-bound corrinoid protein is hypothesized to facilitate extrusion of Na^+ or protons during the transfer of methyl group from methyl-H_4F to CODH/ACS [75]. However, all enzymes involved are predicted to be soluble rather than membrane bound. Recent evidence suggested coupling to an Rnf complex in *A. woodii*, and *C. ljungdahlii* (Figure 3) which acts as ferredoxin:NAD^+-oxidoreductase [62, 91-93]. The Rnf complex is also found in other Clostridia (but not in ABE model organism *C. acetobutylicum*) and bacteria, and was originally discovered in *Rhodobacter capsulatus* where it is involved in nitrogen fixation [93]. Using reduced ferredoxin (Fd^{2-}) generated from CO oxidation, carbohydrate utilization and/or hydrogenase reactions, this membrane-bound electron transfer complex is predicted to reduce NAD^+ with concomitant translocation of Na^+/ H^+. The ion gradient generated from the above processes is harvested by H^+- or Na^+- ATP synthase to generate ATP [33, 93]. The recent genome sequencing of *A. woodii* revealed that Rnf complex is likely to be the only ion-pumping enzyme active during autotrophic growth and the organism's entire catabolic metabolism is optimized to maximize the Fd^{2-}/NAD^+ ratio [42]. Recently, a third mechanism of energy conservation which involves bifurcation of electrons by hydrogenases was proposed for anaerobes [94] and demonstrated for enzymes hydrogenase (see above; [80]), butyryl-CoA dehydrogenase [94, 95], or an iron-sulfur flavoprotein Nfn [96]. A similar mechanism has also been proposed for the methylene-THF reductase of the reductive acetyl-CoA pathway, which would enable this highly exergonic reduction step ($\Delta G^{0'} = -22$ kJ/mol)

to be coupled with the Rnf complex for additional energy conservation [62]. However, no experimental proof to support this hypothesis has been published to date.

In an attempt to generate an autotrophic *E. coli*, the genes encoding MeTr, the two subunits of CODH/ACS, and the two subunits of CoFeSP from *M. thermoacetica* were cloned and heterologously expressed in *E. coli* [97]. Although the MeTr was found to be active, the other subunits misassembled hence no active enzymes were found [97]. Autotrophic capability is clearly a very complex process that involves many genes other than the CODH/ACS complex and tetrahydrofolate pathway, including compatible cofactors, electron carriers, specific chaperones and energy conservation mechanisms. For instance, more than 200 genes are predicted to be involved in methanogenesis and energy conservation from CO_2 and H_2 in methanogens [98]. A recent patent application described the introduction of three Wood-Ljungdahl pathway genes encoding MeTr, CoFeSP subunit α and β from *C. difficile* into *C. acetobutylicum* [99]. The recombinant strain was shown to incorporate more CO_2 into extracellular products than wild-type [99].

8. Products of gas fermentation

Acetyl-CoA generated via the Wood-Ljungdahl pathway serves as key intermediate for synthesis of cell mass as well as products. All acetogens are described to produce acetate, in order to gain energy via SLP to compensate for the energy invested in activating formate in the *Western* branch of the reductive acetyl-CoA pathway. Acetate and ATP are formed via acetyl-phosphate through the successive actions of Pta and Ack. *pta* and *ack* are arranged in the same operon and they were reported to be constitutively expressed [100]. With CO_2 and H_2 as substrate, only acetate has been observed as major product [44], with minor amounts of ethanol produced in rare cases with *C. ljungdahlii* [101], *C. autoethanogenum* [53], or "*Moorella sp.*" [102, 103]. Using the more reduced substrate CO, production of a range of other products have been reported, such as ethanol, butanol, butyrate, 2,3-butanediol [104], and lactate (Figure 4.) [105]. From a biofuel perspective, ethanol and butanol are of particular interest. Ethanol and butanol have even been described as the main fermentation products over acetate in some acetogens under specific conditions. Ethanol producers include *C. ljungdahlii* [62, 63], *C. autoethanogenum* [53], *"C. ragsdalei"* ("*Clostridium* strain P11") [106, 107], "*Moorella sp.*" [102, 103], *Alkalibaculum bacchii* [44], *C. carboxidivorans* ("*Clostridium* strain P7") [54, 55], and *B. methylotrophicum* [49, 108]. The latter two have also been described to produce butanol.

Due to historical roles in ABE fermentation, organisms like *C. acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum*, and *C. saccharoperbutylacetonicum* have been much more extensively characterized than acetogenic Clostridia [95]. Since *C. acetobutylicum* was the first *Clostridium* to be fully sequenced [109] and it remains the most commonly used species for industrial production of solvents to date [110], it provides a model for study of solventogenesis. Although sugar- and starch-utilizing ABE Clostridia and acetogens exhibit clear distinctions in substrate utilization and thus metabolism, they share some similarities in the biochemical

pathway and genetic organization of product synthesis and can be used as model for comparison. Structure of key genes and operons (except for the absence of acetone biosynthetic genes) have been found to be very similar in sequenced acetogen *C. carboxidivorans* [54], and in respect of acetate and ethanol genes to some extent also in *C. ljungdahlii* [62]. For instance, the operon structure of *pta-ack*, *ptb-buk* and the *bcs* cluster of acetogen *C. carboxidivorans* are highly similar to starch-utilizing *C. acetobutylicum* and *C. beijerinckii* [54, 109] (Figure 5). Due to these reasons, solventogenic genes from starch-utilizing Clostridia are ideal targets for heterologous expression in acetogens for improvement of product yield and expansion of product range.

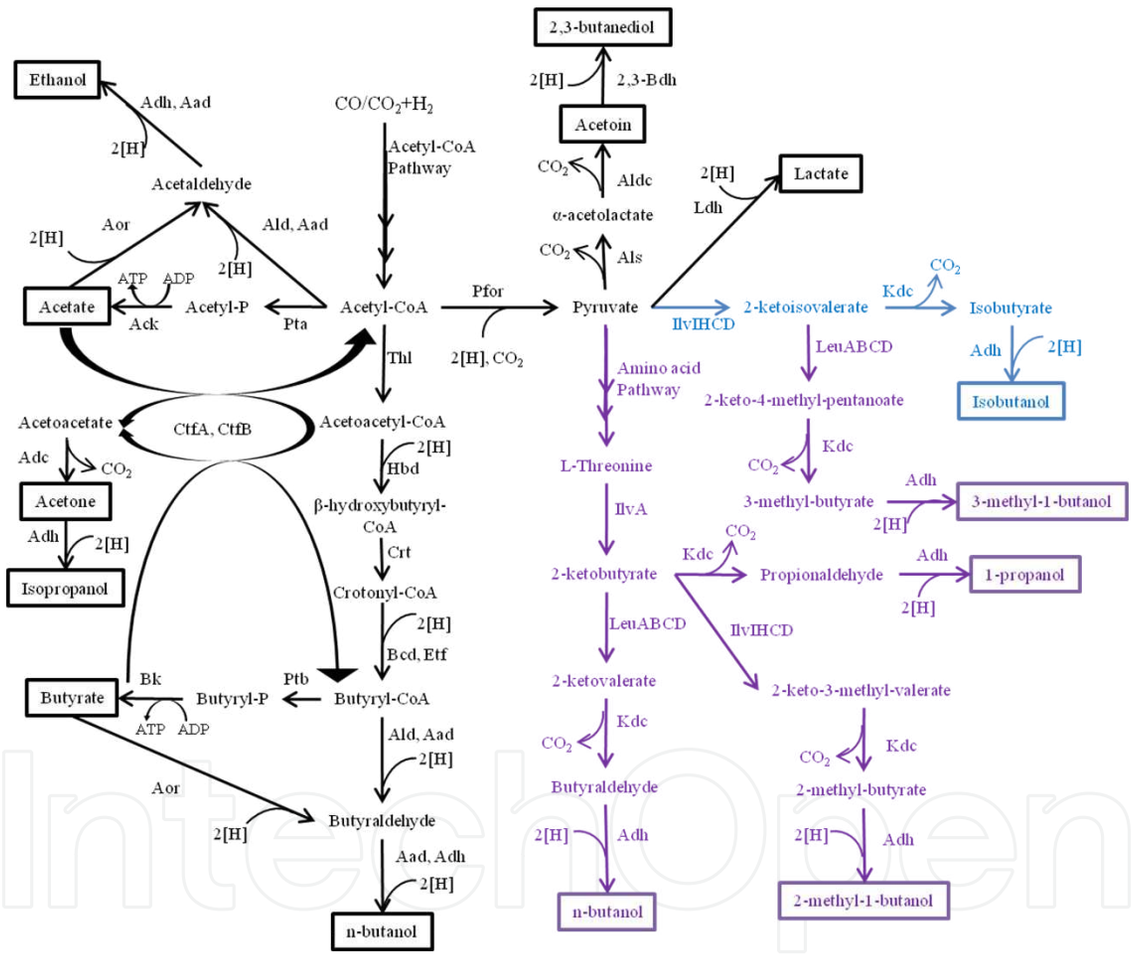

Figure 4. Scheme of metabolite production from gas fermentation using native and genetically modified Clostridia. Black denotes well-characterized pathways in Clostridia. Blue shows demonstrated heterologous pathways that have been engineered into Clostridia. Purple designates hypothetical pathways that can be engineered into Clostridia. Products are highlighted in boxes. Aad, aldehyde/alcohol dehydrogenase; Ack, acetate kinase; Adc, acetoacetate decarboxylase; Adh, alcohol dehydrogenase; Ald, aldehyde dehydrogenase; Aldc, acetolactate decarboxylase; Aor, aldehyde oxidoreductase; Bcd, butyryl-CoA dehydrogenase; Bk, butyrate kinase; Crt, crotonase; CtfA & CtfB, CoA transferase A & B; Etf, electron-transferring flavoprotein; Hbd, hydroxybutyryl-CoA dehydrogenase; IlvA, threonine deaminase; IlvIHCD, valine and isoleucine biosynthesis; Kdc, 2-ketoacid decarboxylase; Ldh, lactate dehydrogenase; LeuABCD, leucine and norvaline biosynthesis; Pfor, Pyruvate ferredoxin oxidoreductase; Pta, phosphotransacetylase; Ptb, phosphotransbutyrylase; Thl, thiolase; 2,3-Bdh, 2,3-butanediol dehydrogenase.

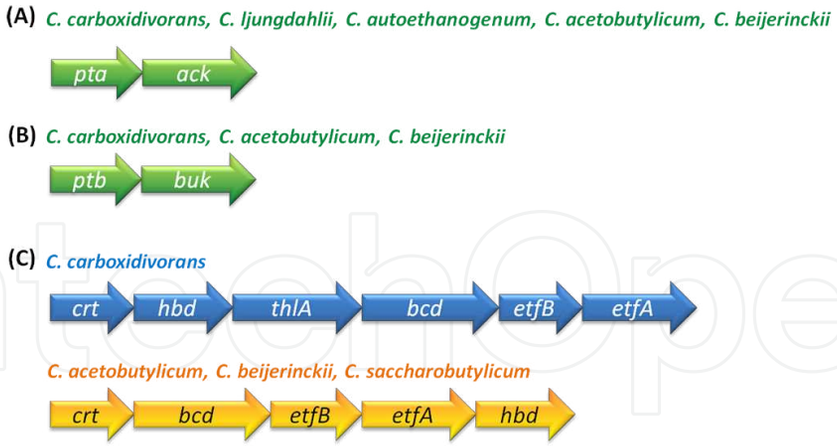

Figure 5. Similarity of acidogenesis and butanol formation gene clusters of acetogens and sugar-utilizing Clostridia. (A) Acetate-forming operon; (B) butyrate-forming operon; (C) butanol-forming operon. *ack*, acetate kinase; *buk,* butyrate kinase; *bcd*, butyryl-CoA dehydrogenase; *crt*, crotonase; *etfA,* electron-transferring flavoprotein subunit A; *etfB*, electron-transferring flavoprotein subunit B; *hbd,* 3-hydroxybutyryl-CoA dehydrogenase; *ptb*, phosphotransbutyrylase; *thlA*, thiolase.

Similar to sugar- and starch-utilizing ABE Clostridia, acetogens such as *C. carboxidivorans* [111, 112], *C. ljungdahlii* [113], and *C. autoethanogenum* [27] also typically undergo biphasic fermentation under autotrophic conditions. The first phase involves the production of carboxylic acids (acidogenic), H_2 and CO_2 during exponential growth. This is followed by the solventogenic phase in which part of the produced acids are reassimilated or reduced into solvents, which usually occurs during stationary growth phase [114]. This shift from acidogenesis to solventogenesis is of industrial importance and several transcriptional analysis on *C. acetobutylicum* [100, 115], and *C. beijerinckii* [116] have been performed to shed light on this process. In both organisms, the onset of solventogenesis coincides with an increase in expression of master sporulation/solventogenesis regulator gene *spo0A*, solventogenic genes such as *ald*, *ctfA-ctfB*, and *adc*, as well as down-regulation of chemotaxis/motility genes [100, 115, 116]. Physiologically, the signals that induce solventogenesis were hypothesized to involve temperature, low pH, high concentrations of undissociated acetic and butyric acids, limiting concentrations of sulphate or phosphate, ATP/ADP ratio and/or NAD(P)H levels [117].

