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PII: S0048-9697(21)05389-4

DOI: <https://doi.org/10.1016/j.scitotenv.2021.150312>

Reference: STOTEN 150312

To appear in: *Science of the Total Environment*

Received date: 23 June 2021

Revised date: 7 September 2021

Accepted date: 9 September 2021

Please cite this article as: A.N. Kumar, O. Sarkar, K. Chandrasekhar, et al., Upgrading the value of anaerobic fermentation via renewable chemicals production: A sustainable integration for circular bioeconomy, *Science of the Total Environment* (2018), <https://doi.org/10.1016/j.scitotenv.2021.150312>

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Upgrading the Value of Anaerobic Fermentation via Renewable Chemicals Production: A Sustainable Integration for Circular Bioeconomy

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Abstract

The single bioprocess approach has certain limitations in terms of process efficiency, product synthesis, and effective resource utilization. Integrated or combined bioprocessing maximizes resource recovery and creates a novel platform to establish sustainable biorefineries. Anaerobic fermentation (AF) is a well-established process for the transformation of organic waste into biogas; conversely, biogas CO₂ separation is a challenging and cost-effective process. Biological fixation of CO₂ for succinic acid (SA) mitigates CO₂ separation issues and produces commercially important renewable chemicals. Additionally, utilizing digestate rich in volatile fatty acid (VFA) to produce medium-chain fatty acids (MCFAs) creates a novel integrated platform by utilizing residual organic metabolites. The present review encapsulates the advantages and limitations of AF along with biogas CO₂ fixation for SA and digestate rich in VFA utilization for MCFA in a closed-loop approach. Biomethane and biohydrogen process CO₂ utilization for SA production is cohesively deliberated along with the role of biohydrogen as an alternative reducing agent to augment SA yields. Similarly, MCFA production using VFA as a substrate and function of electron donors namely ethanol, lactate, and hydrogen are comprehensively discussed. A road map to establish the fermentative biorefinery approach in the framework of AF integrated sustainable bioprocess development is deliberated along with limitations and factors influencing for techno-economic analysis. The discussed integrated approach significantly contributes to promote the circular bioeconomy by establishing carbon-neutral processes in accord with sustainable development goals.

Keywords: Organic waste, CO₂ sequestration, Succinic acid, Fatty acids, Biorefinery, Biogas

1. Introduction

There is an ongoing global search for alternative resources that can effectively substitute the fossil feedstocks and facilitate environmental sustainability (Hoang and Fogarassy, 2020). Organic fractions of municipal waste serve as a potential resource for the production of collective bio-based products and also address the waste management constraints (Venkata Mohan et al., 2016; Yaashikaa et al., 2020). According to the 2020 World Bank report, approximately 2.01 billion tons of municipal solid waste will be produced from households and commercial suppliers, thus amounting to a footprint of nearly 0.74 kg per person per day (<https://datatopics.worldbank.org/what-a-waste/index.html>). Sustainable management of these large quantities of waste is challenging and requires strategic disposal practices to avoid further environmental contamination (Xu and Strømme, 2019). Specifically, urban inhabitants are severely impacted by these unsustainable waste management practices, which cause environmental consequences, ecological imbalance, and deteriorating health conditions (Baran et al., 2016; Venkata Mohan et al., 2020). Conventional waste management practices incidentally eliminating the resource potential of waste and emit greenhouse gases (GHGs) (Sharma et al., 2020). Hence, there is an alarming situation to shift towards the effective waste management practices embedded with effective resource recovery (Laurens et al., 2012)

Development of sustainable waste management technologies could follow the ‘trash to cash’ concept by producing industrially important renewable chemicals, fuels, and materials. In this direction, the global research fraternity is shifting towards the effective mining of renewable resources through an integrated biorefinery framework to produce collective bio-based products (Laurens et al., 2015). A single bioprocess or product-based approach has certain limitations in terms of extensive waste utilization, high product yields, economic viability, residual waste

management, and sustainability. The development of integrated approaches encompasses multiple biological processes in a cascading manner to mine every constituent of waste by maximizing its utilization (Xiong et al., 2019).

Succinic acid (SA) is a four-carbon platform chemical that has wide applications in pharmaceuticals, food, cosmetics, polyesters, polyurethanes, and plasticizers (Ghayur et al., 2019; Patsalou et al., 2017). The United States Department of Energy (DOE) has declared SA to be a value-added chemical, with a global production capacity of 30,000-50,000 tons per year (Dai et al., 2020). Currently, SA production occurs via chemical routes and thus contributing to CO₂ emissions leading towards global warming (Liebal et al., 2018). Therefore, the revolution of biological SA production using organic waste as a resource along with AF produces biogas CO₂ consumption as a carbonate source to address CO₂ emission issues. Additionally, biohydrogen utilization as an electron donor stimulates SA yields. Simultaneously, medium-chain fatty acid (MCFA) (C₆-C₁₀) production integration by utilizing volatile fatty acid (VFA) (C₂-C₅)-rich fermentative effluents as a substrate facilitates complete carbon utilization. Perceptively, MCFAs are superior to VFAs and have wider applications, specifically as fuel blends, in biomedical applications and food additives, etc. Traditionally, MCFAs are produced using fossil-based resources; in a sustainable model, they can be produced from VFA using bio-hydrogen as a reducing agent alternative to ethanol or lactate (Bao et al., 2019; Jankowska et al., 2018). Integrated production of these easily marketable renewable chemicals facilitates sustainability and also improves the economics of the AF process, contemporarily facilitating complete carbon capture, including CO₂.

