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Abstract

Syngas is created through the thermochemical conversion of biomass using gasification or pyrolysis and from CO-rich off-gases obtained from industries such as steel mills. The Wood-Ljungdahl metabolic pathway, or its variations, is used by acetogenic bacteria to convert syngas components (CO, H_2, and CO_2) to alcohols and other compounds. Many factors affect how well syngas is fermented, including the bacteria species used, syngas composition, medium components, bioreactor type, operational parameters used, and the gas-liquid mass transfer rate. These parameters impact carbon and electron flow in the bacteria, influencing the distribution, concentration, and metabolic end-product yield, which determines process feasibility. This article focuses on gas composition, microorganisms, gas-liquid mass transfer fermentation strategies, medium design, and commercialization activities to develop the syngas fermentation processes.

Keywords: Bioethanol; Syngas; Fermentation; Gas-Liquid mass transfer; Medium design;

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1. Introduction

Petroleum and other liquid fuel consumption is expected to increase globally from 90 million to 121 million barrels per day by 2040 [1] (Figure 1). Liquid fuels continue to comprise most of the fossil fuels consumed, with most of the increased use in the transportation sector. Advancements in nonliquid-based transportation systems are expected; however, they will not be sufficient to satisfy the growing global demand for transportation services [1].

This significant reliance on fossil fuels produces increased greenhouse gas emissions, contributing to climate change. Ethanol is heavily promoted as a clean fuel for transportation with cleaner combustion than gasoline [2,3]; it is a high-octane fuel made from waste streams and renewable bio-based resources. Ethanol also has a high oxygen content; therefore, blending it with gasoline enhances hydrocarbon combustion [2]. Gasoline containing 10% or more bioethanol (E10) can achieve complete combustion and release fewer unused hydrocarbons [2]. Factors such as an increase in national energy security concerns, high gasoline costs, and environmental impacts from high petroleum usage within the transportation sector have led to increased advocacy for biofuel production [4]

Figure 1. Production of petroleum and other liquid fuels (million barrels per day) [1].

First-generation bioethanol fuels are made from sugars derived from sugarcane or starches from cereal grains and starchy tubers, such as cassava, all of which are food-based feedstocks [5]. The cost-effectiveness and sustainability of first-generation biofuels have been scrutinized and the concerns about fuel versus food have stimulated research on using feedstocks that are non-food based. These concerns and with various other issues motivated the development of second generation and syngas fermentation technologies [6].

Second-generation bioethanol technology is a biochemical process [7] in which biochemical or thermochemical conversion process methods are used to release sugars from lignocellulosic biomass, which are then used for fermentation to produce ethanol [7].

Lignocellulosic feedstocks contain lignin, which acts as a barrier that significantly hinders the hydrolysis reaction that converts cellulose and hemicellulose into fermentable sugars; this makes syngas fermentation better than the first- and second-generation technologies because the gasification process of syngas fermentation gasifies all of the lignin into fermentable sugars that are made available for microbial conversion into bioethanol [8].

Syngas fermentation is a thermochemical/biochemical hybrid process that uses the flexibility of the gasification process and the uniqueness of the fermentation process to produce ethanol and other chemical compounds [9].

The energy rich lignocellulosic biomass and waste feedstocks are gasified (thermochemical process) to produce syngas comprised largely of CO, H_2, and CO_2. The produced syngas is then chemically transformed into bioethanol [10]. The Fischer-Tropsch (FT) process is the most common thermochemical process that converts syngas into bioethanol using a metallic catalyst such as cobalt or iron [11,12]; albeit, this process requires high pressure and temperature. The catalysts are easily poisoned by contaminants in the gas stream and have a strict CO/H_2 molar ratio requirement [13,12,7]. Research on syngas fermentation for bioethanol production has been ongoing for several years, however these studies have not yet defined a methodology for producing high levels of bioethanol with stable culture [9].

Commercial bioethanol production is restricted by challenges related to the syngas fermentation process, including low productivity rates and limitations due to gas-liquid mass transfer.

This article provides a review of syngas fermentation process development with a focus on gas-liquid mass transfer, microorganisms, fermentation media design, and the effects of temperature and pH on operating conditions.

2. Bioethanol and biofuels derived from syngas

Syngas can be produced by gasifying municipal solid wastes, agricultural residues, biomass, animal wastes, energy crops, coal, petroleum, coke, and other non-food-based feedstocks. Carbon monoxide-rich exhaust gases generated by steel industries can also be converted to syngas components [14,15]. The gasification process converts the entire feedstock, including lignin and glucose, into a mixture of gases primarily comprised of carbon monoxide (CO), carbon dioxide (CO_2), hydrogen (H_2), and some minor compounds such as ethane, methane, ash, nitrogen oxides, and tar [16].