For Clostridia such as acetogen *C. carboxidivorans* [54], which harbour the genes thiolase (*thlA*), 3-hydroxybutyryl-CoA dehydrogenase (*hbd*), crotonase (*crt*) and butyryl-CoA dehydrogenase (*bcd*), the two carbon acetyl-CoA can be converted to four carbon butyryl-CoA [95]. ThlA compete with the activities of Pta, Ald (aldehyde dehydrogenase), and PFOR to condense two acetyl-CoA into one acetoacetyl-CoA, and plays a key role in regulating the C2:C4 acid ratio [110, 118]. Since the formation of acetate yields twice as much ATP per mole of acetyl-CoA relative to butyrate formation, thiolase activity indirectly affects ATP yield [118]. Under physiological conditions, Crt catalyzes dehydration of β-hydroxybutyryl-CoA to crotonyl-CoA [119]. Bcd was shown to require a pair of electron transfer flavoproteins (EtfA and EtfB) to convert crotonyl-CoA to butyryl-CoA [120]. Furthermore, the Bcd was dem-

onstrated to form a stable complex with EtfA and EtfB, and they were shown to couple the reduction of crotonyl-CoA to butyryl-CoA with concomitant generation of reduced ferredoxins, which can be used for energy conservation via Rnf complex [94, 119]. Subsequent actions of phosphotransbutyrylase (*ptb*) and butyrate kinase (*buk*) then generate ATP and butyrate from butyryl-CoA [118].

Under low extracellular pH of 4-4.5, the secreted undissociated acetic acid (pK_a 4.79) and/or butyric acid (pK_a 4.82) diffuse back into cell cytoplasm and then dissociate into the respective salts and protons because of the more alkaline intracellular conditions. Without further interventions, the result of this is abolishment of the proton gradient and inevitable cell death [95]. The conversion of acetate and butyrate into solvents increase the pH, thus provide some time for the organism to sporulate and secure long term survival. However, the solvents produced are toxic because they increase membrane fluidity and disrupt critical membrane-associated functions such as ATP synthesis, glucose uptake and other transport processes [114, 121]. In *C. acetobutylicum*, it has been demonstrated that the addition of 7-13 g/l of butanol, or up to 40 g/l of acetone and ethanol resulted in 50% growth inhibition [122]. The bacterium is likely to experience a different cytotoxic effect from endogenously produced solvents because the organism has time to adapt to increasing amount of solvents.

The reassimilation of acetate and butyrate into the respective acyl-CoA and acetoacetate is catalyzed by acetoacetyl-CoA:acetate/butyrate CoA transferase (CtfA and CtfB) [110, 117, 118]. Acetoacetate is deconstructed by acetoacetate decarboxylase (Adc) into acetone and CO_2. This enzyme is missing in acetogenic *C. carboxidivorans* compared to the ABE strains [54, 123]. Some ABE strains such as *C. beijerinckii* NRRL B593 also possess a primary/secondary alcohol dehydrogenase that converts acetone to isopropanol [124]. In acetogenic "*C. ragsdalei*", reduction of acetone to isopropanol was also observed although the mechanism of this reduction is as yet unknown [124, 125]. Again, *C. carboxidivorans* lacks this activity [125]. The recycled acetyl-CoA and butyryl-CoA can be converted to ethanol and butanol through the actions of coenzyme A-acylating aldehyde dehydrogenase (Ald) and alcohol dehydrogenase (Adh) [110, 118]. Ald converts acyl-CoA into aldehydes, and the enzyme has been purified from *C. beijerinckii* NRRL B593 and was shown to be NADH-specific, exhibit higher affinity with butyraldehyde than acetaldehyde, but possess no Adh activity [126]. In *C. ljungdahlii*, two variants of aldehyde:ferredoxin oxidoreductases (AOR) are present in the genome, and they are hypothesized to couple reduced ferredoxin from CO oxidation via the CODH (see above) to perform the reversible reduction of acetate into acetaldehyde, which can be further reduced into ethanol [62].

The final step of solventogenesis utilizes Adh to reduce acetaldehyde and butyraldehyde into ethanol and butanol, respectively. For ethanol synthesis, transposon mutagenesis and enzymatic assay in *C. acetobutylicum* showed the involvement of a specific Ald that does not interact with butyryl-CoA, and a NAD(P)H-dependent Adh [127, 128]. The production of butanol by *C. acetobutylicum* is mainly due to the action of butanol dehydrogenase A and B (BdhA and BdhB), and bifunctional butyraldehyde/butanol dehydrogenase 1 and 2 (AdhE1 and AdhE2) [95]. In *C. carboxidivorans* [54] and *C. ljungdahlii* [62] both *adhE1* and *adhE2* are arranged in tandem and separated by a 200bp gap which contains a putative terminator [62,

111]. This is likely the result of gene duplication [62]. qRT-PCR analysis from *C. carboxidivorans* fed with syngas showed that the two *adhE* showed differential expression, and the more abundant *adhE2* was significantly upregulated over 1000 fold in a time span that coincided with the greatest rate of butanol production [111].

Pyruvate is a central molecule for anabolism and it is predominantly generated from glycolysis during heterotrophic growth. But under autotrophic growth, this four carbon molecule can be synthesized by PFOR and potentially also the pyruvate-formate lyase (PFL). Two variants of PFOR were reported in *C. autoethanogenum*, and transcriptional analysis showed that they were differentially expressed when grown using industrial waste gases (containing CO, CO_2 and H_2) [104]. Unlike PFL from most other microorganisms that only catalyze the lysis of pyruvate into formate and acetyl-CoA, clostridial PFL (*C. kluyveri, C. butylicum*, and *C. butyricum*) were reported to readily catalyze the reverse reaction (i.e. pyruvate formation) [129]. Apart from roles in anabolism, pyruvate is also a precursor to other products such as lactic acid and 2,3-butanediol. Small amounts of lactic acid are converted from pyruvate in acetogens, a reaction which is catalyzed by lactate dehydrogenase (Ldh) [104, 118]. Recently, Köpke *et al*. (2011) reported the production of 2mM 2,3-butanediol from acetogenic bacteria (*C. autoethanogenum*, *C. ljungdahlii*, and *C. ragsdalei*) using industrial waste gases (containing CO, CO_2 and H_2) as feedstock [104]. Pyruvate is first converted into α-acetolactate by the enzyme acetolactate synthase, followed by acetolactate decarboxylase which split acetolactate into acetoin and CO_2, before a final reduction of acetoin into 2,3-butanediol by 2,3-butanediol dehydrogenase [104] (Figure 4).

9. Strain improvement and metabolic engineering

The genomes of several solventogenic Clostridia, including gas fermenting species, have been sequenced since 2001 [54, 62, 109, 119, 123, 130], and an array of transcriptomic [100, 116, 121, 131, 132], proteomic [132] and systems analysis [133, 134] are being made increasingly available. However, the generation of stable recombinant Clostridia has been severely hindered by the difficulties encountered introducing foreign DNA into cells and a lack of established genetic tools for this genera of bacteria. In comparison to starch-utilizing Clostridia, very little information is available for metabolic engineering of acetogens. Although this section describes recent advances in the development of genetic tools for mostly sugar-utilizing Clostridia, these techniques are highly relevant and applicable to the closely related acetogenic Clostridia for biofuels or chemical production via gas fermentation.

The ideal microbial catalyst for industrial scale gas fermentation might exhibit the following traits: high product yield and selectivity, low product inhibition, no strain degeneration, asporogenous, prolonged cell viability, strong aero-tolerance, high biomass density and efficient utilization of gas substrates. These can be achieved by directed evolution, random mutagenesis and/or targeted genetic engineering. Traditionally, chemical mutagenesis [135-137] and adaption strategies [138, 139] have been deployed to select for these traits. However, these strategies are limited and often come with the expense of unwanted events.

First attempts of targeted genetic modification of Clostridia were made in the early 1990s by the laboratory of Prof. Terry Papoutsakis [140-142]. While these pioneering efforts relied on use of plasmids for (over)expression of genes in *C. acetobutylicum*, more sophisticated tools were later developed for a range of solventogenic and pathogenic Clostridia.

Antisense RNA (asRNA) has been employed to down-regulate genes. Here, single stranded RNA binds to a complementary target mRNA and prevents translation by hindering ribosome-binding site interactions [143]. For instance, this method has been used to knockdown *ctfB* resulting in production of 30 g/l solvents with significantly suppressed acetone yield in *C. acetobutylicum* ATCC 824 [144, 145].

Several homologous recombination methods have been developed for integration or knock-out of genes in a range of sugar-utilizing Clostridia. In early stage, knockout mutants were almost exclusively generated from single crossover events that could revert back to wild-type [146-152], with stable double crossovers only observed in rare cases [153, 154]. For *C. acetobutylicum* [155] and cellulolytic *C. thermocellum* [156] counter selectable markers have been developed to allow more efficient screening for the rare second recombination event.

ClosTron utilizes the specificity of mobile group II intron Ll.*ltrB* from *Lactoccocus lactis* to propagate into a specified site in the genome via a RNA-mediated, retro-homing mechanism which can be used to disrupt genes [157]. This technique has initially been developed by InGex and Sigma-Aldrich under the name 'TargeTron™' and successfully adapted to a range of solventogenic and pathogenic Clostridia including *C. acetobutylicum*, *C. difficile*, *C. sporogenes*, *C. perfringens*, and *C. botulinum* [158-160] by the laboratory of Prof. Nigel Minton.

The same laboratory recently also developed another method for integration of DNA into the genome. Termed Allele-Coupled Exchange (ACE), this approach does not employ a counter selective marker to select for the rare second recombination event. Rather, it utilizes the activation or inactivation of gene(s) that result in a selectable phenotype, and asymmetrical homology arms to direct the order of recombination events [161]. Remarkably, the whole genome of phage lambda (48.5kb minus a 6kb region) was successfully inserted into the genome of *C. acetobutylicum* ATCC 824 in three successive steps using this genetic tool. This technique was also demonstrated in *C. difficile* and *C. sporogenes* [161].

For reverse engineering, mainly transposon mutagenesis has been utilized. Earlier efforts of transposon mutagenesis were demonstrated in *C. acetobutylicum* P262 (now: *C. saccharobutylicum* [162]), *C. acetobutylicum* DSM792, *C. acetobutylicum* DSM1732, and *C. beijerinckii* NCIM 8052, but issues with multiple transposon insertions per mutant, and non-random distribution of insertion were reported [163, 164]. Recent developments have seen the successful generation of mono-copy random insertion of transposon *Tn1545* into cellulolytic *C. cellulolyticum* [165] and mariner transposon *Himar1* into pathogenic *C. difficile* [166].

While there is still a lack of some other essential metabolic engineering tools such as efficient inducible promoters, the array of available tools that enabled significant improvements to the ABE process and cellulolytic Clostridia fermentations as summarized in Table 3.

Organism	Genetic modification	Phenotypes/Effects	Ref
Acetogens			
C. ljungdahlii	Plasmid overexpression of butanol biosynthetic genes from *C. acetobutylicum* (*thlA, crt, hbd, bcd, adhE* and *bdhA*)	Produced 2 mM butanol from syngas	[62]
C. autoethanogenum	Plasmid overexpression of butanol biosynthetic genes from *C. acetobutylicum* (*thlA, crt, hbd, bcd*, *etfA, & etfB*)	Produced 26 mM butanol using steel mill gas	[167]
C. autoethanogenum	Plasmid expression of native *groES* and *groEL*	Increased alcohol tolerance	[168]
C. aceticum	Plasmid overexpression of acetone operon from *C. acetobutylicum* (*adc, ctfAB, thlA*)	Produced up to 140 μM acetone using gas	[169, 170]
Acidogenesis and Solventogenesis			
C. acetobutylicum	Inactivation of *buk* and overexpression of *aad*	Produced same amount of butanol as control but relatively more ethanol, corresponding to a total alcohol tolerance of 21.2 g/l	[171]
C. acetobutylicum	Inactivation of *hbd* using ClosTron	Produced 716 mM ethanol by diverting C4 products	[172]
C. acetobutylicum	Inactivation of *ack* using ClosTron	Reduction in acetate kinase activity by more than 97% resulted in 80% less acetate produced but similar final solvent amount	[173]
C. tyrobutylicum	Inactivation of *ack* and plasmid overexpression of *adhE2* from *C. acetobutylicum*	Produced 216 mM butanol	[174]
C. thermocellum	Inactivation of *ldh* and *pta* via homologous recombination	Showed 4 fold increase in ethanol yield (122 mM instead of 28 mM)	[156]
C. cellulolyticum	Inactivation of*ldh* and *mdh* (malate dehydrogenase) using ClosTron	Generated 8.5 times higher ethanol yield (56.4 mM) than wild type (6.5 mM)	[175]
C. acetobutylicum	Plasmid overexpression of a synthetic acetoneoperon (*adc, ctfA, ctfB*) and primary/secondary *adh* from *C. beijerinckii* NRRL B593	Produced 85 mM isopropanol	[176]

C. acetobutylicum	Genome insertion of *adh* gene from *C.beijerinckii* NRRL B593 using Allele-Coupled Exchange	Converted acetone into 28 mM isopropanol without affecting the yield of other fermentation products	[161]
Biosynthesis of New Products			
C. cellulolyticum	Plasmid overexpression of *kivD, yqhD, alsS, ilvC* and *ilvD*	Produced 8.9 mM isobutanol by diverting 2-ketoacid intermediates	[177]
C. acetobutylicum	Plasmid expression of native *ribGBAH* operon and mutated PRPP amidotransferase	Produced 70 mg/l riboflavin and 190 mM butanol	[178]
Solvent- and Aero-tolerance			
C. acetobutylicum	Plasmid overexpression of glutathione *gshA* and *gshB* from *E. coli*	Improved aero- and solvent-tolerance	[179]
C. acetobutylicum	Plasmid overexpression of chaperone *groESL*	Showed 85% decrease in butanol inhibition and 33% increase in solvent yield	[180]
Substrate Utilization			
C. acetobutylicum	Plasmid expression of *acsC, acsD* and *acsE* from *C. difficile*	Increased incorporation of CO_2 into extracellular products	[99]
C. saccharoperbutylacetonicum strain N1-4	Knockdown hydrogenase *hupCBA* expression using siRNA delivered from plasmid	Significantly reduced hydrogen uptake activity to 13% (relative to control strain)	[181]

Table 3. Genetically modified solventogenic Clostridia

In contrast, to date only a limited number of acetogenic Clostridia have been successfully modified. Pioneering work in this area has been undertaken in the laboratory of Prof. Peter Dürre. *C. ljungdahlii*, a species that does not naturally produce butanol, was modified with butanol biosynthetic genes (*thlA*, *hbd*, *crt*, *bcd*, *adhE* and *bdhA*) from *C. acetobutylicum* ATCC 824 resulting in production of up to 2 mM of butanol using synthesis gas as sole energy and carbon source [62]. By delivering a plasmid with acetone biosynthesis genes *ctfA*, *ctfB*, *adc*, and *thlA* in *C. aceticum*, production of up to 140 μM acetone was demonstrated from various gas mixes (80% H_2/20% CO_2 and 67% H_2/33% CO_2) [169, 170]. Recent patent filings by Lanza-Tech describe the production of butanol as main fermentation product and increased alcohol tolerance in genetically engineered acetogens. Up to 26 mM butanol were produced with genetically modified *C. ljungdahlii* and *C. autoethanogenum* using steel mill gas (composition 44% CO, 32% N_2, 22% CO_2, and 2% H_2) as the only source of carbon and energy when the butanol biosynthetic genes *thlA, hbd, crt, bcd, etfA,* and *etfB* were heterologously expressed [167]. Overexpression of native *groESL* operon in *C. autoethanogenum* resulted in a strain that displayed higher alcohol tolerance relative to wild-type when challenged with ethanol [168].