By keeping this in mind, the present review aimed to summarize the integrated up-gradation opportunities of AF to produce easily marketable SA and MCFA using organic municipal solid waste as a resource in a closed-loop approach (Figure 1). While there are multiple

articles published regarding the various aspects of AF improvements and integrated models, this is the first comprehensive review that covers AF biogas utilization for SA production and fermentative effluents integration for MCFA production. Firstly, the advantages and limitations of the AF are comprehensively discussed along with the scope for sustainable integration. Furthermore, biogas up-gradation for SA production using CO₂ as a carbonate source, along with compounding advantages of biohydrogen and biomethane processes are discussed. Similarly, MCFA production using VFA-rich fermentative effluent as a substrate and the role of various electron donors including hydrogen are covered in the complete carbon turnover framework. The factors that influence the techno-economics of the bioprocess are illustrated by covering aspects such as process economics, issues that need to be prioritized before scaling up, and inherent limitations. This closed-loop concept validates the existing fermentation process by completely utilizing organic waste and produces easily marketable bio-based products in line with the establishment of carbon-neutral societies.

Figure 1

2. Fermentative Valorization of Organic Waste

2.1 Anaerobic Fermentation: Advantages and Limitations

Anaerobic or ‘dark’ fermentation is a well-established process for the biological transformation of organic waste into biogas (Bio-CH₄/H₂) and nutrient or organic-rich intermediates (Venkata Mohan et al., 2019). Traditionally, the process of AF has been visualized as a wastewater treatment plant, in due course recognized as a bioenergy production process (Verbeeck et al., 2018). AF has dual benefits such as waste remediation and bioenergy production and can be broadly classified as acidogenic fermentation and bio-methanation processes; the

terminology may vary based on the desired end product (Fonseca et al., 2021; Ghysels et al., 2020). Figure 2 depicts the various fermentative pathways along with a list of bio-based products that could be potentially synthesized. The biomethanation process produces methane as an end product, wherein intermediate metabolites such as hydrogen and VFAs are utilized for methane synthesis. Theoretically, 1 kg of COD could produce 0.35 m³ methane, which is equivalent to 0.7 m³ of total biogas (Venkata Mohan et al., 2016). In the case of acidogenic fermentation processes, microbial cultures (mixed) are specifically enriched using selective inoculum pretreatments such as heat (70-90 °C), 2-bromoethanesulfonic acid (BESA), and acid (HCl/HNO₃/H₂SO₄) by eliminating non-spore-forming or VFA-consuming methanogenic microbiomes. While, acidogenic fermentation produces the 4 moles of hydrogen per mole of glucose via the acetate pathway and 2 moles with butyrate pathway (Fra-Vázquez et al., 2020; Sarkar et al., 2016).

The bio-methanation process is also called anaerobic digestion, has certain limitations in terms of high retention time requirements, low biogas yields, the minimal economic value of biogas, and classification of methane as a GHG (Majeed et al., 2018). Nevertheless, amendments to the anaerobic digestion process have been implemented for the production of biohydrogen (Bio-H₂). Hydrogen is known as an eco-friendly energy carrier with a high calorific value (122 kJ/g); it has 2.75 times more energy than conventional hydrocarbon fuels (Jung et al., 2011). Additionally, hydrogen has a good conversion efficiency of usable power which thus flags hydrogen as a carbon-neutral energy carrier. Conventionally, hydrogen is mainly produced from natural gas, accounting for 70 million tonnes per year. Alternatively, renewable hydrogen (biomass/organic waste-based) can replace natural gas utilization (Sharma et al., 2020). In this direction, investments in renewable hydrogen are increasing, with the European Union is planning to invest up to €180-470 billion by 2050. The current estimated cost of hydrogen derived from

fossil resources is approximately 1.5 €/kg (EU context); thus, the cost mainly depends on the natural gas price and availability. The cost of renewable hydrogen could be lower; the estimated cost of carbon capture and storage is approximately 2.5–5.5 €/kg (<https://ec.europa.eu/energy>). A dialogue over international trade concerning hydrogen and its downstream products is essential to decrease costs with strategic objectives and decisions. Additionally, the market penetration of hydrogen is expanding; it is estimated that by 2030, the consumption rate will increase specifically towards the automobile industrial sector, along with chemicals, petrochemicals, and others (Castelló et al., 2020).