Syngas composition is usually influenced by a variety of factors, including feedstock type; oxidizing agents such as steam, air, or oxygen; gasification process conditions such as pressure and residence time, temperature, and heating rate; and type of gasifier used, such as fluidized or fixed bed [17]. Syngas is converted into bioethanol and various biofuels using the Fischer Tropsch and syngas fermentation processes. The syngas fermentation process uses acetogenic bacteria, which can use CO and CO_2 as carbon sources, for metabolism and growth in the presence of H_2 and synthesize bioethanol and other useful biofuels in bioreactors operated at ambient pressure and temperature and with flexible CO:H_2 molar ratios [18].

3. Syngas fermentation

Acetogenic bacteria transform CO, CO_2, and H_2 into syngas under anaerobic conditions, which is then used to create bioethanol and other valuable compounds [19]. The Wood-Ljungdahl metabolic pathway (WLP) is the process that dictates the conversion of these gases using acetogenic bacteria to produce bioethanol and acetic acid. Literature reports over 60 acetogen strains, and majority of them grow with CO_2 and H_2, while a handful of them just needs CO or both substrates.

Examples of some acetogenic bacteria include *Clostridium autoethanogenum* [20], *Clostridium ljungdahlii* [21], *Clostridium ragsdalei* [21], *Clostridium aceticum* [22], *Clostridium carboxidivorans* [23,24],

Acetobacterium woodii [25]*, Blautia producta, Clostridium magnum, Moorella Thermoacetica, Eubacterium limosum,* and *Clostridium coskatii* [21]

Equations 1 and 2 represent the conversion to acetic acid, while Equations 3 and 4 represent the conversion to bioethanol [26].

$$2H_2O + 4CO \rightarrow CH_3COOH + 2CO_2 \quad (1)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (2)$$

$$3H_2O + 6CO \rightarrow C_2H_5OH + 4CO_2 \quad (3)$$

$$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O \quad (4)$$

Certain acetogens, such as *C. carboxidivorans*, can transform CO, CO_2, and H_2 into butanoic acid, butanol, hexanoic acid, and hexanol, while also producing ethanoic acid, or acetic acid, and ethanol [27–29]. Several of these compounds, along with additional chemicals such as octanol, n-propanol, and hexanoic acid, can also be generated by fermenting defined or undefined mixed culture syngas [30].

3.1. Acetogenic microorganisms

Syngas fermentation can be accomplished with either pure or mixed cultures [31]. The medium is first sterilized prior to inoculating with only one strain of bacteria in the pure culture fermentation process, including *C. ljungdahlii* [32], *C. ragsdalei* [33], *C. carboxidivorans* [34,35], and *C. autoethanogenum* [36] [31]. *Moorella thermoacetica* [8] and *Butyribacterium methylotrophicum* [8,37] in a pure culture can synthesize ethanol, hexanol, butanol, acetic acid, hexanoic acid, and butanoic acid, among other compounds (Table 1). Various yields and selectivities differ according to microbe type, medium component, gaseous substrate, bioreactor type, and culture parameters such as pressure, temperature, and pH.

Mixed culture syngas fermentation processes may use wastewater sludge and manure as inoculum since they are rich in microbes that produce alcohols from syngas [38,39,30]. Mixing pure cultures can also result in co-cultures, which extends product range and yields longer carbon-chain carboxylic acids and

alcohols [40,41]; however, the productivities and selectivities of mixed culture syngas fermentation are comparatively low compared to pure culture syngas fermentation.

Table 1. A summary of microorganisms used to ferment syngas.

3.2. Wood-Ljungdahl metabolic pathway

The Wood-Ljungdahl pathway (WLP) is used by bacteria that ferment syngas (Figure 2). This process converts CO_2 and CO to Acetyl Coenzyme A (CoA), an essential precondition for biomass components and fermentation products [50]. Acetyl-CoA Synthetase (ACS) uses the end product of the Carbon Monoxide Dehydrogenase (CODH) reaction, with carbon monoxide (CO) as the substrate to produce Acetyl-CoA [51,52]. The production of ethanol and acetic acid from the syngas components CO, H_2, and CO_2 involves a series of simple chemical processes [18,53] (Figure 2). The simple inorganic chemical substrates, CO, H_2, and CO_2, are transformed into Acetyl-CoA and ultimately into compounds such as acetic acid and ethanol [18]. A portion of the Acetyl-CoA is rerouted so it can be used to produce more complicated organic cell components such as lipids, proteins, and carbohydrates. Most of the consumed gas is used to supply energy for the cell functions, which results in the accumulation of acetic acid and ethanol [18].