Besides the classical Clostridial butanol pathway (which constitutes genes *thlA, crt, hbd, bcd, etfA and etfB*; see earlier section), a non-fermentative approach has been described and demonstrated in *E. coli* for branched chain higher alcohol production [182]. This alternative approach requires a combination of highly active amino acid biosynthetic pathway and artificial diversion of 2-keto acid intermediates into alcohols by introduction of two additional genes: broad substrate range 2-keto-acid decarboxylase (*kdc*) which converts 2-keto acids into aldehydes, followed by *Adh* to form alcohols [182]. Engineered strains of *E. coli* have been shown to produce alcohols such as isobutanol, n-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol via this strategy [182]. For instance, the overexpression of *kivD* (KDC from *Lactococcus lactis*), *adh2, ilvA,* and *leuABCD* operon, coupled with deletion of *ilvD* gene and supplementation of L-threonine, increased n-butanol yield to 9 mM while producing 10 mM of 1-propanol [182].An even more remarkable yield of 300 mM isobutanol was achieved through introduction of k*ivD, adh2, alsS* (from *B. subtilis*), and *ilvCD* into *E. coli* [182]. Like butanol, isobutanol exhibits superior properties as a transportation fuel when compared to ethanol [177]. By applying similar strategy into *C. cellulolyticum,* 8.9 mM isobutanol was produced from cellulose when k*ivD, yqhD, alsS, ilvC,* and *ilvD* were overexpressed [177]. This result suggests that such non-fermentative pathway is suitable target for metabolic engineering of acetogens for the biosynthesis of branched chain higher alcohols. Via synthetic biology and metabolic engineering, production of additional potential liquid transportation fuels like farnesese or fatty acid based fuels has successfully been demonstrated in *E. coli* or yeast from sugar [183, 184]. Given the unsolved energetics in acetogens, it is unclear if production of such energy dense liquid fuels could be viable via gas fermentation.

10. Fermentation and product recovery

10.1. Bioreactor design

An optimum gas fermentation system requires efficient mass transfer of gaseous substrates to the culture medium (liquid phase) and microbial catalysts (solid phase). Gas-to-liquid mass transfer has been identified as the rate-limiting step and bottleneck for gas fermentation because of the low aqueous solubility of CO and H_2, respectively at only 77% and 68% of that of oxygen (on molar basis) at 35°C [185]. Hence, a bioreactor design that delivers sufficient gas-to-liquid mass transfer in an energy-efficient manner at commercial scale for gas fermentation represents a significant engineering challenge. A brief overview of reactor configurations reported in gas fermentation operations is given below.

10.2. Continuous Stirred Tank Reactor (CSTR)

In continuous stirred tank reactor (CSTR), gas substrates are continuously fed into the reactor and mechanically sheared by baffled impellers into smaller bubbles, which has greater interfacial surface area for mass transfer [16]. In addition, finer bubbles have a slower rising velocity and a longer retention time in the aqueous medium, resulting in higher gas-to-liq-

uid mass transfer [24]. Fermentation reactions using *C. ljungdahlii* have been successfully maintained in a 2 litre CSTR under autotrophic conditions for more than a month, while achieving peak ethanol level of 6.5 g/l and CO conversion rate of 93% [186]. The production of 49 g/l of ethanol from gas substrates using *C. ljungdahlii* was demonstrated using CSTR [113]. In another example, a 100 litre stirred tank reactor was demonstrated to produce up to 24.57 g/l ethanol, 9.25 g/l isopropanol and 0.47 g/l n-butanol during a 59-day semi-batch gas fermentation using "*C. ragsdalei*" strain P11 as biocatalysts [112]. An improved version of CSTR incorporates microbubble sparger to generate finer bubbles to achieve higher mass transfer coefficient [187]. Although CSTR offers complete mixing and uniform distribution of gas substrates to the microbes, the high power per unit volume required to drive the stirrer are thought to make this approach economically unviable for commercial scale gas fermentation systems [187].

10.3. Bubble column reactor

In contrast to CSTR, gas mixing in bubble column reactor is achievable by gas sparging, without mechanical agitation. This reactor configuration has fewer moving parts, and consequently has a lower associated capital and operational costs while exhibiting good heat and mass transfer efficiencies, making it a good candidate for large scale gas fermentation [17]. However, excessive level of gas inflow for enhanced mixing have been cited as an issue that leads to heterogeneous flow and back-mixing of the gas substrates [16, 17]. *C. carboxidivorans* strain P7 was cultured in a 4 litre bubble column reactor for 20 days using a combination of producer gas and synthetic syngas, generating a peak ethanol concentration of 6 g/l [13].

10.4. Immobilized cell column reactor

One of the key challenges of gas fermentation is cell density. Immobilization of microbes through crosslinking or adsorption to insoluble biosupport materials and the subsequent packing within the column offers a range of benefits [14]. These include high cell densities, plug flow operation, high mass transfer rate via direct contact between microbe and gas, reduction of retention time, and operation without mechanical agitation [14, 16]. However, channelling issues may arise when the microbe overgrows and completely fill the interstitial space. Due to limitations in column dimensions and packing, this reactor configuration lacks flexibility to operate in various gas fermentation conditions [14, 16].

10.5. Trickle-bed reactor

Trickle-bed reactor is a gas- or liquid- continuous reactor consisting of packed bed, which liquid culture trickles down through packing media containing suspended or immobilized cells [16, 24, 187]. The gas substrate is delivered either co-currently or counter-currently to the liquid flow, and no mechanical agitation is required [187].In this reactor format, low gas and liquid flow rates are typically applied, generating relatively low pressure drops [187]. Trickle-bed reactor was found to exhibit excellent gas conversion rates and higher productivities than CSTR and bubble column reactor [15].

11. Gas fermentation parameters

11.1. Gas composition

The gas composition and its impurities can have an impact on the productivity of the gas fermentation process. Greater molar ratio of H_2:CO allows greater efficiency in the conversion of the carbon from CO into products such as ethanol, because reducing equivalents are generated from oxidation of H_2 (rather than CO). However, CO is also a known inhibitor of hydrogenase which can affect utilization of H_2 during fermentation. In *B. methylotrophicum*, H_2 utilization was inhibited until CO was exhausted [108]. When CO is consumed, acetogens are able to grow using CO_2 and H_2. Common impurities from biomass gasification or other waste gases are tar, ash, char, ethane, ethylene, acetylene, H_2S, NH_3 and NO [17, 22, 24, 188].These have been shown to cause cell dormancy, inhibition of hydrogen uptake, low cell growth and shift between acidogenesis and solventogenesis in acetogens [13, 188]. For instance, NH_3 from the feed gas readily convert into NH_4^+ in the culture media and these ions were recently shown to inhibit hydrogenase and cell growth of acetogen "*C. ragsdalei*" [189]. A number of strategies to mitigate the impact of such impurities have been proposed, for example installing 0.025 mm filters, or the use of gas scrubbers or cyclones, and improvement in gasification efficiency and scavenging for contaminants in the gas stream using agents such as potassium permanganate, sodium hydroxide or sodium hypochlorite [24, 190-192]. H_2S does not have a negative effect on acetogens such as *C. ljungdahlii* up to 5.2% (v/v) [193].

11.2. Substrate pressure

The partial pressure of syngas components have a major influence on microbial growth and product profiles because the enzymes involved are sensitive to substrate exposure [194]. Due to the low solubility of CO and H_2 in water, the growth of dense bacterial cell cultures can face mass transfer limitations, so increasing the partial pressure of gaseous substrates can help alleviate this problem. For instance, studies in which the CO partial pressure (P_{CO}) increased from 0.35 to 2.0 atm showed that this resulted in a 440% increase in maximum cell density, a significant increase in ethanol productivity and a decrease in acetate production in *C. carboxidivorans* strain P7 [195]. In another study involving *C. ljungdahlii*, the increase of P_{CO} from 0.8 to 1.8 atm had a positive effect on ethanol production, and the microbe did not exhibit any substrate inhibition at high P_{CO} [196].In less CO-tolerant microorganisms, the effect of increasing P_{CO} partial pressure range from non-appreciable in the case of *Rhodospirillum rubrum* [197], to negative impact on doubling time of *Peptostreptococcus productus* (now: *Blautia product*) [194] and *Eubacterium limosum* [198]. Similar to CO, the increase in partial pressure of H_2 (pH_2) to 1700 mbar enhanced acetate productivity of *A. woodii* to 7.4g acetate/l/day [199].

11.3. Medium formulation

Although acetogens are able of utilizing CO and CO_2/H_2 as carbon and energy source, other constituents such as vitamins, trace metal elements, minerals and reducing agents are also required for maintenance of high metabolic activity [16, 113]. Studies indicated that formation of ethanol in solventogenic Clostridiais non-growth associated and limitation of growth

by reducing availability of carbon-, nitrogen- and phosphate- nutrients shift the balance from acidogenesis to solventogenesis [113, 200, 201]. Optimization of medium formulation for *C. ljungdahlii* through reduction of B-vitamin concentrations and elimination of yeast extract significantly enhanced the final ethanol yield to 48 g/l in a CSTR with cell recycling (23 g/l without cell recycling) [113]. Another study by Klasson *et al.* showed thatthe replacement of yeast extract with cellobiose not only increased maximum cell concentration, but also enhanced ethanol yield by 4-fold [14]. Media formulation for *C. autoethanogenum* was investigated using Plackett-Burman and central composite designs, but only low ethanol yield was recorded overall [202]. In an attempt to reduce the cost of fermentation medium and improve process economics, 0.5 g/l of cotton seed extract without other nutrient supplementation was shown to be a superior medium for *C. carboxidivorans* strain P7 in producing ethanol from syngas fermentation [203]. A recent study showed that increasing concentrations of trace metal ions such as Ni^{2+}, Zn^{2+}, SeO_4^-, WO_4^-, Fe^{2+} and elimination of Cu^{2+} from medium improved enzymatic activities (FDH, CODH, and hydrogenase), growth and ethanol production in "*C. ragsdalei*" under autotrophic conditions [107].

A low redox potential is necessary for strict anaerobes to grow, hence reducing agents such as sodium thioglycolate, ascorbic acid, methyl viologen, benzyl viologen, titanium (III)–citrate, potassium ferricyanide, cysteine-HCl and sodium sulfide are commonly added to fermentation medium [14, 16, 204]. Furthermore, the addition of reducing agent directs the electron and carbon flow towards solventogenesis by enhancing the availability of reducing equivalents to form NADH for alcohol production [16, 205]. Excessive addition of reducing agents can cause slower microbial growth due to reduced ATP formation from acetogenesis so it is important to determine the optimum concentration of reducing agents [14, 16]. The sulfur containing gases (e.g. H_2S) present in syngas are toxic to chemical catalysts but can be beneficial for microbial catalysts by reducing medium redox potential, stimulate redox sensitive enzymes such as CODH, and promote alcohol formation [206, 207].

11.4. Medium pH

Like other organisms, acetogens have a limited range of pH for optimal growth so the pH of the fermentation medium needs to be closely controlled. The extracellular pH directly influences the intracellular pH, membrane potential, proton motive force, and consequently substrate utilization and product profile [208, 209]. In most studies, lowering pH medium divert carbon and electron flow from cell and acid formation towards alcohol production [113, 209-211]. By applying this knowledge, Gaddy and Clausen performed a two-stage CSTR syngas fermentation systems using *C. ljungdahlii* where they set the first reactor at pH 5 to promote cell growth, and pH 4 - 4.5 in the second reactor to induce ethanol production [212]. One recent study with *C. ljungdahlii* showed conflicting results in which cell density and ethanol production were both higher at pH 6.8 when compared to pH 5.5 [213].

11.5. Temperature

The optimum temperature for mesophilic acetogens are between 30-40˚C, while thermophilic acetogens grow best between 55 and 58˚C. The fermentation temperature not only affects

substrate utilization, growth rate and membrane lipid composition of the acetogens, but also gas substrate availability because gas solubility increases with decreasing temperature [24, 211]. "*C. ragsdalei*"was reported to produce more ethanol at 32˚C than at the optimum growth temperature of 37˚C [211].