Extensive studies have evaluated the production of bio-hydrogen using various organic wastes as feedstocks in different reactor configurations, such as continuous stirred tank reactors (CSTR), anaerobic dynamic membrane bioreactors (AnDMBR), up-flow anaerobic sludge blanket reactors (UASB), anaerobic sequencing batch reactors (ASBR), and fixed bed reactors (FBR) (Bakonyi et al., 2014; Jung et al., 2011; Preethi et al., 2019; Sivagurunathan et al., 2016). Continuous hydrogen has several advantages over batch production in terms of production rates, waste utilization, and ease of operation (Bhatia et al., 2021; Palazzi et al., 2000). A study reported by Schmidt and Ahring, 1996 reported that CSTR operation rapidly flocculates and granulates hydrogen-producing bacteria. As an improvement to CSTR, AnDMBR restricts microbial discharge and facilitates solid material for *in situ* biofilm formation (Anburajan et al., 2019). Additionally, factors such as initial reversible substrate adoption, cell surface transportation to other cells, and irreversible adhesion lead to rapid multiplication along with granule formation stimulation. Moreover, reactor configuration, operation type, pH, temperature, accumulation of organic acids, and enrichment of hydrogen consumers govern the hydrogen process efficiency.

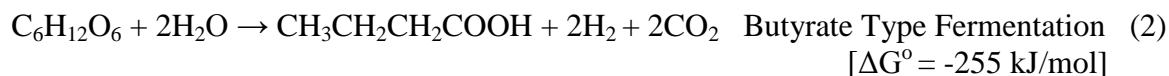
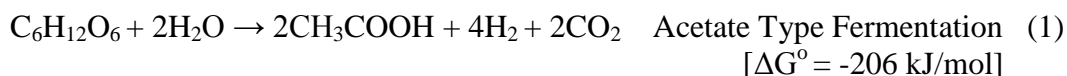
However, optimization studies are still in progress to overcome limitations such as hydrogen yield, production cost, effective carbon utilization, buffering maintenance, and design factors. The rate limiting steps in acidogenic fermentation is (i) hydrolysis: improving the hydrolysis step can increase the readily available substrate required for its conversion to biohydrogen and volatile fatty acids production, (ii) H₂ and fatty acid consumer: dominance of methanogens hampers overall production of H₂ and fatty acids affecting its net production. Thus elimination of methanogens is crucial towards achieving a high concentration of products, (iii) Elevated H₂ partial: H₂ partial pressure in the acidogenic reactors directly influences the metabolic shift and influences the formation of acetate, butyrate, propionate, and ethanol by altering the electron flow of respective pathways, (iv) feedback inhibition: the undissociated acids due to their lipophilic nature are known to cross the cell membrane of biocatalyst causing cell medium acidification and finally leading to hindered process performance (Sarkar et al., 2020; Venkata Mohan et al., 2016). Most of the studies resulted in yields < 50%, which could be due to thermodynamic limitations, acid shock, buffering maintenance, the existence of non-hydrogen producers, and promotion of hydrogen consumers (Lalman et al., 2013).

Lactic acid bacterial growth in acidogenic fermentation also suppresses the activity of hydrogen producers and reduces yield. Approaches such as heat, acid, and alkali pretreatments of inoculums have been studied to minimize the lactic acid-producing microbiomes (Sarkar et al., 2016; Dahiya et al., 2015; Naresh Kumar and Mohan, 2018b). However, achieving 2 moles of hydrogen per mole of hexose is considered a success, thus corresponds to 5% of the energy content of food waste which has the 30% carbohydrate content. A study published by Jang et al. (2015) reported that 1 kg COD was equivalent to 1.4 m³ of H₂ yields (133 mL/H₂ COD_{added}), which is only 10% of the energy content of food waste was transformed into hydrogen. Although various types of organic waste can be used for the production of biohydrogen, the production

costs are still higher than the current selling market price (0.5-3.2 USD/kg H₂) (Bartels et al., 2010). Overall, the economic feasibility of waste-based hydrogen production via anaerobic or dark fermentation remains challenging. Recently, electrochemical modifications and biochar augmentation in the AF process have evolved to induce process efficiency with minimal amendments (Kumar et al., 2021).

Organic acid accumulation in the AF process leads to acid shock specifically during biohydrogen production, thus hampering metabolic efficiency and limiting product yield. The undissociated form of these acids is lipophilic and can consequently cross the cell membrane (Castelló et al., 2020). Upon transformation into the cytoplasm, these acids dissociate and release protons, which leads to a drop in pH. Simultaneously, the demand for metabolic energy necessitates the maintenance of the internal pH and thus regulates substrate utilization along with minimizing the product yields (Shelef, 1994). A study published by Wang et al. (2008) investigated the inhibitory effect of acetic acid, propionic acid, butyric acid, and ethanol during the process of mixed microbial hydrogen production. The inhibitory influence of ethanol was relatively lower than that of acetic acid, propionic acid, and butyric acid at 35°C and an initial pH of 7.0. On the other hand, butyric acid has a reasonably high inhibition of NAD⁺ regeneration compared to the corresponding acetic acid. Butyric acid production allows NAD⁺ restoration through hydrogen production by reducing butyryl phosphate, which leads to unfavorable hydrogen production and thus possibly drives the bioprocess toward solventogenesis (Van Ginkel and Logan, 2005). Conventionally, butyric acid is produced by the hydroformylation of propene and syngas, wherein butyraldehyde forms as intermediates and are finally oxidized into butyric acid. Biological butyric acid production occurs via glycolytic breakdown of glucose into pyruvate, followed by acetyl-CoA, acetoacetyl-CoA, butyryl-CoA, and finally butyric acid as an end product. Here, 2 moles of hydrogen are produced per mole of glucose and 4 moles of hydrogen in