Figure 2. Wood-Ljungdahl Pathway: the process for fermenting CO and CO_2 to produce ethanol and acetic acid [18].

3.3. Substrates and energy source

Acetogenic bacteria use CO and H_2 as their primary substrate and energy source and use CO_2 as a carbon source if CO and H_2 are present [54]. The feedstock type and production methods determine the H_2,

CO_2, and CO ratios present in the syngas [55]. Cell growth and end-product synthesis patterns are influenced by the H_2 and CO composition in the substrate [55,56]. Researchers observed that increasing the partial pressure of CO to 202.7 from 35.5 kPa enhanced *C. carboxidivorans'* cell mass by 3.4-times and the concentration of ethanol by 20-times [56]. *C. carboxidivorans'* ethanol synthesis transitioned to growth from non-growth in response to an increased CO partial pressure [56,57].

Syngas derived from biomass gasification typically has various types and amounts of impurities, depending on the oxidizing agent, feedstock type, and operating conditions [8,58]. Tar, Ammonia (NH_3), Hydrogen Cyanide (HCN), and Nitric Oxide (NO) are the most prevalent biomass gasification by-products [8]. Many of these contaminants, including those at extremely low concentrations, can limit the acetogenic bacteria activity [8]. Benevenuti et al. [8] reported that syngas fermentation could be negatively impacted by contaminants in various ways, including cell growth inhibition and enzyme activity, as well as the change in physiochemical parameters such as pH, redox potential, and osmolarity. Syngas-fermenting acetogens can tolerate some pollutants; however, cleaning the gas before fermentation is crucial to produce a steady and consistent bioprocess [59].

3.4. Media components and formulation

The syngas fermentation medium must include all of the essential nutrients and have an acceptable reductive-oxidative potential (ORP) to sustain the growth and metabolic processes of the anaerobic syngas fermenting acetogens [18]. Amino acids, such as those found in yeast extracts, mineral salts, vitamins, and trace metals, are the typical components present in an acetogen medium [60,61]. Co-factors, typically supplied in very small amounts, are essential vitamins for enzyme function [61]. Researchers investigated the impact of mineral salts on syngas fermentation and reported that the metalloenzymes of the Wood-Ljungdahl pathway rely heavily on trace metals for their function [62]. The effects of minerals and trace metals on syngas fermentation are summarized in Table 2.

Table 2. Minerals and trace element effects on product formation, cell development and growth, and their functions during syngas fermentation.

Removing the elements calcium (Ca), potassium (K), and sodium (Na) from the medium did not influence syngas fermentation in *C. ragsdalei* [61]. The researchers reported that the organism's cell mass and ethanol production decreased significantly when phosphate (PO_4^{3+}), ammonium ion (NH^{4+}), magnesium (Mg^{2+}), and sulfide (S_2^-) were removed from the *C. ragsdalei* media [61]. They also reported that ethanol formation decreased by 97% when tungstate (W) was removed from the medium [61]. Ethanol formation decreased by 82% when iron (Fe) was removed from the medium [61]. Ethanol formation decreased by 38% when molybdate (Mo) was removed from the medium [48,61] and decreased by 24% when cobalt (Co) was removed from the medium [61].

Li et al., [63] established that increasing the zinc (Zn) concentration to 280 from 7 μM resulted in both cell concentration of *C. carboxidivorans* and ethanol production increasing by almost 200%. Furthermore, butanol production was increased by 660%, and hexanol production increased by over 4300%. Increased alcohol production has been associated with higher ADH gene expression and carbon fixation. Limiting nutrients may also boost the yield of the desired products from syngas fermentation.

Formulating a fermentation medium that is efficient and cost-effective is another significant area of research for syngas fermentation [64]. A low-cost medium can be created by decreasing the number of components present in the medium or removing any unnecessary components [65]. The medium's existing nutrients can be replaced with cheaper alternatives, which is vital for commercialization [64].

Gao et al. [26] formulated a low-cost *C. ragsdalei* fermentation medium in which the 4 – morpholineethanesulfonic acid (MES) buffer was removed. MES is the most expensive medium component, accounting for 90% of the total cost. The authors lowered the trace metal and mineral

concentrations and used defined chemicals to replace the yeast extract (YE). The overall cost of the medium after modification was lowered by 95%, with a 36% increase in ethanol yield using CO, compared to the more expensive YE medium [26]. Phillips et al. [48] created a low-cost *C. carboxidivorans* medium by minimizing mineral and trace metal use, eliminating YE and MES, and replacing KOH with low-cost ammonium hydroxide to adjust pH [48].