12. Cell separation and product recovery

To retain high cell densities in reactor, microbes can be grown as biofilm attached to carrier material. Planktonic cells can be retained in the fermentation broth by installing solid/liquid separators such as membranous ultra-filtration units, spiral wound filtration systems, hollow fibres, cell-recycling membranes and centrifuges [214-216]. The concentrations of solvents from gas fermentation rarely exceed 6% [w/v] so a cost- and energy- efficient product recovery process is required. Furthermore, acetogens also exhibit low resistance towards solvents like ethanol [217, 218] and butanol [219, 220] so an *in situ*/online product recovery system can enhance solvent productivity by decreasing solvent concentrations (and hence toxicity) in the fermentation broth. Distillation has been the traditional method of product recovery but the associated high energy costs have led to the development of alternative methods such as liquid-liquid extraction, pervaporation, perstraction, and gas stripping [24, 221].

12.1. Liquid-liquid extraction

In liquid-liquid extraction, a water-insoluble organic extractant is mixed with the fermentation broth [222]. Because solvents are more soluble in the organic phase than in the aqueous phase, they get selectively concentrated in the extractant.Although this technique does not remove water or nutrients from the fermentation broth, some gaseous substrates might be removed because CO and H_2 have much higher solubility in organic solvents than water [222, 223]. Oleyl alcohol has been the extractant of choice due to its relatively non-toxicity [224].

12.2. Perstraction

Liquid-liquid extraction is associated with several problems including toxicity to the microbes, formation of emulsion, and the accumulation of microbes at the extractant and fermentation broth interphase [222]. In an attempt to remediate these problems, perstraction was developed and this technique employs membrane to separate the extractant from the fermentation broth. This physical barrier prevent direct contact between the microbe and the toxicity of extractant, but it can also limit the rate of solvent extraction and is susceptible to fouling [219, 221]

12.3. Pervaporation

In a product recovery technique termed pervaporation, a membrane that directly comes in contact with fermentation broth is used to selectively remove volatile compounds such as ethanol and butanol [219, 222]. The volatile compounds diffuse through the membrane as

vapour and are then collected by condensation. To facilitate volatilization of permeates into vapour, a partial pressure difference across the membrane is usually maintained by applying a vacuum or inert gas (e.g. N_2) across the permeate side of the membrane [219]. Polydimethylsiloxane (PDMS) is the current material of choice for the membrane, but other materials such as poly(1-trimethylsilyl-1-propyne) (PTMSP), hydrophobic zeolite membranes, and composite membranes have also been investigated [225].

12.4. Gas stripping

Gas stripping is an attractive product recovery method for gas fermentation because the exit gas stream from the bioreactor can be used for *in situ*/online product recovery [219]. Following product recovery via condensation, the effluent and gas can be recycled back into the bioreactor. In sugar-based fermentation using *C. beijerinckii* mutant strain BA101, *in situ* gas stripping was shown to improve ABE productivity by 200%, complete substrate utilization and also complete acid conversion into solvents, when compared to non-integrated process [226].

13. Commercialization

The growing commercial interests in using gas fermentation as a platform for biofuels production is evident in the recent spike in patent fillings within the field [105]. A 2009 report compared mass and energy conversion efficiencies from a process engineering standpoint between enzymatic hydrolysis fermentation of lignocellulose, syngas fermentation and FTP [227]. The authors concluded that while syngas fermentation offers a range of advantages such as low pretreatment requirement and low energy requirement for bioconversion, the technology is severely limited by low ethanol productivity [227]. Another report documented the techno-economic analysis of gas fermentation and concluded that the selling price of ethanol using this technology would still be significantly higher than gasoline in 2009 [228]. In contrast, Griffin and Schultz recently compared the production of ethanol from CO-rich gas using thermo-chemical route and biological gas fermentation route [22]. The authors concluded that gas fermentation offers superior fuel yield per volume of biomass feed, carbon conversion to fuel, energy efficiency and lower carbon emissions relative to the thermochemical approach to bioethanol production.

Ethanol and butanol are the most attractive fuel products from current gas fermentation but other by-products such as 2,3-butanediol, acetic acid and butyric acid are also valuable commodities that have the potential to provide significant additional revenue streams, setting off costs for biofuel production. 2,3-butanediol is a high value commodity which can be used to synthesize chemical products such as 1,3-butanediane, methyl ethyl ketone, and gamma butyrolactone, with a combined potential market value of $43 billion [104]. Acetic acid is an important precursor for synthesis of polymers while butyric acid can be used as a flavouring agent in the food industry [229, 230]. With the development of advanced genetic

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tools for expansion of product range, the industry might witness an increasing emphasis on the production of high-value commodities in addition to biofuels.

Several companies are actively engaged in the development of the gas fermentation technology and some are approaching commercialization. Bioengineering Resources Inc (BRI) founded by Prof. James Gaddy of University of Arkensas, Fayetteville, an early pioneer in the investigation of gas fermentation at scale, was the first company to explore the potential of gas fermentation for industrial bioethanol production. BRI was acquired by chemical company INEOS and rebranded as INEOS Bio (www.ineosbio.com). A pilot-scale facility in Arkansas has been operated since 2003 using several isolates of *C. ljungdahlii* [231] and is building a US$130 million commercial facility in Florida with its joint venture partner New Planet Energy Florida [232]. The commercial facility is expected to start operation in the second quarter of 2012 and is aiming to generate 8 million gallon of cellulosic ethanol per annum and 6 MW of power to the local communities [232]. INEOS Bio also announced design of a second plant, the Seal Sands Biorefinery in Teeside, UK [233].

Founded in 2006, Coskata Inc. (www.coskata.com) is a US-based company that has reported achieving ethanol yields of 100 gallons per dry ton of wood biomass in a semi-commercial facility in Pennsylvania [234]. The company licensed several microbial strains from the University of Oklahoma [235], which has filed patents and journal publications for acetogens such as "*C. ragsdalei*" [211, 236, 237] and *C. carboxidivorans* [55, 112]. A patent documenting a new ethanologenic species, "*C. coskatii*" was also recently filed by Coskata [238]. Backed by a conditional US$250 million loan guarantee from the US Department of Agriculture (USDA), Coskata has announced that it is planning to build a commercial plant with the capacity to produce 55 million gallon fuel grade ethanol per annum in Alabama [234, 239]. While the initial strategy saw biomass as feedstock, the company recently announced its first commercial plant will be switched to 100% natural gas as feedstock [240]. A planned IPO with the aim to tap into private investors to finance the plant was put on hold [241]. In 2012, Coskata and INEOS Bio were involved in a trade secret dispute which culminated in a settlement that see INEOS Bio receiving US$2.5 million cash payment, shares and right to receive 2.5% of future ethanol royalties from Coskata [242].

LanzaTech is a NZ/US based company that has developed a gas fermentation technology to utilize industrial off-gases from steel making and other sources, as well as syngas produced from biomass as feedstocks. The company has reported the development of a proprietary Clostridial biocatalyst that is able to convert the CO-rich waste gas with minimal gas conditioning into bioethanol and the platform chemical 2,3-butanediol. The use of industrial off-gases as feedstock not only helps to reduce the carbon footprint of the steel-making operations but also allows the production of valuable commodities without the costs associated with feedstock gasification. The company has estimated that up to 30 billion gallon of bioethanol per year can be produced from the CO-rich off gases produced through steel manufacturers globally [243]. Founded in 2005, LanzaTech has successfully demonstrated bioethanol production at a pilot plant at BlueScope Steel in Glenbrook, NZ, since 2008 and the company has recently started operating its 100,000 gallon bioethanol per year demonstration facility in Shanghai, China, using waste gas collected from an ad-

jacent steel mill plant owned by its partner Baosteel Group [243, 244]. LanzaTech is planning to build a commercial facility with the capacity to produce 50 million gallon of bioethanol per annum in China by 2013 [243]. The recent acquisition of a biorefinery facility developed by the US-based gasification technology company Range Fuels in Georgia, and a milestone signing of its first commercial customer, Concord Enviro Systems (India), highlighted LanzaTech's intention to utilize MSW and lignocellulosic waste as feedstocks for biofuel and chemical production [243, 244].

14. Conclusion

One of the fundamental factors that govern the environmental and economical sustainability of biofuel production is feedstock. Through gasification, a spectrum of renewable non-food feedstock such as agricultural wastes, dedicated energy crops, forest residues, and MSW can be converted into syngas. This article presents a detailed examination of gas fermentation technology in capturing the carbon and energy from syngas and produce biofuels and chemicals. In comparison to indirect fermentation of lignocellulose via enzymatic hydrolysis, and thermo-chemical FTP, gas fermentation offers several advantages such as good product yield and selectivity, operation in ambient conditions, high tolerance to gas impurities, and elimination of expensive pre-treatment steps and costly enzymes. Furthermore, some industries such as steel mill, natural gas steam reforming, oil refining and chemical production generate large volumes of CO-rich off-gas. Gas fermentation can access these existing feedstocks and generate valuable products from these while reducing carbon emissions. Pivotal to gas fermentation is acetogens such as *C. ljungdahlii*, *C. carboxidivorans*, "*C. ragsdalei*" and *C. autoethanogenum*, which are able to metabolize CO, and CO_2/H_2 into a range of products such as ethanol, butanol, isopropanol, acetone, 2,3-butanediol, acetic acid and butyric acid. Sustained effort in studying the physiology and biochemistry using advanced molecular techniques such as genomics, transcriptomics, proteomics, metabolomics and systems biology are essential to further the understanding of these microbes. Furthermore, recent advances in Clostridial genetic tools offer endless opportunities to engineer strains that have improved product yield, substrate utilization, no strain degeneration, and synthesis of new products.

The main challenges associated with commercialization of gas fermentation have been identified as gas-to-liquid mass transfer limitation, product yield, substrate utilization efficiency, low biomass density and product recovery. Further development of bioreactor is necessary to improve the availability of gas substrates and maintain high cell density for higher productivity. Improvement in integrated product recovery technology is also essential to lower the costs of product recovery and alleviate product inhibition. Gas fermentation appears to be mature enough for commercialization since several companies have already demonstrated their technologies at pilot scale and are moving towards commercialization in the near future.

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References

[1] Vasudevan PT, Gagnon MD, Briggs MS. Environmentally sustainable biofuels – The case for biodiesel, biobutanol and cellulosic ethanol. In: Singh OV, Harvey SP, editors. Sustainable Biotechnology: Sources of Renewable Energy: Springer Science +Business Media B.V.; 2010. p. 43-62.

[2] Appenzeller T. The end of cheap oil. National Geographic Magazine. 2004.

[3] European_Union. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. 2009.

[4] REN21. Renewables 2011 Global Status Report. Paris: REN21, 2011.

[5] Demirbas A. Political, economic and environmental impacts of biofuels: A review. Applied Energy. 2009 Nov;86:S108-S17. PubMed PMID: WOS:000271170300013.

[6] Köpke M, Noack S, Dürre P. The past, present, and future of biofuels – Biobutanol as Promising Alternative. In: dos Santos Bernades MA, editor.: InTech; 2011. p. 451-86.

[7] Pernick R, Wilder C, Winnie T. Clean Energy Trends 2012. Clean Edge Inc., 2012.

[8] Dürre P. Biobutanol: an attractive biofuel. Biotechnology journal. 2007;2(12):1525-34.

[9] OECD-FAO. OECD-FAO Agricultural Outlook 2011-2020. 2011.

[10] Murse T. Understanding the ethanol subsidy 2011. Available from: http://usgovinfo.about.com/od/moneymatters/a/The-Federal-Ethanol-Subsidy.htm.

[11] Pear R. After three decades, tax credit for ethanol expires. The New York Times. 2nd January 2012.

[12] Carere CR, Sparling R, Cicek N, Levin DB. Third generation biofuels via direct cellulose fermentation. International Journal of Molecular Sciences. 2008 Jul;9(7):1342-60. PubMed PMID: WOS:000257986800014.

[13] Datar RP, Shenkman RM, Cateni BG, Huhnke RL, Lewis RS. Fermentation of biomass-generated producer gas to ethanol. Biotechnology and bioengineering. 2004;86(5):587-94.

[14] Klasson KT, Ackerson MD, Clausen EC, Gaddy JL. Bioreactor design for synthesis gas fermentations. Fuel. 1991 May;70(5):605-14. PubMed PMID: WOS:A1991FL49400009.

[15] Klasson KT, Ackerson MD, Clausen EC, Gaddy JL. Bioconversion of synthesis gas into liquid or gasesous fuels. enzyme and microbial technology. 1992 Aug;14(8):602-8. PubMed PMID: WOS:A1992JE44900001.

[16] Mohammadi M, Najafpour GD, Younesi F, Lahijani P, Uzir MH, Mohamed AR. Bioconversion of synthesis gas to second generation biofuels: A review. Renewable & Sustainable Energy Reviews. 2011 Dec;15(9):4255-73. PubMed PMID: WOS: 000298764400005.

[17] Abubackar HN, Veiga MC, Kennes C, Coruña L. Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol. Biofuels, Bioproducts & Biorefining. 2011:93-114.

[18] Siedlecki M, de Jong W, Verkooijen AHM. Fluidized bed gasification as a mature and reliable technology for the production of bio-syngas and applied in the production of liquid transportation fuels-A review. Energies. 2011 Mar;4(3):389-434. PubMed PMID: WOS:000288788000002.

[19] Wei L, Pordesimo LO, Haryanto A, Wooten J. Co-gasification of hardwood chips and crude glycerol in a pilot scale downdraft gasifier. Bioresource Technology. 2011 May; 102(10):6266-72. PubMed PMID: WOS:000291125800097.

[20] Dudynski M, Kwiatkowski K, Bajer K. From feathers to syngas - Technologies and devices. Waste Management. 2012 Apr;32(4):685-91. PubMed PMID: WOS: 000302822500007.