the acetic acid pathway (Eq. 1 and 2). Castro-Villalobos et al. (2012) reported that a concentration of non-dissociated acids > 30 mM influences the kinetics and stoichiometry of the process and thus inhibits production efficiency. However, the process of VFA separation is not economically feasible due to its high carbon-oxygen ratio; therefore, studies have been directed towards the integrated utilization of VFA as a substrate for the production of biopolymers, MCFAs, substrates for algal biomass growth, mixed alcohols, as well as biological nutrient removal (Duber et al., 2020; Venkata Mohan et al., 2016; Wu et al., 2020). The integrated utilization of biogas CO₂ for SA production and residual effluent rich in VFA for MCFA production facilitates the complete carbon turnover into renewable chemicals and maximizes carbon recovery, simultaneously promoting a circular bio-economy.



Figures 2

3. Anaerobic Fermentation Integration for Succinic Acid Synthesis

3.1 CO₂ Utilization and Biogas Fixation for Succinic Acid Production

Biological SA production necessitates CO₂ fixation through anaplerotic reactions, wherein 1 mole of CO₂ is required per mole of SA produced (Lu et al., 2009; Tan et al., 2017). According to reports, the global market for SA is estimated as 136.5 million USD in 2020 and will reach up to 200.2 million USD by 2027 (www.researchandmarkets.com). 1,4-butanediol (BDO) is industrials using solvent and has large-scale industrial chemical for polymers, elastic fibers and polyurethanes production, wherein SA is used as raw material for BDO synthesis. Also, the

polybutylene subset material (PBS) and poly(butyl-substance co-butylene terephthalate), which are two biodegradable plastics with a number of potential applications, can be used as the solvent base material for several other major industrial chemicals, including μ -Butyrolacin (GBI), tetrahydrofuran (THF), 2-pyrrolidone (2-P). Conventionally, SA is produced via fossil resources using maleic acid hydrogenation, while biological SA production utilizes organic waste as carbon and CO₂ as carbonate sources (Tan et al. (2017)). Biological SA is produced as an intermediate in the TCA cycle or as a product in anaerobic metabolism using CO₂ as a carbonate source (Figure 3). SA production occurs via three different routes, namely the fermentative pathway or reductive branch of the TCA cycle, the oxidative branch of the TCA cycle, and the glyoxylate pathway (McKinlay and Vieille, 2008). In the fermentation pathway, oxaloacetate is transformed into malate, followed by fumarate and succinate as end products. The biological SA production pathway requires 2 moles of NADH per mole of succinate produced, wherein the glycolytic pathway of 1 mole of glucose metabolism provides only 2 moles of NADH. Therefore, NADH limitation acts as a regulating factor in SA production, and the maximum SA theoretical production is limited to 1.71 moles of SA per mole of glucose in the presence of CO₂ with complete carbon flux (glyoxylate pathway). This theoretical yield could be increased to 2 moles by supplying hydrogen as an external electron donor (Amulya and Venkata Mohan, 2019; Dai et al., 2020).

Carbonate availability and the carboxylation process also play a key role in SA production, wherein phosphoenolpyruvate (PEP) or pyruvate is transformed into oxaloacetate in the presence of CO₂ (Ong et al., 2020). A study by Zou et al. (2011) reported that a minimal impact of CO₂ partial pressure was observed when gaseous CO₂ was used as a carbonate source. However, the supply and solubility of CO₂ limit SA production, which could lead to the formation

of formate, lactate, and acetate by lowering SA levels. Therefore, carbonate salts have been used as carbonate sources, namely MgCO_3 , CaCO_3 , NaHCO_3 , ZnCO_3 , and NH_4CO_3 . The reducing equivalents of each carbonate salt were different, with Ca having the highest value (+2.87), followed by Na (+2.71), Mg^{2+} (+2.37), Zn (+0.76), and NH_4^+ (-0.27). Tan et al. (2017) studied the role of various carbonate salts in *Actinobacillus succinogenes*, wherein MgCO_3 showed relatively high SA production (18.7 g/L) followed by NaHCO_3 (17.9 g/L), CaCO_3 (12.8 g/L), ZnCO_3 (3.3 g/L), and NH_4CO_3 (2.4 g/L). This is attributed to MgCO_3 providing Mg^{2+} ions for induced phosphoenolpyruvate carboxykinase activity, which is likely a key enzyme for SA production. Moreover, Mg^{2+} promotes the reservation of intracellular high-energy compounds, thus offering significant support for material transport inside and outside cells, along with elevated energy support for intracellular metabolism (Lu et al., 2020). Zou et al. (2011) reported that CO_2 (159.22 mM dissolved) along with MgCO_3 (40 g/L) resulted in good carbonate availability with a maximum SA production of 61.92 g/L compared to CO_2 only conditions (10.97 g/L, 20.22 mM dissolved).