Syrona et al. [66] conducted an experiment in a CSTR using NH_4OH as Nitrogen source. Syngas was introduced into the reactor vessel that contains fermentation medium of *Clostridium ljungdahlii* C01 culture. The pH of the culture was set to about 4.5 to 4.6 and controlled with 5% solution of $NaHCo_3$. NH_4CL was used as the starting Nitrogen source and was later changed to NH_4OH by removing the starting NH_4CL from the medium. The researchers found that changing to NH_4OH increased the culture pH by about 4.6%, while increasing productivity of ethanol by 13% from 16.2 g/L.day to 18.3 g/L.day. They observed that acetic acid concentrations initially increased but later decreased steadily. They reported no significant change in cell density and gas uptake as a result of the change to NH_4OH nitrogen source [66].

Kundiyana et al. [67] demonstrated that adding cotton seed extract (CSE) to the basal medium provided all of the essential nutrients for *C. ragsdalei* without affecting the amount of produced ethanol, despite the removal of MES, YE, minerals, vitamins, and trace metals. Maddipati et al. [68] replaced other nutrients in corn steep liquor (CSL) to ferment syngas using *C. ragsdalei*. Adding a CSL medium of approximately 20 g L^{-1} to a 7.5 L fermenter increased ethanol production by approximately 60% and increased CO consumption by approximately 35% [68].

Shen et al. [69] used the Box-Behnken design to optimize a medium formulated with *C. carboxidivorans*. These researchers raised the amounts of Co^{2+}, Ni^{2+}, SeO_4^{2+}, WO_4^{2+}, and Cu^{2+} and decreased the amounts of MoO_4^{2+}, Fe^{2+}, and Zn^{2+} while adding Fe^{3+}. The authors revealed that this increased the amount of ethanol, hexanol, and butanol produced to 89.8% from 47.7% [69].

Recent scientific advances have indicated that biochar can improve syngas fermentation efficiency [54,54,70,71]. Biochar's physical and chemical characteristics are primarily influenced by its feedstock and production processes [54,54,70,71]. Biochar is a low-cost pH buffer as well as trace metal nutrients and mineral source and using biochar to buffer a medium increases ethanol production and could reduce commercial syngas fermentation costs [72].

3.5. Effect of temperature and pH on syngas fermentation

Syngas fermentation is strongly influenced by operating conditions such as temperature and pH. The media's pH influences the shift between acetogenesis and solventogenesis [73]. Biofuel production during syngas fermentation consists of two stages: Acetogenesis, which is the synthesis and accumulation of acetic acid, and Solventogenesis, which is the synthesis of alcohols such as ethanol and butanol [74]. A pH drop during acetogenesis causes acid accumulation, and acids that do not dissociate can diffuse through the microorganism's cytoplasmic membrane. Microorganisms convert acids into neutral solvents, which prevents cell damage or death; therefore, solventogenesis is a method for avoiding an additional pH decrease [74].

Undissociated fatty acid accumulation reduces the pH to a thermodynamic limit during acetogenesis, after which solventogenesis occurs, leading to an acid transition to alcohols and a pH increase [75]. Abubackar et al. [76] established that *C. autoethanogenum* did not accumulate acetic acid during syngas fermentation with a regulated pH of 4.5; however, the same quantity of ethanol and acetic acid accumulated when fermentation was conducted at a steady pH of 6.0, suggesting that ethanol yield was promoted by low pH. The effects of pH during syngas fermentation have also been reported for *C. ljungdahlii* [20], *C. ragsdalei* [77], *C. carboxidivorans* [78], and *C. autoethanogenum* [76]. These studies revealed that a low pH ranging from 4.5 to 5.0 favored solventogenesis, while a high pH range of 5.0 to 6.0 favored acetogenesis. Liu et al. [45] determined that *Alkalibaculum bacchi*, a syngas-fermenting strain that is

slightly alkaliphilic, could produce up to 2 g L^{-1} of ethanol and up to 3.0 g L^{-1} of acetic acid within a pH range of 6.0–8.0.

Syngas fermentation using cultures with only one strain, or pure cultures, was studied using a two-stage reactor that included a growth reactor for producing carboxylic acids at a higher pH and a product reactor for converting carboxylic acids to alcohols at lower pH [60]. Richter et al. reported ethanol concentrations of approximately 5.5 g L^{-1} in *C. ljungdahlii* and acetate concentrations of 18 g L^{-1} with a growth reactor at a constant pH of 5.5, and ethanol concentrations of approximately 20.7 g L^{-1} with a product reactor at a pH range between 4.5 and 4.8 [60]. Atiyeh et al. [77] developed a novel approach for controlling the syngas supply based on the culture's pH. The novel pH control approach enhanced operational stability, concentration, ethanol selectivity, and doubled ethanol production during continuous syngas fermentation.