[21] Sipma J, Henstra AM, Parshina SM, Lens PN, Lettinga G, Stams AJM. Microbial CO conversions with applications in synthesis gas purification and bio-desulfurization. Critical reviews in biotechnology. 2006;26(1):41-65.

[22] Griffin DW, Schultz MA. Fuel and chemical products from biomass syngas: A comparison of gas fermentation to thermochemical conversion routes. Environmental Progress & Sustainable Energy. 2012 Jul;31(2):219-24. PubMed PMID: WOS: 000302794000008.

[23] McKendry P. Energy production from biomass (part 3): gasification technologies. Bioresource Technology. 2002 May;83(1):55-63. PubMed PMID: WOS:000175355300006.

[24] Munasinghe PC, Khanal SK. Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. Bioresour Technol. 2010;101(13):5013-22.

[25] Bartish CM, Drissel GM. Carbon monoxide. In: Othmer K, editor. Encyclopedia of Chemical Technology. New York: John Wiley and Sons; 1978.

[26] van Houten RT, Lettinga G. Biological sulphate reduction with synthesis gas: Microbiology and technology. Immobilized Cells: Basics and Applications. 1996;11:793-9. PubMed PMID: WOS:A1996BF78D00106.

[27] Köpke M, Mihalcea C, Liew FM, Tizard JH, Ali MS, Conolly JJ, et al. 2,3-butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. Applied and Environmental Microbiology. 2011 Aug;77(15): 5467-75. PubMed PMID: WOS:000293224500045.

[28] van der Drift A, van Doorn J, Vermeulen JW. Ten residual biomass fuels for circulating fluidized-bed gasification. Biomass & Bioenergy. 2001;20(1):45-56. PubMed PMID: WOS:000167001400006.

[29] Gordillo G, Annamalai K. Adiabatic fixed bed gasification of dairy biomass with air and steam. Fuel. 2010 Feb;89(2):384-91. PubMed PMID: WOS:000271295400017.

[30] Boateng AA, Banowetz GM, Steiner JJ, Barton TF, Taylor DG, Hicks KB, et al. Gasification of Kentucky bluegrass (Poa pratensis l.) straw in a farm-scale reactor. Biomass & Bioenergy. 2007 Feb-Mar;31(2-3):153-61. PubMed PMID: WOS:000244170800006.

[31] Bridgwater T. Biomass for energy. Journal of the Science of Food and Agriculture. 2006 Sep;86(12):1755-68. PubMed PMID: WOS:000240412500004.

[32] Swanson RM, Platon A, Satrio JA, Brown RC. Techno-economic analysis of biomass-to-liquids production based on gasification. Fuel. 2010 Nov;89:S2-S10. PubMed PMID: WOS:000282368600002.

[33] Drake HL, Gössner AS, Daniel SL. Old acetogens, new light. Annals of the New York Academy of Sciences. 2008;1125:100-28.

[34] Thauer RK. Microbiology - A fifth pathway of carbon fixation. Science. 2007 Dec; 318(5857):1732-3. PubMed PMID: WOS:000251616800025.

[35] Drake HL, Küsel K, Matthies C. Acetogenic prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, editors.: Springer New York; 2006. p. 354-420.

[36] Lindahl PA, Chang B. The evolution of acetyl-CoA synthase. Origins of Life and Evolution of the Biosphere. 2001;31(4-5):403-34. PubMed PMID: WOS:000170975700006.

[37] Ragsdale SW. Enzymology of the Wood-Ljungdahl pathway of acetogenesis. Incredible Anaerobes: from Physiology to Genomics to Fuels. 2008;1125:129-36. PubMed PMID: WOS:000255395600007.

[38] Ragsdale SW. Life with carbon monoxide. Critical Reviews in Biochemistry and Molecular Biology. 2004 May-Jun;39(3):165-95. PubMed PMID: WOS:000223849300003.

[39] Ragsdale SW, Clark JE, Ljungdahl LG, Lundie LL, Drake HL. Properties of purified carbon monoxide dehydrogenase from Clostridium thermoaceticum, a nickel, iron-sulfur protein. Journal of Biological Chemistry. 1983;258(4):2364-9. PubMed PMID: WOS:A1983QD12900054. English.

[40] Morsdorf G, Frunzke K, Gadkari D, Meyer O. Microbial growth oncarbon monoxide. Biodegradation. 1992;3:61-82.

[41] Genthner BRS, Bryant MP. Additional characteristics of one-carbon-compound utilization by Eubacterium limosum and Acetobacterium woodi. Applied and Environmental Microbiology. 1987 Mar;53(3):471-6. PubMed PMID: WOS:A1987G206800001.

[42] Poehlein A, Schmidt S, Kaster AK, Goenrich M, Vollmers J, Thurmer A, et al. An ancient pathway combining carbon dioxide fixation with the generation and utilization of a sodium ion gradient for ATP synthesis. Plos One. 2012 Mar;7(3). PubMed PMID: WOS:000304523400022.

[43] Kane MD, Breznak JA. Acetonema longum gen nov SP-nov, an H2/CO2 acetogenic bacterium from the termite, Pterotermes occidentis. Archives of Microbiology. 1991 Aug;156(2):91-8. PubMed PMID: WOS:A1991GB27400002.

[44] Allen TD, Caldwell ME, Lawson Pa, Huhnke RL, Tanner RS. Alkalibaculum bacchi gen. nov., sp. nov., a CO-oxidizing, ethanol-producing acetogen isolated from livestock-impacted soil. International journal of systematic and evolutionary microbiology. 2010;60(Pt 10):2483-9.

[45] Liu K, Atiyeh HK, Tanner RS, Wilkins MR, Huhnke RL. Fermentative production of ethanol from syngas using novel moderately alkaliphilic strains of Alkalibaculum bacchi. Bioresource Technology. 2012 Jan;104:336-41. PubMed PMID: WOS: 000301155800045.

[46] Lorowitz WH, Bryant MP. Peptostreptococcus productus strain that grows rapidly with CO as the energy source. Applied and Environmental Microbiology. 1984;47(5): 961-4. PubMed PMID: WOS:A1984SQ91300014.

[47] Lynd L, Kerby R, Zeikus JG. Carbon monoxide metabolism of the methylotrophic acidogen Butyribacterium methylotrophicum. Journal of bacteriology. 1982;149(1): 255-63.

[48] Zeikus JG, Lynd LH, Thompson TE, Krzycki Ja, Weimer PJ, Hegge PW. Isolation and characterization of a new, methylotrophic, acidogenic anaerobe, the marburg strain. Current Microbiology. 1980;3(6):381-6.

[49] Grethlein AJ, Worden RM, Jain MK, Datta R. Evidence for production of n-butanol from carbon monoxide by Butyribacterium methylotrophicum. Journal of Fermentation and Bioengineering. 1991;72(1):58-60.

[50] Lux MF, Drake HL. Reexamination of the metabolic potentials of the acetogens Clostridium aceticum and Clostridium formicoaceticum - chemolithoautotrophic and aromatic-dependent growth. Fems Microbiology Letters. 1992 Aug;95(1):49-56. PubMed PMID: WOS:A1992JJ52500008.

[51] Adamse AD. New isolation of Clostridium aceticum (Wieringa). Antonie Van Leeuwenhoek Journal of Microbiology. 1980;46(6):523-31. PubMed PMID: WOS:A1980LA02100002.

[52] Braun M, Mayer F, Gottschalk G. Clostridium aceticum (Wieringa), A microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Archives of Microbiology. 1981;128(3):288-93. PubMed PMID: WOS:A1981LB03200006.

[53] Abrini J, Naveau H, Nyns EJ. Clostridium autoethanogenum, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. Archives of microbiology. 1994;161(4):345-51.

[54] Bruant G, Lévesque M-J, Peter C, Guiot SR, Masson L. Genomic analysis of carbon monoxide utilization and butanol production by Clostridium carboxidivorans strain P7. PloS one. 2010;5(9):e13033.

[55] Liou JSC, Balkwill DL, Drake GR, Tanner RS. Clostridium carboxidivorans sp nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp nov. International Journal of Systematic and Evolutionary Microbiology. 2005 Sep;55:2085-91. PubMed PMID: WOS:000232239600055.

[56] Küsel K, Dorsch T, Acker G, Stackebrandt E, Drake HL. Clostridium scatologenes strain SL1 isolated as an acetogenic bacterium from acidic sediments. International journal of systematic and evolutionary microbiology. 2000;50 Pt 2:537-46.

[57] Gössner AS, Picardal F, Tanner RS, Drake HL. Carbon metabolism of the moderately acid-tolerant acetogen Clostridium drakei isolated from peat. FEMS microbiology letters. 2008;287(2):236-42.

[58] Andreese.Jr, Gottscha.G, Schlegel HG. Clostridium formicoaceticumnov spec - isolation, description and distinction from C aceticum and C thermoaceticum. Archiv Fur Mikrobiologie. 1970;72(2):154. PubMed PMID: WOS:A1970G704100007.

[59] Diekert GB, Thauer RK. Carbon monoxide oxidation by Clostridium thermoaceticum and Clostridium formicoaceticum. Journal of bacteriology. 1978;136(2):597-606.

[60] Kusel K, Karnholz A, Trinkwalter T, Devereux R, Acker G, Drake HL. Physiological ecology of Clostridium glycolicum RD-1, an aerotolerant acetogen isolated from sea grass roots. Applied and Environmental Microbiology. 2001 Oct;67(10):4734-41. PubMed PMID: WOS:000171237700045.

[61] Ohwaki K, Hungate RE. Hydrogen utilization by Clostridia in sewage sludge. Applied and Environmental Microbiology. 1977;33(6):1270-4.

[62] Köpke M, Held C, Hujer S, Liesegang H, Wiezer A, Wollherr A, et al. Clostridium ljungdahlii represents a microbial production platform based on syngas. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(29): 13087-92.

[63] Tanner RS, Miller LM, Yang D. Clostridium ljungdahlii sp. nov., an acetogenic species in clostridial rRNA homology group I. International journal of systematic bacteriology. 1993;43(2):232-.

[64] Bomar M, Hippe H, Schink B. Lithotrophic growth and hydrogen metabolism by Clostridium magnum. Fems Microbiology Letters. 1991;83(3):347-50.

[65] Schink B. Clostridium magnum sp nov, a non-autotrophic homoacetogenic bacterium. Archives of Microbiology. 1984;137(3):250-5.

[66] Kane MD, Brauman A, Breznak JA. Clostridium mayombei sp nov, an H2/CO2 acetogenic bacterium from the gut of the african soil-feeding termite, Cubitermes speciosus. Archives of Microbiology. 1991;156(2):99-104.

[67] Mechichi T, Labat M, Patel BKC, Woo THS, Thomas P, Garcia JL. Clostridium methoxybenzovorans sp nov., a new aromatic o-demethylating homoacetogen from an olive mill wastewater treatment digester. International Journal of Systematic Bacteriology. 1999 Jul;49:1201-9. PubMed PMID: WOS:000081448000032.

[68] Huhnke R, Lewis RS, Tanner RS, inventors. Isolation and characterization of novel Clostridial species. 2008.

[69] Genthner BRS, Davis CL, Bryant MP. Features of rumen and sewage sludge strains of Eubacterium limosum, a methanol-utilizing and H2-CO2-utilizing species. Applied and Environmental Microbiology. 1981;42(1):12-9. PubMed PMID: WOS:A1981LX19300003.

[70] Krumholz LR, Bryant MP. Clostridium pfennigii sp nov uses methoxyl groups of monobenzenoids and produces butyrate. International Journal of Systematic Bacteriology. 1985;35(4):454-6. PubMed PMID: WOS:A1985ASV9200009.

[71] Pierce E, Xie G, Barabote RD, Saunders E, Han CS, Detter JC, et al. The complete genome sequence of Moorella thermoacetica (f. Clostridium thermoaceticum). Environmental microbiology. 2008;10(10):2550-73.

[72] Daniel SL, Hsu T, Dean SI, Drake HL. Characterization of the H2-dependent and CO-dependent chemolithotrophic potentials of the acetogens Clostridium thermoaceticum and Acetogenium kivui. Journal of Bacteriology. 1990 Aug;172(8):4464-71. PubMed PMID: WOS:A1990DR47100043.

[73] Kerby R, Zeikus JG. Growth of Clostridium thermoaceticum on H2/CO2 or CO as energy source. Current Microbiology. 1983;8(1):27-30. PubMed PMID: WOS:A1983QB38000006.

[74] Savage MD, Wu ZR, Daniel SL, Lundie LL, Drake HL. Carbon monoxide-dependent chemolithotrophic growth of Clostridium thermoautotrophicum. Applied and Environmental Microbiology. 1987 Aug;53(8):1902-6. PubMed PMID: WOS:A1987J392000033.

[75] Müller V. Energy conservation in acetogenic bacteria. Applied and environmental microbiology. 2003;69(11):6345-53.

[76] Calusinska M, Happe T, Joris B, Wilmotte A. The surprising diversity of clostridial hydrogenases: a comparative genomic perspective. Microbiology-Sgm. 2010 Jun; 156:1575-88. PubMed PMID: WOS:000279798900001.

[77] Vignais PM, Colbeau A. Molecular biology of microbial hydrogenases. Current Issues in Molecular Biology. 2004;6:159-88. PubMed PMID: WOS:000228691500013.

[78] Vignais PM. Hydrogenases and H^+-reduction in primary energy conservation. In: Schafer G, Penefsky HS, editors. Bioenergetics: Energy Conservation and Conversion. 45: Springer Berlin / Heidelberg; 2007. p. 223-52.

[79] Demuez M, Cournac L, Guerrini O, Soucaille P, Girbal L. Complete activity profile of Clostridium acetobutylicum FeFe -hydrogenase and kinetic parameters for endogenous redox partners. Fems Microbiology Letters. 2007 Oct;275(1):113-21. PubMed PMID: WOS:000249428700015.