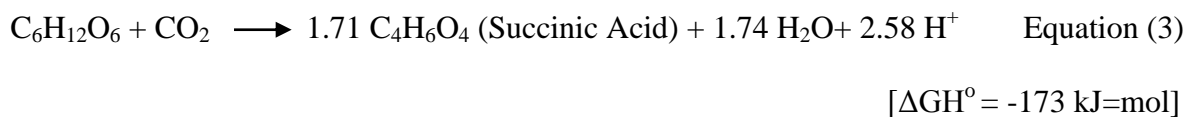


Figure 3

3.1.1 Bio-Methane CO_2 Utilization for Succinic Acid Generation

SA production integrated with biogas offers great advantages for CO_2 fixation and low-cost biogas upgradation. Figure 4 illustrates the opportunities for biogas integration with SA production, and Table 1 presents biogas and CO_2 utilization potentials for SA production. This approach entails the biological fixation of CO_2 for SA and simultaneously producing the purified

methane. Gunnarsson et al. (2014) were the first to report the utilization of biogas (60% CH₄ and 40% CO₂) for SA production using *A. succinogenes* 130Z, wherein biogas pressure of 101-140 kPa showed high carbonate availability and thus positively influenced SA production. The SA productivity of 14.4 g.L⁻¹ (140 kPa) was documented along with a biogas CO₂ fixation rate of 2.60 CO₂⁻¹ d⁻¹, wherein biogas methane purity reached up to 95% after 24 h of fermentation. Babaei et al. (2019) studied SA production using organic waste-derived sugars as substrates and biogas CO₂ as a carbonate source. The biomass growth (optical density₆₀₀) with MgCO₃ (5.5 ± 0.4) and biogas CO₂ (5.6 ± 0.3) as carbonate sources showed negligible differences. However, the MgCO₃ final titer value (5.5 g/L g_{SA}/g_{glucose}) of SA productivity was relatively higher than that of biogas (3.8 g/L g_{SA}/g_{glucose}). This was attributed to the positive influence of Mg²⁺ ions on PEP activity, along with higher carbonate availability compared to biogas. However, biogas CO₂ utilization has produced encouraging results, and further detailed investigations are needed to understand biogas CO₂ pressure, solubility, supply, and interference by H₂S (Amulya and Venkata Mohan, 2021). Recently, the European Union H2020-EU.3 funded a project called “NEOSUCCESS”, which aims to develop biogas CO₂ fixation for SA production along with biomethane upgradation. The project demonstrated biomethane production capacities ranging from 10,000 to 100,000 Nm³/year and integrated SA production around 350 t/years (<https://neosuccess-project.eu>). The project will exhibit effective resource utilization, cost-effectiveness, and environmental friendliness (biological CO₂ fixation for SA: 0.4 kg CO₂ per kg SA). This is the first integrated bioprocess technology that maximizes resource recovery from waste along with biogas CO₂ fixation for easily marketable SA along with industrially usable biomethane.

3.1.2 Bio-Hydrogen Integration

Biohydrogen utilization as an electron donor in SA production has dual advantages: firstly, hydrogen improves the NADH mobility and secondly, the CO₂ content of biogas is utilized as a carbonate source (Eq. 4). Werf et al. (1997) studied the role of hydrogen and fumarate as electron donors using *Actinobacillus 130Z*, wherein hydrogen significantly promoted SA yields, while formate did not exert much influence on SA yields, and no formate-hydrogen lyase activity was identified. SA is a highly reduced fermentative product, wherein redox couples such as NADH/NAD⁺ play a vital role in directing yields. Additionally, hydrogen supply lowers the formation of acetic acid and induces biomass growth along with elevated SA production rates (McKinlay and Vieille, 2008). A study published by Lee et al. (1999) investigated the role of hydrogen and CO₂ supply in SA production using *Anaerobiospirillum succiniciproducens*. They concluded that an external supply of hydrogen accelerated cell growth and resulted in high SA production rates, wherein an SA yield of 1.8 g/L⁻¹ h⁻¹ was documented with an optimum H₂/CO₂ ratio of 5:95 (v/v) with 20 g/L glucose.