The temperature during fermentation is crucial and influences the proliferation and metabolic activities of the acetogenic bacteria. Ramió-Pujol et al. [79] established that the optimal temperature for alcohol synthesis and carbon chain elongation by *C. carboxidivorans* is 25 °C. Shen et al. [69] reported that *C. carboxidivorans* produces more ethanol, butyrate, acetate, caproate, hexanol, and butanol when the fermentation temperature is reduced from approximately 37 °C to 25 °C after 24 hours.

Microbial activity can be altered by the pH of the fermentation as well as the incubation temperature and media buffer's existence or no-existence [80]. The solubility of carbon monoxide and hydrogen in syngas decreases as temperature increases. Clostridium species selectively shift to solventogenesis phase, from acetogenesis, at pH values below 5.0. Also, the addition of morpholinoethanesulfonic acid (MES) acting as a media buffer has been found to prolong the lag time for the production of ethanol [80].

Kundiyana et al. [80] investigated the impact of temperature, pH and MES buffer on *Clostridium ragsdalei's* ability to produce ethanol, and according to the researchers they found *Clostridium ragsdalei* syngas fermentation at 32 °C with media devoid of a buffer was linked to higher ethanol concentrations

and a shorter lag time before transitioning to solventogenesis. Temperature beyond 40 °C and pH level under 5.0 were beyond the optimal range of bacterial growth and metabolism [80].

Shen et al. [81] investigated the influence of culture temperature on high alcohol production and biomass growth using *C. carboxidivorans* P7 cultivated at constant temperature and two-step temperatures within the range of 25 to 37 °C [81].

The researchers reported that the use of two-step temperature culture contributed significantly to increased production of alcohol. Additionally, while 37 °C encouraged significant gene expression associated with the Wood-Ljungdahl pathway, genes that encode enzymes initiating acyl-condensation processes linked to higher-alcohol synthesis were abundantly expressed at 25 °C [81].

3.6. Gas-Liquid mass transfer

Gas-liquid mass transfer is challenging because CO and H_2 are poorly soluble in water: 83% and 71%, respectively, relative to O_2 (~10^{-4} g/g) at 37 °C [18]. Numerous studies have investigated various reactor designs and configurations to enhance gas-liquid mass transfer. The reactors include hollow fiber membrane reactors (HFMR) [82], constant stirred tank reactors (CSTR) [82], trickle bed reactors (TBR) [83,84], gas-to-atomized-liquid-contactors [85], gas lift reactors [86], and bubble column reactors [87–89].

Yasin et al. [90] reported that the HFMR volumetric mass transfer coefficient k_{La} improved with the surface area of the membrane per unit working volume and pressure. The researchers proposed using submerged HFMR that can achieve high CO mass transfer rate in microbial syngas systems. The fermentation was performed using *Eubacterium limosum* KIST612 [90]. They fabricated a membrane module using hydrophobic polyvinylidene fluoride (PVDF) membrane fibres, that they used to pressurize CO in water phase. The gas-liquid volumetric mass transfer coefficient (kLa) was determined by the pressure (P) through the hollow fibre lumen and the surface area of the membrane per unit working volume of the liquid (AS/VL).

It was found that when pressure was 93.76 kPa and surface area of membrane per unit working volume of liquid was set at 27.5 m-1, the volumetric mass transfer coefficient kLa was 135.72h-1. Increasing the surface area of the membrane per unit working volume AS/VL to 62.5 m-1, and reducing the pressure to 37.23 kPa, achieved a higher volumetric mass transfer coefficient kLa of 155.16 h-1 [90]

Elisiario et al. [91] reported that silicone membranes are highly resistant to mechanical and chemical stress, unlike microporous membranes, and they are not susceptible to pore clogging, biofouling or liquid entry in the pores [91].

The simplicity of operation and control has made CSTR the most used reactor in research laboratories and industrial settings [92]. Table 3 provides a summary of some widely used bioreactors, presenting how they perform during syngas fermentation.

Other significant factors known to affect gas-liquid mass transfer include liquid-gas contact and gas diffusion. Orgill et al. [82] studied the volumetric mass transfer coefficient (k_{La}) of different types of hollow fiber membrane reactors (HFMR), constant stirred tank reactors (CSTR), and trickle bed reactors (TBR) with varying packing sizes and discovered that HFMR containing non-porous polydimethylsiloxane yielded the highest volumetric mass transfer (k_{La}) at 1062 h^{-1}, with CSTR yielding 114 h^{-1} and TBR containing 6 mm of beads at 421 h^{-1}.