[80] Schut GJ, Adams MWW. The iron-hydrogenase of Thermotoga maritima utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. Journal of bacteriology. 2009;191(13):4451-7.

[81] Menon S. Unleashing hydrogenase activity in carbon monoxide dehydrogenase/ acetyl-CoA synthase and pyruvate: ferredoxin oxidoreductase. Biochemistry. 1996;2960(96):15814-21.

[82] Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anerobic bacteria. Bacteriological Reviews. 1977;41(1):100-80. PubMed PMID: WOS:A1977DC99200003.

[83] Oelgeschläger E, Rother M. Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archaea. Archives of microbiology. 2008;190(3):257-69.

[84] Rohde M, Mayer F, Meyer O. Immunocytochemical localization of carbon-monoxide oxidase in Pseudomonas carboxydovorans - The enzyme is attached to the inner aspect of the cytoplasmic membrane. Journal of Biological Chemistry. 1984;259(23): 4788-92. PubMed PMID: WOS:A1984TV66900080.

[85] Rohde M, Mayer F, Jacobitz S, Meyer O. Attachement of CO dehydrogenase to the cytoplasmic membrane is limiting the respiratory rate of Pseudomonas carboxydovorans. Fems Microbiology Letters. 1985;28(2):141-4. PubMed PMID: WOS:A1985AMV6400003.

[86] Kumar M, Lu WP, Ragsdale SW. Binding of carbon disufide to the site of acetyl-CoA synthesis by the nickel-iron-sulfur protein, carbon monoxide dehydrogenase, from Clostridium thermoaceticum. Biochemistry. 1994 Aug;33(32):9769-77. PubMed PMID: WOS:A1994PC54500048.

[87] Lindahl PA, Munck E, Ragsdale SW. CO dehydrogenase from Clostridium thermoaceticum - EPR and electrochemical studies in CO2 and argon atmosphere. Journal of Biological Chemistry. 1990 Mar;265(7):3873-9. PubMed PMID: WOS:A1990CQ48000047.

[88] Ragsdale SW, Pierce E. Acetogenesis and the Wood-Ljungdahl pathway of CO(2) fixation. Biochimica et biophysica acta. 2008;1784(12):1873-98.

[89] Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou JH, Woodard TL, et al. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. Applied and Environmental Microbiology. 2011 May; 77(9):2882-6. PubMed PMID: WOS:000289773100009.

[90] Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. Microbial electrosynthesis : Feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. mBio. 2010;1(2).

[91] Biegel E, Schmidt S, Müller V. Genetic, immunological and biochemical evidence for a Rnf complex in the acetogen Acetobacterium woodii. Environmental microbiology. 2009;11(6):1438-43.

[92] Li F, Hinderberger J, Seedorf H, Zhang J, Buckel W, Thauer RK. Coupled ferredoxin and crotonyl coenzyme A (CoA) reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from Clostridium kluyveri. Journal of bacteriology. 2008;190(3):843-50.

[93] Müller V, Imkamp F, Biegel E, Schmidt S, Dilling S. Discovery of a ferredoxin:NAD+-oxidoreductase (Rnf) in Acetobacterium woodii: a novel potential coupling site in acetogens. Annals of the New York Academy of Sciences. 2008;1125:137-46.

[94] Herrmann G, Jayamani E, Mai G, Buckel W. Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. Journal of Bacteriology. 2008 Feb;190(3): 784-91. PubMed PMID: WOS:000252616800002.

[95] Dürre P. Handbook on Clostridia. Dürre P, editor: CRC Press Taylor & Francis Group; 2005.

[96] Wang SN, Huang HY, Moll J, Thauer RK. NADP(+) reduction with reduced ferredoxin and NADP(+) reduction with NADH are coupled via an electron-bifurcating enzyme complex in Clostridium kluyveri. Journal of Bacteriology. 2010 Oct;192(19): 5115-23. PubMed PMID: WOS:000281866900032.

[97] Roberts DL, Jameshagstrom JE, Garvin DK, Gorst CM, Runquist JA, Baur JR, et al. Cloning and expression of the gene-cluster encoding key proteins involved in acetyl-CoA synthesis in Clostridium thermoaceticum - CO dehydrogenase, the corrinoid Fe-S protein, and methyltransferase. Proceedings of the National Academy of Sciences of the United States of America. 1989 Jan;86(1):32-6. PubMed PMID: WOS:A1989R820200008.

[98] Kaster AK, Goenrich M, Seedorf H, Liesegang H, Wollherr A, Gottschalk G, et al. More than 200 genes required for methane formation from H-2 and CO2 and energy conservation are present in Methanothermobacter marburgensis and Methanothermobacter thermautotrophicus. Archaea-an International Microbiological Journal. 2011. PubMed PMID: WOS:000292403100001.

[99] Papoutsakis ET, AL-Hinai MA, Jones SW, Indurthi DC, Mitchell DK, Fast A, inventors. Recombinant Clostridia that fix CO2 and CO and uses thereof. U.S.2012.

[100] Alsaker KV, Papoutsakis ET. Transcriptional program of early sporulation and stationary-phase events in Clostridium acetobutylicum. Journal of bacteriology. 2005;187(20):7103-18.

[101] Köpke M. Genetische Veränderung von Clostridium ljungdahlii zur produktion von 1-butanol aus synthesegas.: Ulm University; 2009.

[102] Sakai S, Nakashimada Y, Yoshimoto H, Watanabe S, Okada H, Nishio N. Ethanol production from H-2 and CO2 by a newly isolated thermophilic bacterium, Moorella sp HUC22-1. Biotechnology Letters. 2004 Oct;26(20):1607-12. PubMed PMID: WOS: 000225691400013.

[103] Sakai S, Nakashimada Y, Inokuma K, Kita M, Okada H, Nishio N. Acetate and ethanol production from H-2 and CO2 by Moorella sp using a repeated batch culture. Journal of Bioscience and Bioengineering. 2005 Mar;99(3):252-8. PubMed PMID: WOS:000229052300009.

[104] Köpke M, Mihalcea C, Liew FM, Tizard JH, Ali MS, Conolly JJ, et al. 2,3-butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. Applied and Environmental Microbiology. 2011 Aug;77(15): 5467-75. PubMed PMID: WOS:000293224500045.

[105] Köpke M, Mihalcea C, Bromley JC, Simpson SD. Fermentative production of ethanol from carbon monoxide. Current Opinion in Biotechnology. 2011 Jun;22(3):320-5. PubMed PMID: WOS:000292228300003.

[106] Kundiyana DK, Wilkins MR, Maddipati P, Huhnke RL. Effect of temperature, pH and buffer presence on ethanol production from synthesis gas by "Clostridium ragsdalei". Bioresource technology. 2011;102(10):5794-9.

[107] Saxena J, Tanner RS. Effect of trace metals on ethanol production from synthesis gas by the ethanologenic acetogen, Clostridium ragsdalei. Journal of Industrial Microbiology & Biotechnology. 2011 Apr;38(4):513-21. PubMed PMID: WOS: 000288673700004.

[108] Heiskanen H, Virkajärvi I, Viikari L. The effect of syngas composition on the growth and product formation of Butyribacterium methylotrophicum. Enzyme and Microbial Technology. 2007;41(3):362-7.

[109] Noelling J. Genome sequence and comparative analysis of the solvent-producing bacterium Clostridium acetobutylicum. Journal of. 2001;183(16):4823-38.

[110] Huang H, Liu H, Gan Y-R. Genetic modification of critical enzymes and involved genes in butanol biosynthesis from biomass. Biotechnology advances. 2010;28(5): 651-7.

[111] Ukpong MN, Atiyeh HK, De Lorme MJM, Liu K, Zhu X, Tanner RS, et al. Physiological response of Clostridium carboxidivorans during conversion of synthesis gas to

solvents in a gas-fed bioreactor. Biotechnology and bioengineering. 2012;Accepted article.

[112] Kundiyana DK, Huhnke RL, Wilkins MR. Syngas fermentation in a 100-L pilot scale fermentor: Design and process considerations. Journal of Bioscience and Bioengineering. 2010 May;109(5):492-8. PubMed PMID: WOS:000279089600011.

[113] Phillips JR, Klasson KT, Claussen EC, Gaddy JL. Biological production of ethanol from coal synthesis gas. Applied Biochemistry And Biotechnology. 1993;39(1):0-7.

[114] Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by clostridia. Biotechnology and Bioengineering. 2008 Oct;101(2):209-28. PubMed PMID: WOS:000259512900001.

[115] Sauer U, Durre P. Differential induction of genes related to solvent formation during the shift from acidogenesis to solventogenesis in continuous culture of Clostridium acetobutylicum. Fems Microbiology Letters. 1995 Jan;125(1):115-20. PubMed PMID: WOS:A1995QA11200018.

[116] Shi Z, Blaschek HP. Transcriptional analysis of Clostridium beijerinckii NCIMB 8052 and the hyper-butanol-producing mutant BA101 during the shift from acidogenesis to solventogenesis. Applied and Environmental Microbiology. 2008 Dec;74(24): 7709-14. PubMed PMID: WOS:000261513700031.

[117] Köpke M, Dürre P. Biochemical production of biobutanol. In: Luque R, Campelo J, Clark JH, editors. Camebridge, UK: Woodhead Publishing Ltd; 2011. p. 221-57.

[118] Gheshlaghi R, Scharer JM, Moo-young M, Chou CP. Metabolic pathways of clostridia for producing butanol. Biotechnology advances. 2009;27(6):764-81.

[119] Seedorf H, Fricke WF, Veith B, Bruggemann H, Liesegang H, Strittimatter A, et al. The genome of Clostridium kluyveri, a strict anaerobe with unique metabolic features. Proceedings of the National Academy of Sciences of the United States of America. 2008 Feb;105(6):2128-33. PubMed PMID: WOS:000253261900064.

[120] Inui M, Suda M, Kimura S, Yasuda K, Suzuki H, Toda H, et al. Expression of Clostridium acetobutylicum butanol synthetic genes in Escherichia coli. Applied microbiology and biotechnology. 2008;77(6):1305-16.

[121] Tomas CA, Beamish J, Papoutsakis ET. Transcriptional analysis of butanol stress and tolerance in Clostridium acetobutylicum. Journal of Bacteriology. 2004 Apr;186(7): 2006-18. PubMed PMID: WOS:000220363200011.

[122] Jones DT, Woods DR. Acetone-butanol fermentation revisited. Microbiological reviews. 1986;50(4):484-524.

[123] Paul D, Austin FW, Arick T, Bridges SM, Burgess SC, Dandass YS, et al. Genome sequence of the solvent-producing bacterium Clostridium carboxidivorans strain P7T. Journal of bacteriology. 2010;192(20):5554-5.

[124] Ismaiel aa, Zhu CX, Colby GD, Chen JS. Purification and characterization of a primary-secondary alcohol dehydrogenase from two strains of Clostridium beijerinckii. Journal of bacteriology. 1993;175(16):5097-105.

[125] Ramachandriya KD, Wilkins MR, Delorme MJM, Zhu X, Kundiyana DK, Atiyeh HK, et al. Reduction of acetone to isopropanol using producer gas fermenting microbes. Biotechnology and bioengineering. 2011;108(10):2330-8.

[126] Toth J, Ismaiel aa, Chen JS. The ald gene, encoding a coenzyme A-acylating aldehyde dehydrogenase, distinguishes Clostridium beijerinckii and two other solvent-producing clostridia from Clostridium acetobutylicum. Applied and environmental microbiology. 1999;65(11):4973-80.

[127] Bertram J, Kuhn A, Durre P. Tn916-induced mutants of Clostridium acetobutylicum defective in regulation of solvent formation. Archives of Microbiology. 1990 Mar; 153(4):373-7. PubMed PMID: WOS:A1990CW24900012.

[128] Durre P, Fischer RJ, Kuhn A, Lorenz K, Schreiber W, Sturzenhofecker B, et al. Solventogenic enzymes of Clostridium acetobutylicum - catalytic properties, genetic organization, and transcriptional regulation. Fems Microbiology Reviews. 1995 Oct;17(3): 251-62. PubMed PMID: WOS:A1995TA64700005.

[129] Thauer RK, Jungerma.Ka, Kirchnia.Fh. Properties and function of pyruvate-formate-lyase reaction in Clostridiae. European Journal of Biochemistry. 1972;27(2):282-90. PubMed PMID: WOS:A1972M614500011.

[130] Hemme CL, Mouttaki H, Lee Y-J, Zhang G, Goodwin L, Lucas S, et al. Genome Announcement - Sequencing of multiple Clostridia genomes related to biomass conversion and biofuels production. Journal of bacteriology. 2010;192(24):6494-6.

[131] Wang Y, Li X, Mao Y, Blaschek HP. Genome-wide dynamic transcriptional profiling in Clostridium beijerinckii NCIMB 8052 using single-nucleotide resolution RNA-Seq. BMC genomics. 2012;13(1):102-.

[132] Janssen H, Döring C, Ehrenreich A, Voigt B, Hecker M, Bahl H, et al. A proteomic and transcriptional view of acidogenic and solventogenic steady-state cells of Clostridium acetobutylicum in a chemostat culture. Applied microbiology and biotechnology. 2010;87(6):2209-26.

[133] Haus S, Jabbari S, Millat T, Janssen H, Fischer R-J, Bahl H, et al. A systems biology approach to investigate the effect of pH-induced gene regulation on solvent production by Clostridium acetobutylicum in continuous culture. BMC systems biology. 2011;5(1):10-.

[134] Alsaker KV, Paredes C, Papoutsakis ET. Metabolite stress and tolerance in the production of biofuels and chemicals: gene-expression-based systems analysis of butanol, butyrate, and acetate stresses in the anaerobe Clostridium acetobutylicum. Biotechnology and bioengineering. 2010;105(6):1131-47.