Apart from biohydrogen, bioethanol fermentation-derived CO₂ could also be used as the SA carbonate source. Zhang et al. (2017) reported that ethanol fermentation plants in the United States released approximately 25.9 million tons of CO₂ for 9 billion gallons of ethanol production. This CO₂ could be effectively utilized for SA production upon its successful integration in a combined bioprocess approach. The obtained results revealed fixation of 388.8 g/L-d CO₂ with *A. succinogenes*, which is 188 times higher than that for the *Chlorella vulgaris* sequestration process for the same reactor volumes. A recent study by Amulya and Mohan, (2019) evaluated SA production using a novel isolated strain, *Citrobacter amalonaticus*, and studied the influence of various factors, namely carbon source type, buffering agent concentrations (NaHCO₃), different

pH conditions, and the role of H₂ as an electron donor. It was concluded that *C. amalonaticus* could be used as a potent strain for SA production (0.36 g.L⁻¹ h⁻¹) with an initial sucrose concentration of 30 g/L with 1 bar CO₂ pressure and partial H₂ supply. SA production integrated with biohydrogen production offers great advantages in augmenting SA productivity and simultaneously fixing biogas CO₂.

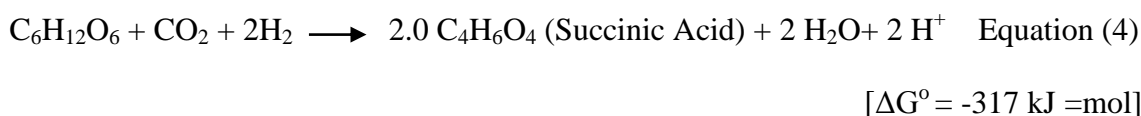


Figure 4 and Table 1

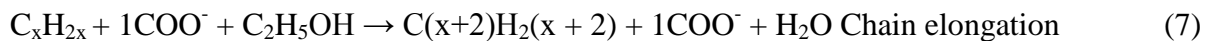
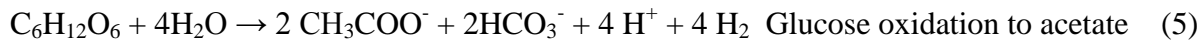
4. Medium Chain Fatty Acids Production

4.1 Volatile Fatty Acids as Electron Acceptors

MCFAs (C₆-C₁₀) are superior to VFAs and act in a process complementary to AF. MCFAs are mainly composed of caproic acid (C₆), oenanthic acid (C₇), caprylic acid (C₈), pelargonic acid (C₉), and capric acid (C₁₀) (Cavalcante et al., 2020; Q. Wu et al., 2020). MCFA has a market value of USD 2,000-3,000/t and market demand of 25,000 tons per year (De Groof et al., 2019). Ease of separation from the fermentation broth and high energy density position MCFA as a platform chemical. MCFAs have a higher energy efficiency than ethanol, wherein the 1 mole of caproic acid heating value is 3,452 kJ and which is higher than ethanol (2,638 kJ) (Dahiya et al., 2018). Additionally, MCFAs have wider applications as fuel additives, antibiotic substitutes, corrosion inhibitors, and fragrances. The biological production of MCFAs occurs via C₂ to C₄ or C₆ through chain elongation in the reverse β-oxidation pathway (Figure 5). In each loop, two carbon atoms, acetyl-CoA (C₂) are attached to a carboxylate and initiate the synthesis of MCFA

(Venkateswar et al., 2018). Extensive studies have been performed using *Clostridium kluyveri*, *Ruminococci*, *Megasphaera aelsdenii*, and enriched mixed microbiomes using ethanol or lactate as electron donors (Steinbusch et al., 2011).

A reverse β -oxidation mechanism initiates ethanol oxidation to acetate, while acetate activates acetyl Co-A. Further, the next loop continues by elongating the butyrate moiety using NADH, FADH₂ and further caproate via butyryl Co-A (Wang et al., 2020). In every cycle of the reverse β -oxidation pathway, the primary carboxylate becomes attached to the two carbon atoms, and thus the initial step requires metabolic energy (ATP). However, oxidation of electron donors such as hydrogen, ethanol, or lactate is essential to supply acetyl Co-A to maintain the required metabolic energy and supply reducing equivalents, namely NADH or ATP (Eq. 5-7) (De Groof et al., 2019; Wu et al., 2021). In the case of ethanol as an electron donor, it is oxidized into acetate for all five carboxylates elongated with a pair of carbon atoms. The process of energy transport phosphorylation controls the energetic coupling of the oxidation reactions. Fermentative production of MCFAs integrated with VFAs facilitates valorization and minimizes VFA consumption for methane production (Eerten-jansen et al., 2013). To attain high MCFA yields, various fermentation strategies have been studied with an enriched mixed culture (Steinbusch et al., 2008), bio-electrochemical production (Eerten-jansen et al., 2013), and two-stage integrations (Grootscholten et al., 2014).