Atiyeh et al.[93] also discovered that adding activated carbon and nanoparticles to the fermentation of a syngas medium increased gas solubility and product formation. Nanoparticles are very small particles that range in diameter from 1 to 100 nm [94]. Kim et al. [95] investigated introducing six different kinds of nanoparticles to a *C. ljungdahlii* syngas fermentation medium to observe the effects on H_2, CO, and CO_2 solubility, as well as the cell mass and acetic acid and ethanol production. The researchers discovered that silica nanoparticles significantly enhanced the solubility of H_2, CO, and CO_2 in comparison to various nanoparticles such as carbon and alumina iron oxide. The researchers also discovered that methyl-functionalized silica improved H_2, CO, and CO_2 solubility more than untreated silica. The cell mass

increased by 34%, and ethanol and acetic acid production increased by 166% and 29%, respectively, when methyl-functionalized silica nanoparticles were added to the fermentation media [95].

Tecante and Chopin [96] completed a study on using helical ribbon screw (HRS) impeller for mixing in a stirred tank reactor. Their results show that the helical ribbon screw impellers have a greater effect on the volumetric mass transfer kLa performance than the power density [96].

Lines [97] carried out a study which examined the effect of different kinds of baffle geometries and non-standard impeller designs on the volumetric mass transfer coefficient. The dynamic approach was used to determine mass transfer using a polarographic sensor. Beavertail baffles offered a modest benefit in enhanced volumetric mass transfer coefficient over standard full baffles at low speeds (N 275 RPM), but there was essentially no advantage at higher speeds [97].

Table 3. Summary of fermentation parameters in different bioreactor systems during syngas fermentation.

4. Safety considerations

Safety needs to be considered in syngas fermentation due to large amounts of H_2 and CO being used. H_2 is highly flammable and needs to be handled with care. It is colourless, odourless and difficult to detect. Similar to H_2, CO is colourless, odourless and highly flammable. It is highly toxic to humans even with small exposure. A proper design to handle these gases needs to include preventing gas leaks, detecting gas leaks and having an alarm system in place in case leaks occur, dealing with gas leaks emergency in order to avoid impact to human health [101].

Specific safety measure designs include having bioreactors in fume hoods to ensure leakages of H_2 and CO are evacuated if they occur. Gas pipes should be used to transport H_2, CO, N_2 and CO_2 into the laboratory from cylinders outside of the building. The cylinders need to have manual pressure regulators and safety shutoff electrovalves. Fumehood should be equipped with detectors for H2 and CO, and there should be visual and sound alarms as warnings both inside and outside of the laboratory in case of any issue [101].

5. Challenges facing syngas fermentation and major scientific advances

One of the major obstacles to the commercialization of syngas fermentation technology is the limitation of gas-to-liquid mass transfer [82,102]. The volumetric mass transfer coefficient (kLa), which is a measurement of the reactor's hydrodynamic state, has been utilized as a reliable criterion when comparing the mass transfer rates of different reactor designs [82,102]. There have been scientific advancements in impeller designs, aerated power efficiency, fluid flow patterns, baffle designs, use of microbubble dispensers, and mixing time, to help improve the limitations of gas-to-liquid mass transfer [102].

Adding activated carbon and nanoparticles to the fermentation of a syngas medium was reported by Atiyeh et al.[99] as a method of improving gas solubility and consequently increasing product formation.

Munasinghe and Khanal [102] also reported syngas impurities as one of the challenges. Syngas impurities include ash, tars, ethane, ethene, ethyne, NOx and SOx. Various gas clean-up technologies have been developed to address the issue of impurities during syngas fermentation. These include rotating particle separators, water scrubbers, filters such as ceramic, fabric and bag, cyclones and wet electrostatic precipitators.

The isolation of anaerobic bacteria that can convert syngas into ethanol with a higher product yield is another challenge towards commercialization. There are scientific advances towards genetic modification of existing syngas fermenting microbes to enable them produce high product yields [102,103].

Syngas fermentation is invariably connected with acid generation, which decreases the pH of the culture. Clostridia cannot produce solvent in an adverse environment due to low pH. Rerouting the metabolic pathway away from acid synthesis and more towards solvent generation may increase ethanol production. Further research in this area is required [102].

Product recovery is another area of challenge due to high energy costs associated with the distillation which is the traditional separation technique for ethanol from a mixture of syngas fermentation byproducts and water. Several methods like vapor reuse, liquid-liquid extraction, and ultrasonic atomization have been investigated in order to lower the cost of ethanol recovery [103,102]

6. Commercialization

A viable commercial production syngas fermentation process needs flexible operations requiring a wide variety of feedstocks, reliable processes to accommodate for differences in syngas quality,

productivity improvements, and selectivity with better syngas component usage at low operating and capital costs.