[135] Allcock ER, Reid SJ, Jones DT, Woods DR. Autolytic activity of an autolysin-deficient mutant of Clostridium acetobutylicum. Appllied Environmental Microbiology. 1981;42:929-35.

[136] Hermann M, Fayolle F, Marchal R, Podvin L, Sebald M, Vandecasteele JP. Isolation and characterization of butanol-resistant mutants of Clostridium acetobutylicum. Applied Environmental Microbiology. 1985;50:1238-43.

[137] Vanderwesthuizen A, Jones DT, Woods DR. Autolytic activity and butanol tolerance of Clostridium acetobutylicum. Applied and Environmental Microbiology. 1982;44(6):1277-81. PubMed PMID: WOS:A1982PT27700005.

[138] Baer SH, Blaschek HP, Smith TL. Effect of butanol challenge and temperature on lipid composition and membrane fluidity of butanol-tolerant Clostridium acetobutylicum. Applied and environmental microbiology. 1987;53(12):2854-61.

[139] Soucaille P, Joliff G, Izard A, Goma G. Butanol tolerance and autobacteriocin production by Clostridium acetobutylicum. Current Microbiology. 1987;14(5):295-9. PubMed PMID: WOS:A1987G253100011.

[140] Mermelstein LD, Welker NE, Bennett GN, Papoutsakis ET. Expression of cloned homologous fermentative genes in Clostridium acetobutylicum ATCC 824. Bio-Technology. 1992 Feb;10(2):190-5. PubMed PMID: WOS:A1992HJ61200027.

[141] Mermelstein LD, Papoutsakis ET, Petersen DJ, Bennett GN. Metabolic engineering of Clostridium acetobutylicum ATCC 824 for increased solvent production by enhancement of acetone formation enzyme activities using a synthetic acetone operon. Biotechnology and Bioengineering. 1993 Nov;42(9):1053-60. PubMed PMID: WOS:A1993MC12100005.

[142] Walter KA, Mermelstein LD, Papoutsakis ET. Studies of recombinant Clostridium acetobutylicum with increased dosages of butyrate formation genes. Recombinant DNA Technology Ii. 1994;721:69-72. PubMed PMID: WOS:A1994BA43Z00006.

[143] Desai RP, Papoutsakis ET. Antisense RNA strategies for metabolic engineering of Clostridium acetobutylicum. Applied and Environmental Microbiology. 1999 Mar; 65(3):936-45. PubMed PMID: WOS:000078882000010.

[144] Sillers R, A-Hinai MA, Papoutsakis ET. Aldehyde-alcohol dehydrogenase and/or thiolase overexpression coupled with CoA transferase downregulation lead to higher alcohol titers and selectivity in Clostridium acetobutylicum fermentations. Biotechnology and Bioengineering. 2009 Jan 1;102(1):38-49. PubMed PMID: WOS: 000261826000006.

[145] Tummala SB, Junne SG, Papoutsakis ET. Antisense RNA downregulation of coenzyme A transferase combined with alcohol-aldehyde dehydrogenase overexpression leads to predominantly alcohologenic Clostridium acetobutylicum fermentations. Journal of Bacteriology. 2003 Jun;185(12):3644-53. PubMed PMID: WOS: 000183364700019.

[146] Green EM, Boynton ZL, Harris LM, Rudolph FB, Papoutsakis ET, Bennett GN. Genetic manipulation of acid formation pathways by gene inactivation in Clostridium acetobutylicum ATCC 824. Microbiology-Uk. 1996 Aug;142:2079-86. PubMed PMID: WOS:A1996VB82900020.

[147] Green EM, Bennett GN. Inactivation of an aldehyde/alcohol dehydrogenase gene from Clostridium acetobutylicum ATCC 824. Applied biochemistry and biotechnology. 1996;57-58:213-21.

[148] Wilkinson SR, Young M. Targeted integration of genes into the Clostridium acetobutylicum genome. Microbiology-Uk. 1994 Jan;140:89-95. PubMed PMID: WOS:A1994NE68400013.

[149] Shimizu T, Bathein W, Tamaki M, Hayashi H. The virr gene, a member of a class of 2-component response regulators, regulates the production of perfringolysin-o, collagenase, and hemagglutinin in Clostridium perfringens. Journal of Bacteriology. 1994 Mar;176(6):1616-23. PubMed PMID: WOS:A1994NB99400008.

[150] Liyanage H, Kashket S, Young M, Kashket ER. Clostridium beijerinckii and Clostridium difficile detoxify methylglyoxal by a novel mechanism involving glycerol dehydrogenase. Applied and Environmental Microbiology. 2001 May;67(5):2004-10. PubMed PMID: WOS:000168488400003.

[151] O'Connor JR, Lyras D, Farrow KA, Adams V, Powell DR, Hinds J, et al. Construction and analysis of chromosomal Clostridium difficile mutants. Molecular Microbiology. 2006 Sep;61(5):1335-51. PubMed PMID: WOS:000239701400020.

[152] Wilkinson SR, Young DI, Morris JG, Young M. Molecular genetics and the initiation of solventogenesis in Clostridium beijerinckii (formerly Clostridium acetobutylicum) NCIMB-8052. Fems Microbiology Reviews. 1995 Oct;17(3):275-85. PubMed PMID: WOS:A1995TA64700007.

[153] Harris LM, Welker NE, Papoutsakis ET. Northern, morphological, and fermentation analysis of spo0A inactivation and overexpression in Clostridium acetobutylicum ATCC 824. Journal of Bacteriology. 2002 Jul;184(13):3586-97. PubMed PMID: WOS: 000176237400020.

[154] Sarker MR, Carman RJ, McClane BA. Inactivation of the gene (cpe) encoding Clostridium perfringens enterotoxin eliminates the ability of two cpe-positive C-perfringens type A human gastrointestinal disease isolates to affect rabbit ileal loops. Molecular Microbiology. 1999 Sep;33(5):946-58. PubMed PMID: WOS:000082779700005.

[155] Soucaille P, Rainer F, Christian C, inventors. Process for chromosomal integration and DNA sequence replacement in clostridia. EU. 2008.

[156] Argyros DA, Tripathi SA, Barrett TF, Rogers SR, Feinberg LF, Olson DG, et al. High ethanol titers from cellulose by using metabolically engineered thermophilic, anaerobic microbes. Applied and Environmental Microbiology. 2011 Dec;77(23):8288-94. PubMed PMID: WOS:000297164100012.

[157] Karberg M, Guo HT, Zhong J, Coon R, Perutka J, Lambowitz AM. Group II introns as controllable gene targeting vectors for genetic manipulation of bacteria. Nature Biotechnology. 2001 Dec;19(12):1162-7. PubMed PMID: WOS:000172524400030.

[158] Heap JT, Pennington OJ, Cartman ST, Carter GP, Minton NP. The ClosTron: A universal gene knock-out system for the genus Clostridium. Journal of Microbiological Methods. 2007 Sep;70(3):452-64. PubMed PMID: WOS:000249573800008.

[159] Heap JT, Kuehne Sa, Ehsaan M, Cartman ST, Cooksley CM, Scott JC, et al. The ClosTron: Mutagenesis in Clostridium refined and streamlined. Journal of microbiological methods. 2010;80(1):49-55.

[160] Camiade E, Peltier J, Bourgeois I, Couture-Tosi E, Courtin P, Antunes A, et al. Characterization of Acp, a Peptidoglycan hydrolase of Clostridium perfringens with N-acetylglucosaminidase activity That is implicated in cell separation and stress-induced autolysis. Journal of Bacteriology. 2010 May;192(9):2373-84. PubMed PMID: WOS:000276685800009.

[161] Heap JT, Ehsaan M, Cooksley CM, Ng YK, Cartman ST, Winzer K, et al. Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker. Nucleic Acids Res. 2012 Apr 1;40(8):e59. PubMed PMID: 22259038. Pubmed Central PMCID: 3333862. Epub 2012/01/20. eng.

[162] Keis S, Shaheen R, Jones DT. Emended descriptions of Clostridium acetobutylicum and Clostridium beijerinckii, and descriptions of Clostridium saccharoperbutylacetonicum sp nov and Clostridium saccharobutylicum sp nov. International Journal of Systematic and Evolutionary Microbiology. 2001 Nov;51:2095-103. PubMed PMID: WOS:000172374400020.

[163] Bertram J, Durre P. Conjugal transfer and expression of Streptococcal transposons in Clostridium acetobutylicum. Archives of Microbiology. 1989 May;151(6):551-7. PubMed PMID: WOS:A1989AA14000015.

[164] Woolley RC, Pennock A, Ashton RJ, Davies A, Young M. Transfer of Tn1545 and Tn916 to Clostridium acetobutylicum. Plasmid. 1989 Sep;22(2):169-74. PubMed PMID: WOS:A1989CK56900010.

[165] Blouzard JC, Valette O, Tardif C, de Philip P. Random mutagenesis of Clostridium cellulolyticum by using a Tn1545 derivative. Applied and Environmental Microbiology. 2010 Jul;76(13):4546-9. PubMed PMID: WOS:000279082800049.

[166] Cartman ST, Minton NP. A mariner-based transposon system for in vivo random mutagenesis of Clostridium difficile. Appl Environ Microbiol. 2010 Feb;76(4):1103-9. PubMed PMID: 20023081. Pubmed Central PMCID: 2820977. Epub 2009/12/22. eng.

[167] Koepke M, Liew FM, inventors. Recombinant microorganism and methods of production thereof. U.S.2011.

[168] Simpson SD, Koepke M, Liew FM, inventors. Recombinant microorganisms and methods of use thereof. U.S.2011.

[169] Lederle S. Heterofermentative acetonproduktion: University of Ulm, Germany; 2011.

[170] Becker U, Grund G, Orschel M, Doderer K, Löhden G, Brand G, et al., inventors. Zellen und Verfahren zur Herstellung von Aceton. Germany. 2009.

[171] Harris LM, Desai RP, Welker NE, Papoutsakis ET. 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. 2000;67(1):1-11.

[172] Lehmann D, Lutke-Eversloh T. Switching Clostridium acetobutylicum to an ethanol producer by disruption of the butyrate/butanol fermentative pathway. Metabolic Engineering. 2011 Sep;13(5):464-73. PubMed PMID: WOS:000294291200002.

[173] Kuit W, Minton NP, Lopez-Contreras AM, Eggink G. Disruption of the acetate kinase (ack) gene of Clostridium acetobutylicum results in delayed acetate production. Applied Microbiology and Biotechnology. 2012 May;94(3):729-41. PubMed PMID: WOS: 000302283700015.

[174] Yu MR, Zhang YL, Tang IC, Yang ST. Metabolic engineering of Clostridium tyrobutyricum for n-butanol production. Metabolic Engineering. 2011 Jul;13(4):373-82. PubMed PMID: WOS:000291471500003.

[175] Li YC, Tschaplinski TJ, Engle NL, Hamilton CY, Rodriguez M, Liao JC, et al. Combined inactivation of the Clostridium cellulolyticum lactate and malate dehydrogenase genes substantially increases ethanol yield from cellulose and switchgrass fermentations. Biotechnology for Biofuels. 2012 Jan;5. PubMed PMID: WOS: 000299821600001.

[176] Lee J, Jang YS, Choi SJ, Im JA, Song H, Cho JH, et al. Metabolic engineering of Clostridium acetobutylicum ATCC 824 for isopropanol-butanol-ethanol fermentation. Applied and Environmental Microbiology. 2012 Mar;78(5):1416-23. PubMed PMID: WOS:000300537400012.

[177] Higashide W, Li YC, Yang YF, Liao JC. Metabolic Engineering of Clostridium cellulolyticum for Production of isobutanol from cellulose. Applied and Environmental Microbiology. 2011 Apr;77(8):2727-33. PubMed PMID: WOS:000289459300020.

[178] Cai XP, Bennett GN. Improving the Clostridium acetobutylicum butanol fermentation by engineering the strain for co-production of riboflavin. Journal of Industrial Microbiology & Biotechnology. 2011 Aug;38(8):1013-25. PubMed PMID: WOS: 000293002000015.

[179] Zhu LJ, Dong HJ, Zhang YP, Li Y. Engineering the robustness of Clostridium acetobutylicum by introducing glutathione biosynthetic capability. Metabolic Engineering. 2011 Jul;13(4):426-34. PubMed PMID: WOS:000291471500008.

[180] Tomas CA, Welker NE, Papoutsakis ET. Overexpression of groESL in Clostridium acetobutylicum results in increased solvent production and tolerance, prolonged me-

tabolism, and changes in the cell's transcriptional program. Applied and Environmental Microbiology. 2003 Aug;69(8):4951-65. PubMed PMID: WOS: 000184672500078.

[181] Nakayama S-i, Kosaka T, Hirakawa H, Matsuura K, Yoshino S, Furukawa K. Metabolic engineering for solvent productivity by downregulation of the hydrogenase gene cluster hupCBA in Clostridium saccharoperbutylacetonicum strain N1-4. Applied Microbiology and Biotechnology. 2008 Mar;78(3):483-93. PubMed PMID: WOS: 000253132500012.

[182] Atsumi S, Hanai T, Liao J. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature. 2008;451(7174):86-9.

[183] Clomburg JM, Gonzalez R. Biofuel production in Escherichia coli: the role of metabolic engineering and synthetic biology. Applied Microbiology and Biotechnology. 2010 Mar;86(2):419-34. PubMed PMID: WOS:000274707600003.

[184] Peralta-Yahya PP, Keasling JD. Advanced biofuel production in microbes. Biotechnology Journal. 2010 Feb;5(2):147-62. PubMed PMID: WOS:000275275500005.