4.2 Ethanol or Lactate as Electron Donor

Mixed microbiome fermentation offers a stable platform with a long operational period while using waste as feedstock and reduces the raw material cost along with maintenance. Multiple studies have evaluated MCFA production using ethanol, lactic acid, and hydrogen as electron donors with pure and mixed microbiomes (Reddy et al., 2018a,b; Wu et al., 2019a). A study published by Yin et al. (2017) examined the influence of acetate and ethanol ratios on caproate production using *C. kluyveri* as a biocatalyst, which resulted in a maximum caproate production of 8.42 g/L, while the ethanol and acetate ratio was 10:1 (550 mM total carbon). Candry et al. (2020) recently investigated the influence of pH on the chain elongation process, wherein a pH < 6 evidenced a predominance of butyric, valeric, and caproic acids. In contrast, pH > 6 shifted the product spectrum toward the mixture of acetic and propionic acids. Changes in the microbial community are also reported to be related to pH change, and it was concluded that the system pH acts as a driving force to induce the chain elongation process. Another study reported by Xu et al. (2018) evaluated Greek yogurt waste for fermentative lactic acid production followed by MCFA, wherein primarily 1.54 g L⁻¹ h⁻¹ lactic acid was produced in a thermophilic reactor (50°C). Thereafter, mesophilic MCFA production was studied using lactic acid-rich effluent as a substrate, and a volumetric caproic acid production rate of 81 mmol C L⁻¹ day (0.07 g L⁻¹ hr⁻¹) was documented.

4.3 Bio-Hydrogen as an Electron Donor

Fermentative hydrogen can be used as an alternative electron donor for MCFA production, thus reduces the MCFA production cost and renders sustainability. Partial hydrogen pressure in the process of fermentation leads to the transformation of acetate into ethanol, which subsequently undergoes MCFA production (Reddy and Mohan, 2017). Wu et al. (2019b) reported

that H₂ supply induces the chain elongation process and results in a 28% increase in MCFA production rates compared to the corresponding non-hydrogenic operation. Additionally, high *Clostridium sp.* abundance was observed under H₂-supplied conditions and was not enriched in the absence of hydrogen. In addition to hydrogen, syngas can also be used as a renewable substrate for MCFA production. A study published by Diender et al. (2016) reported MCFA production using carbon monoxide (CO) and syngas with acetate, *Clostridium autoethanogenum*, and *C. kluyveri* co-culture conditions. The co-culture system was capable of utilizing CO or syngas as a substrate along with acetate, resulting in 2.5 ± 0.63 mmol/L/day caproate production. In contrast, *C. kluyveri* monoculture was not able to utilize CO and bacterial metabolism was inhibited, which could be attributed to *C. autoethanogenum* diminishing *C. kluyveri* growth by minimizing available CO concentrations. Two-stage or integrated production of MCFAs with existing biohydrogen processes offers great advantages in terms of effective waste utilization and complete carbon turnover into commercially important renewable chemicals. Grootscholten et al. (2014) studied two-stage MCFA production using fractions of organic municipal solid waste (OMSW), wherein the first stage consists of OMSW acidification, with further utilization for MCFA production by secondary fermentation. A maximal MCFA production rate of 1.9 g L day^{-1} ($0.5 \text{ mol eq l}^{-1} \text{ d}^{-1}$), has been reported, which is approximately two-fold higher than that of single-stage systems (Grootscholten et al., 2013). In addition, the partial pressure of hydrogen plays a key role in directing chain elongation toward butyrate or caproate synthesis.

Figure 5

5. Road Map for Complete Carbon Turnover

Complete carbon turnover or zero waste approach can be achieved through combined processing, wherein waste is utilized in its entirety without any primary or secondary carbon

emissions, including CO₂. For instance, acidogenic fermentation is aimed at biohydrogen production and integration with SA and MCFA production offers the complete carbon (including CO₂) turnover into renewable products. First, organic waste is acidogenically fermented for the production of eco-friendly Bio-H₂, wherein VFAs are produced as a liquid discharge and CO₂ as a co-product. While there are two ways to proceed, firstly, Bio-H₂ could be separated from the biogas mixture by using hydrogen as a fuel and CO₂ as a carbonate source for SA production. Secondly, the unseparated biogas mixture (CO₂/H₂) could be directed to SA production, wherein CO₂ fixation occurs along with H₂ utilization as an electron donor. As mentioned above, the theoretical yield of 1.71 moles of SA per mole glucose could be increased up to 2 moles by supplying hydrogen as an electron donor. Usually, an individual hydrogen production process requires the separation of CO₂ for the effective utilization of hydrogen as a fuel. SA integration can overcome such separation module fixation, and this will increase the economic value of the process. Furthermore, utilization of VFA discharge for MCFA and/or mixed alcohol production transforms a significant quantity of carbon into commercially available renewable chemicals. Overall, carbon or CO₂ emissions are zero from this fermentative combined bioprocess, thus offering environmental sustainability and, simultaneously, creating a novel platform for easily marketable renewable chemical production. Table 2 illustrates the various combined bioprocess approaches for the synthesis of collective bio-based products.