Several organizations, including IneosBio (Vero Beach, Florida, USA [104]), Coskata (Warrenville, Illinois, USA [105]), and LanzaTech (Georgia, Chicago, USA [106]), have been working toward the goal of scaling-up and commercializing syngas fermentation technologies [46]. Organizations such as Genomatica (San Diego, California, USA [107]) and Kiverdi (Pleasanton, California, USA [108]) are also working toward the goal of commercializing syngas fermentation. IneosBio and Coskata are no longer in business since they were unable to overcome the related financial and operational challenges.

INEOS Bio was a division of INEOS and founded in 2008. INEOS Bio utilized patented isolates of *C.Ljungdahlii* in a pilot plant and reported 100 gallons of ethanol as their production rate per dry ton of feedstock [103].

INEOS Bio commissioned their first commercial scale plant, the Indian River BioEnergy Center in Florida, in July 2013. The plant produced ethanol from waste biomass and also produced six megawatts of renewable electricity per year from unused syngas and recovered heat. The plant had a planned capacity of about 250 thousand tons of waste biomass per year, and producing 8 million gallons of ethanol per year [109,104]

Coskata Inc., established in 2006 employed technologies and microorganisms such as *C.ragsdalei* and *C. carboxidivorans* which were licensed from University of Oklahoma and the Oklahoma State University [110,103]. Coskata Inc. identified and developed a proprietary bacterium called "*Clostridium coskatii*", for production of ethanol [111]. Coskata Inc. operated their gas fermentation plant utilizing syngas obtained from municipal solid wastes and wood biomass, and reported construction of a commercial plant to produce ethanol from wastes and wood chips with 16 million gallons of ethanol per year as planned production capacity, which will be scaled up every year to 78 million gallons [110,103]. Coskata

Inc., announced in July 2012, their change of directions with plans to build a commercial plant financed by private investors that exclusively used natural gas as a feedstock[112]

LanzaTech was established in 2005 and they have focused their commercialization on the use of syngas and CO-rich off-gases from industries to produce 2,3 – butanediol and ethanol utilizing their propriety strain of *C.autoethanogenum* [113].

LanzaTech is collaborating with the United States Department of Energy's Pacific Northwest National Laboratory (PNNL) to produce aviation fuels using syngas fermentation, then converting the produced ethanol into jet fuel through catalytic conversion [106,114]. Shougang Group, a renowned Chinese steel maker, has partnered with LanzaTech to launch a commercial facility that converts industrial emissions to 100,000 gallon per year of ethanol from steel mill off gases [106,114].

7. Future directions

Significant progress has been made toward commercially viable syngas fermentation technology. Further research and improvement are required to increase syngas fermentation technology productivity, yield, and production costs. The evolution of genetically altered microorganisms will help with bioprocess development and allow for the development of resilient microorganisms and processes that have efficient mass transfer and process control. Utilizing abundant carbon-based waste and renewable resources can advance syngas fermentation technology and produce biofuels and chemicals that have a neutral or even negative carbon footprint, assisting the energy, fuel, chemical, environmental, and agricultural industries.

Scientific advances in raw material development lower the cost of producing biofuel. This entails improving the production and sustainability of the present biomass resources, as well as developing new non-food feedstocks that are able to flourish amid adverse environmental circumstances with greater energy density. Advancements in biomass treatment and conversion techniques will improve overall

process efficiency while reducing negative impacts on the environment. Future research considerations include enhancing separation methods, microbial fermentation optimization and developing

8. Conclusion

Syngas fermentation is a versatile method for creating fuels and chemicals. Autotrophic bacteria use CO, H_2, and CO_2 from syngas to grow and produce alcohols and volatile fatty acids, based on the Wood-Ljungdahl pathway model and variations. Developing pathways or techniques for producing value-added chemicals, improving gas-liquid mass transfer performance and efficiency, and designing media that use low-cost substrates are essential for decreasing product costs and improving the viability of syngas fermentation. Using genetically modified strains, efficient process control design, appropriate fermentation media, and optimal bioreactor design can increase product generation while simultaneously lowering production costs.

Competing Interests

The authors would like to acknowledge that there are no competing interests with this work.

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Table 1. A summary of microorganisms used to ferment syngas.

Organism	pH	Temperature	Products Formed	References
Clostridium ljungdahlii	4.0 – 6.0	30 °C – 40 °C	Ethanol, Ethanoic acid (Acetic acid), 2,3-butanediol (2,3-butylene glycol), Methanoic acid (Formic acid),	42
Butyribacterium methylotrophicum	5.5 – 6.0	37 °C	Ethanol, Ethanoate (Acetate), Butanoic acid (Butyrate), Butanol	43
Clostridium autoethanogenum	4.5 – 6.5	20 °C – 44 °C	Ethanol, Ethanoic acid (Acetic acid), 2,3-butanediol (2,3-butylene glycol),	44
Moorella thermoacetica	6.5	55 °C	Ethanoate (Acetate)	45
Clostridium ragsdalei	5.0 – 7.5	25 °C – 40 °C	Ethanol, Ethanoic acid (Acetic acid), 2,3-butanediol (2,3-butylene glycol),	46
(Genetically modified) *Acetobacterium woodii [pMTL84151_actthlA]*	7.0	30 °C	Ethanoate (Acetate), Acetone,	47
Clostridium carboxidivorans	4.4 – 7.6	24 °C – 42 °C	Ethanol, Ethanoic acid (Acetic acid), Butanol, Hexanol	48
Alkalibaculum bacchi	6.5 – 10.5	15 °C – 40 °C	Ethanol, Ethanoic acid (Acetic acid)	49