[185] Kapic A, Jones ST, Heindel TJ. Carbon monoxide mass transfer in a syngas mixture. Industrial & Engineering Chemistry Research. 2006 Dec;45(26):9150-5. PubMed PMID: WOS:000242786200049.

[186] Mohammadi M, Younesi H, Najafpour G, Mohamed AR. Sustainable ethanol fermentation from synthesis gas by Clostridium ljungdahlii in a continuous stirred tank bioreactor. Journal of Chemical Technology and Biotechnology. 2012 Jun;87(6): 837-43. PubMed PMID: WOS:000304190300018.

[187] Bredwell MD, Srivastava P, Worden RM. Reactor design issues for synthesis-gas fermentations. Biotechnology Progress. 1999 Sep-Oct;15(5):834-44. PubMed PMID: WOS:000082936200007.

[188] Ahmed A, Cateni BG, Huhnke RL, Lewis RS. Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of Clostridium carboxidivorans P7T. Biomass and Bioenergy. 2006;30(7):665-72.

[189] Xu D, Lewis RS. Syngas fermentation to biofuels: Effects of ammonia impurity in raw syngas on hydrogenase activity. Biomass and Bioenergy. 2012.

[190] Ahmed A, Lewis RS. Fermentation of biomass-generated synthesis gas: Effects of nitric oxide. Biotechnology and Bioengineering. 2007 Aug;97(5):1080-6. PubMed PMID: WOS:000247960300008.

[191] Brogren C, Karlsson HT, Bjerle I. Absorption of NO in an alkaline solution of KMnO4. Chemical Engineering & Technology. 1997 Aug;20(6):396-402. PubMed PMID: WOS:A1997XX28400006.

[192] Chu H, Chien TW, Li SY. Simultaneous absorption of SO2 and NO from flue gas with KMnO4/NaOH solutions. Science of the Total Environment. 2001 Jul;275(1-3): 127-35. PubMed PMID: WOS:000169969700011.

[193] Klasson KT, Ackerson MD, Clausen EC, Gaddy JL. Biological conversion of coal and coal-derived synthesis gas. Fuel. 1993 Dec;72(12):1673-8. PubMed PMID: WOS:A1993MJ23700016.

[194] Vega JL, Holmberg VL, Clausen EC, Gaddy JL. Fermentation parameters of Peptostreptococcus productus on gaseous substrates (CO, H2/CO2). Archives of Microbiology. 1989;151(1):65-70. PubMed PMID: WOS:A1989R169500012.

[195] Hurst KM, Lewis RS. Carbon monoxide partial pressure effects on the metabolic process of syngas fermentation. Biochemical Engineering Journal. 2010;48(2):159-65.

[196] Najafpour G, Younesi H. Ethanol and acetate synthesis from waste gas using batch culture of Clostridium ljungdahlii. Enzyme and Microbial Technology. 2006 Jan; 38(1-2):223-8. PubMed PMID: WOS:000234199300033.

[197] Gaddy JL, Chen GJ, inventors. Bioconversion of waste biomass to useful products. 1998.

[198] Genthner BRS, Bryant MP. Growth of Eubacterium limosum with carbon monoxide as the energy source. Applied and Environmental Microbiology. 1982;43(1):70-4. PubMed PMID: WOS:A1982MX20500009.

[199] Demler M, Weuster-Botz D. Reaction Engineering analysis of hydrogenotrophic production of ccetic ccid by Acetobacterium woodii. Biotechnology and Bioengineering. 2011 Feb;108(2):470-4. PubMed PMID: WOS:000285393000024.

[200] Cotter JL, Chinn MS, Grunden AM. Ethanol and acetate production by Clostridium ljungdahlii and Clostridium autoethanogenum using resting cells. Bioprocess Biosyst Eng. 2009;32(3):369-80.

[201] Kanchanatawee S, Maddox IS. Effect of phosphate and ammonium ion concentrations on solvent production in defined medium by Clostridium acetobutylicum. Journal of Industrial Microbiology. 1990 Jul;5(5):277-82. PubMed PMID: WOS:A1990DU68500002.

[202] Guo Y, Xu JL, Zhang Y, Xu HJ, Yuan ZH, Li D. Medium optimization for ethanol production with Clostridium autoethanogenum with carbon monoxide as sole carbon source. Bioresource Technology. 2010 Nov;101(22):8784-9. PubMed PMID: WOS: 000281262900044.

[203] Kundiyana DK, Huhnke RL, Maddipati P, Atiyeh HK, Wilkins MR. Feasibility of incorporating cotton seed extract in Clostridium strain P11 fermentation medium during synthesis gas fermentation. Bioresource Technology. 2010 Dec;101(24):9673-80. PubMed PMID: WOS:000282201200037.

[204] Sim JH, Kamaruddin AH. Optimization of acetic acid production from synthesis gas by chemolithotrophic bacterium - Clostridium aceticum using statistical approach.

Bioresource Technology. 2008 May;99(8):2724-35. PubMed PMID: WOS: 000254072200003.

[205] Vega JL, Prieto S, Elmore BB, Clausen EC, Gaddy JL. The biological production of ethanol from synthesis gas. Applied Biochemistry and Biotechnology. 1989 Jan-Aug; 20-1:781-97. PubMed PMID: WOS:A1989U642400058.

[206] Hu P, Jacobsen LT, Horton JG, Lewis RS. Sulfide assessment in bioreactors with gas replacement. Biochemical Engineering Journal. 2010 May;49(3):429-34. PubMed PMID: WOS:000277782900018.

[207] Do YS, Smeenk J, Broer KM, Kisting CJ, Brown R, Heindel TJ, et al. Growth of Rhodospirillum rubrum on synthesis gas: Conversion of CO to H-2 and poly-beta-hydroxyalkanoate. Biotechnology and Bioengineering. 2007 Jun;97(2):279-86. PubMed PMID: WOS:000246434700008.

[208] Devi MP, Mohan SV, Mohanakrishna G, Sarma PN. Regulatory influence of CO2 supplementation on fermentative hydrogen production process. International Journal of Hydrogen Energy. 2010 Oct;35(19):10701-9. PubMed PMID: WOS: 000283977100086.

[209] Grethlein AJ, Worden RM, Jain MK, Datta R. Continuous production of mixed alcohol and acids from carbon monoxide. Applied Biochemistry and Biotechnology. 1990 Spr-Sum;24-5:875-84. PubMed PMID: WOS:A1990DE57300079.

[210] Girbal L, Croux C, Vasconcelos I, Soucaille P. Regulation of metabolic shifts in Clostridium acetobutylicum ATCC 824. Fems Microbiology Reviews. 1995 Oct;17(3): 287-97. PubMed PMID: WOS:A1995TA64700008.

[211] Kundiyana DK, Wilkins MR, Maddipati P, Huhnke RL. Effect of temperature, pH and buffer presence on ethanol production from synthesis gas by "Clostridium ragsdalei". Bioresource Technology. 2011 May;102(10):5794-9. PubMed PMID: WOS: 000291125800033.

[212] Gaddy JL, Clausen EC, inventors. Clostridium ljungdahlii, an anaerobic ethanol and acetate producing microorganism. 1992.

[213] Cotter JL, Chinn MS, Grunden AM. Influence of process parameters on growth of Clostridium ljungdahlii and Clostridium autoethanogenum on synthesis gas. Enzyme and Microbial Technology. 2009 May;44(5):281-8. PubMed PMID: WOS: 000264908000006.

[214] Frohlich H, Villian L, Melzner D, Strube J. Membrane technology in bioprocess science. Chemie Ingenieur Technik. 2012 Jun;84(6):905-17. PubMed PMID: WOS: 000304438600024.

[215] Rushton A, Ward AS, Holdich RG. Solid-liquid filtration and separation technology. Germany: VCH Publishers, Inc.; 1996.

[216] Kargupta K, Datta S, Sanyal SK. Analysis of the performance of a continuous membrane bioreactor with cell recycling during ethanol fermentation. Biochemical Engineering Journal. 1998 Jan;1(1):31-7. PubMed PMID: WOS:000077894400004.

[217] Brown SD, Guss AM, Karpinets TV, Parks JM, Smolin N, Yang S, et al. Mutant alcohol dehydrogenase leads to improved ethanol tolerance in Clostridium thermocellum. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(33):13752-7.

[218] Shao XJ, Raman B, Zhu MJ, Mielenz JR, Brown SD, Guss AM, et al. Mutant selection and phenotypic and genetic characterization of ethanol-tolerant strains of Clostridium thermocellum. Applied Microbiology and Biotechnology. 2011 Nov;92(3):641-52. PubMed PMID: WOS:000295673800020.

[219] Ezeji T, Milne C, Price ND, Blaschek HP. Achievements and perspectives to overcome the poor solvent resistance in acetone and butanol-producing microorganisms. Applied Microbiology and Biotechnology. 2010 Feb;85(6):1697-712. PubMed PMID: WOS:000273978400007.

[220] Borden JR, Papoutsakis ET. Dynamics of genomic-library enrichment and identification of solvent tolerance genes for Clostridium acetobutylicum. Applied and environmental microbiology. 2007;73(9):3061-8.

[221] Ezeji TC, Li Y. Advanced product recovery technologies. Hoboken: Wiley; 2009.

[222] Ezeji TC, Qureshi N, Blaschek HP. Bioproduction of butanol from biomass: from genes to bioreactors. Current opinion in biotechnology. 2007;18(3):220-7.

[223] Jauregui-Haza UJ, Pardillo-Fontdevila EJ, Wilhelm AM, Delmas H. Solubility of hydrogen and carbon monoxide in water and some organic solvents. Latin American Applied Research. 2004 Apr;34(2):71-4. PubMed PMID: WOS:000220679200001.

[224] Karcher P, Ezeji TC, Qureshi N, Blaschek HP. Microbial production of butanol: product recovery by extraction. New Delhi: IK International Publishing House Pvt Ltd; 2005.

[225] Ezeji TC, Li Y. Advanced product recovery technologies. Hoboken: Wiley; 2009.

[226] Ezeji TC, Qureshi N, Blaschek HP. Production of acetone, butanol and ethanol by Clostridium beijerinckii BA101 and in situ recovery by gas stripping. World Journal of Microbiology & Biotechnology. 2003 Aug;19(6):595-603. PubMed PMID: WOS: 000184717400007.

[227] Wei L, Pordesimo LO, Igathinathane C, Batchelor WD. Process engineering evaluation of ethanol production from wood through bioprocessing and chemical catalysis. Biomass & Bioenergy. 2009 Feb;33(2):255-66. PubMed PMID: WOS:000263995200011.

[228] Piccolo C, Bezzo F. A techno-economic comparison between two technologies for bioethanol production from lignocellulose. Biomass & Bioenergy. 2009 Mar;33(3): 478-91. PubMed PMID: WOS:000264433600016.

[229] Yoneda N, Kusano S, Yasui M, Pujado P, Wilcher S. Recent advances in processes and catalysts for the production of acetic acid. Applied Catalysis a-General. 2001 Nov;221(1-2):253-65. PubMed PMID: WOS:000173000700019.

[230] Zigova J, Sturdik E, Vandak D, Schlosser S. Butyric acid production by Clostridium butyricum with integrated extraction and pertraction. Process Biochemistry. 1999 Oct;34(8):835-43. PubMed PMID: WOS:000083166300009.

[231] Gaddy JL, inventor. Biological production of ethanol from waste gases with Clostridium ljungdahlii. US. 2000.

[232] Guzman DD. INEOS Bio looks to start US cellulosic ethanol in 2012. ICIS Chemical Business. 2011:22-3.

[233] Bio I. INEOS Bio - Pioneering waste-to-biofuel technology attracts cross-party support in UK 2011. Available from: http://www.ineosbio.com/76-Press_releases-16.htm.

[234] Coskata. Coskata, Inc.'s semi-commercial facility demonstrates two years of successful operation 2011. Available from: http://www.coskata.com/company/media.asp?story=504B571C-0916-474E-BFFA-ACB326EFDB68.

[235] Coskata. About us 2012. Available from: http://www.coskata.com/company/.

[236] Huhnke RL, Lewis RS, Tanner RS, inventors. Isolation and characterization of novel Clostridial species. US. 2010.

[237] Kundiyana DK, Huhnke RL, Wilkins MR. Effect of nutrient limitation and two-stage continuous fermentor design on productivities during "Clostridium ragsdalei" syngas fermentation. Bioresource Technology. 2011 May;102(10):6058-64. PubMed PMID: WOS:000291125800069.

[238] Zahn JA, Saxena J, inventors. Novel ethanologenic Clostridium species, Clostridium coskatii. US. 2012.

[239] Kuo I. Coskata grabs biggest slice of USDA's $405M in biofuels loan guarantees: Reuters; 2011. Available from: http://www.reuters.com/article/2011/01/21/idUS126007139820110121.

[240] Lane J. Coskata switches focus from biomass to natural gas; to raise $100M in natgas-oriented private placement: Biofuels Digest; 2012. Available from: http://www.biofuelsdigest.com/bdigest/2012/07/20/coskata-switches-from-biomass-to-natural-gas-to-raise-100m-in-natgas-oriented-private-placement/.

[241] Wernau J. Biofuel firm Coskata drops initial public offering plans amid volatile IPO market: Chicago Tribute Business; 2012. Available from: http://www.chicagotribune.com/business/ct-biz-0721-ipos-20120721,0,1092013.story.

[242] Lane J. The intrepid investor: How are biofuels IPOs performing and why? : Biofuels Digest; 2012. Available from: http://www.biofuelsdigest.com/bdigest/2012/02/09/the-intrepid-investor-part-i/.

[243] Guzman DD, Chang J. Bio-based 2,3 BDO set for 2014 sales. ICIS Chemical Business. 2012:24.

[244] LanzaTech. Media Releases. Available from: http://lanzatech.com/media/media-releases.

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