Table 2. Minerals and trace element effects on product formation, cell development and growth, and their functions during syngas fermentation.

Minerals/Ion	Effects on fermentation of syngas	Functions	References
Na / Na^+	Not essential for ethanol and acetate production or cell development	Critical for ATP synthesis	61
K / K^+	Not essential for ethanol and acetate production or cell development	Activates the enzyme Formyl-H_4folate synthase	61
Ca / Ca_2^+	Production of ethanol and acetate and cell development were unaffected by absence	Critical for cell membrane stability and ATPase activity	61
N / NH_4^+	Absence reduced ethanol production by 41% and cell mass by 33%	Source of inorganic Nitrogen necessary for cell growth	61
S / S_2^-	Absence of sulfur-containing cysteine sulfide produced no ethanol or acetate, or cell mass	Utilized by H_2ase and corrinoid enzyme to reduce fermentation medium for anaerobic conditions	61
P / PO_4^{3+}	Absence reduced ethanol production by 85% and cell mass by 58%	Part of nucleotides, phospholipids, and nucleic acids	61
Trace metals / Ions			
Mo / Mo^{6+}	8.3 µM Mo caused a 34% ethanol decrease, while 0 µM Mo caused a 38% decrease with *C. ragsdalei*	Part of Formate dehydrogenase (FDH)	61 48
Se / SeO_4^-	10.6 µM Se caused an increase in ethanol production by 52%	Part of FDH	61
W / WO_4^-	6.8 µM W caused an increase in ethanol production by 102% for *C. ragsdalei*	Part of FDH	61
Zn / Zn^{2+}	66.9 µM Zn caused ethanol to increase by 4.2-fold for *C. ragsdalei.* 280 µM Zn caused ethanol to increase by 3.0-fold and cell mass to double for *C. carboxidivorans*	Part of Alcohol dehydrogenase (ADH)	61 63
Cu / Cu^{2+}	Caused ethanol production inhibition	Impacts ACS activity negatively	61
Fe / Fe^{2+}	Elimination reduced ethanol production by 82%; however, there was no effect on product formation and cell mass with 204 µM Fe	Part of ADH, FDH,CODH, and H_2ase	61
Co / Co^2+	Elimination reduced ethanol production by 24%; however, there was no effect on product formation and cell mass with 84 µM Co	Used to synthesize the methyl group in acetyl-CoA by Corrinoid enzyme	61

Table 3. Summary of fermentation parameters in different bioreactor systems during syngas fermentation.

Bioreactor	Microorganism	Gas Compositions (% by volume)	Product Concentrations (g L^{-1})	References
CSTR (with 100 L)	*C. ragsdalei strain*	CO:12-18, H_2:7-12, CO_2:10-17, N_2:55-60	Ethanol: 15.0 Acetate: 2.8	98
CSTR (with 7.5 L)	*C. ragsdalei*	CO_2: 15, H_2: 5, N_2: 60, CO: 20	Ethanol: 9.6 Acetate: 3.4	68
CSTR (with 3 L)	*C. ragsdalei* in a medium with activated carbon	H_2: 30, CO: 40, N_2: 0 CO_2: 30 or H_2: 5, CO: 20, N_2: 60, CO_2: 15	Ethanol: 19.0 Acetate: < 1.0	99
HFMR (with 8 L reservoir)	*C. carboxidivorans*	H_2: 5, CO: 20, N_2: 60, CO_2: 15	Ethanol:23.9 Acetate: 7.0	34
TBR (0.5 L)	*C. ragsdalei*	CO: 38, H_2: 29, CO_2: 28, N_2: 5	Ethanol:13.2 Acetate: 4.3	84
CSTR with cell recycle	*C. ljungdahlii*	H_2: 20, CO: 55, Ar: 15, CO_2: 10	Ethanol: 48 Acetate: 3	100
CSTR without cell recycle	*C. ljungdahlii*	H_2: 20, CO: 55, Ar: 15, CO_2: 10	Ethanol: 6.5 Acetate: 5.43	32