Artificial intelligence (AI) technologies can also be integrated to achieve effective zero-waste technologies, wherein AI-based bioprocess controllers help stabilize product quality (Tan et al., 2021). Moreover, AI helps to identify waste dumping and real-time management tracking. The use of AI could minimize the involvement of people and eliminate manual sorting of waste, along with waste production quantification. Canadian company Intuitive has recently launched an AI approach target to achieve a zero-waste framework, thus offering decentralized waste

collection and/or segregation points equipped with artificial intelligence (<https://intuitiveai.ca>). Their product called “Oscar” helps to sort waste during disposal based on location or regional specifications. The use of AI technology in bioprocess monitoring and/or control reduces the cost of operation by offering real-time monitoring employing an appropriate database (Aynsley et al., 1993). Also, AI maintains the database of standard operating procedures, maintenance and troubleshooting of the reactors. However, the most work in artificial agents and has focused in a static environment on learning a single complex problem. For instance, Greyparrot AI technology has initiated the implementation of AI in the 21st century waste management sector and was awarded Best Startup Company in Climate Tech/Green Tech Startup-2020 at Europa Tech Startup Awards. The AI of the Winnow Vision company assigns a currency value to every scrapped platform dumped in its intelligent wastebasket and properly identifies the refining scope of waste along with quantity. In these perspectives, it is believed as AI technology helps in reaching the adequate waste database to the industrial communities for establishing waste resource based companies and offers the demand-based supply of resources.

Table 2

6. Environmental Sustainability and Techno-Economic Analysis

The economic viability and environmental sustainability of bio-based technologies are primarily governed by process efficiency, resource utilization, and carbon footprint emission (Katakojwala and Mohan, 2021). Factors such as selection of feedstock, process integration, manufacturing, and end-life utilization facilitate sustainable establishments (Figure 6). The present investor and industrial sector community are seeing AF biogas as an energy carrier, still considering it as a high-risk investment because of several factors such as resource availability,

waste segregation, product efficiency, operational issues, and economic viability (Kim et al., 2019). Approaches such as combined bioprocessing offer a sustainable framework through integrated decarbonization along with easily marketable high-value bio-based products analogous to fossil-based outcomes (Ncube et al., 2021). However, before commercialization, assaying techno-economic (TE) and life-cycle assessment facilitates the effectiveness of the process or product along with market feasibility with counterparts. TE analysis offers the feasibility and essentiality of the bioprocess, along with bottlenecks, to identify economic viability. TE analysis of the AF process can be broadly categorized into three areas: feedstock collection/transportation, biogas production, and product separation/utilization (as biogas or effluent). First, the collection and transportation fee is called a tipping fee, which is usually imposed on waste treatment units. For instance, the transportation cost in the USA is charged at USD 2.2/ton/km (curbside collection and transportation), which may vary based on geographical location. The transportation of waste is not economically viable if distances involved are > 25 km; hence, decentralized AF reduces transportation costs (Rajendran et al., 2014).

Biogas production from AF involves multiple processing steps, and the first collected organic waste composition is evaluated (composition, moisture, and VS content) and milled into small sizes. Thereafter, the feedstock is subjected to fermentation, as discussed above there are so many factors that govern the biogas yields, such as feedstock composition, microbial consortia, organic load, retention time, and temperature (Rajendran and Murthy, 2019). Kabir et al. (Kabir et al., 2015) conducted an economic assessment of forest biomass microbial transformation into biogas using organosolv pretreatment methods. 2.5 ton/h forest biomass was used as feedstock, which resulted in methane yields of 0.23 to 0.34 m³/kg VS, the capital expenditure (CAPEX) of the process was up to USD 60 M with a payback period of > 8 years. SA is a potential chemical

building block and is extensively used for the production of polybutyl succinate or other platform chemicals. A study published by Klein et al. (2017) investigated SA production from pentoses integrated with sugarcane biorefinery processing.

SA integration with bioethanol processing exhibits a relatively lower internal rate of return, whereas SA production has a strong probability of succeeding, perhaps greater than 12%. From an eco-friendly point of view, integration of SA with ethanol utilizes residual pentose sugars as a resource and CO₂ as a carbonate source, thus facilitating complete carbon turnover along with commercially interesting renewable chemicals (Amulya and Venkata Mohan, 2021; Herselman et al., 2017). SA production TE analysis using pure glycerol (98% w/w) indicates a price range of USD 2.01-2.95/kg, which includes downstream separation processing with a plant capacity of 460 kg/h. A study by Vlysidis et al. (2011) reported the possible integration of SA production with glycerol, wherein different schemes were investigated for glycerol utilization, such as crude glycerine disposal, purification of glycerin, and SA production using glycerine. Integration of SA production is a profitable approach and enhances earnings by up to 60% over a 20-year lifetime. MCFAs are monocarboxylic acids with high market value due to their widespread application in the production of dyes, pharmaceuticals, rubbers, antimicrobial agents, and fuel blends. Integration of the AF process with SA and MCFA production facilitates the production of easily marketable products and simultaneously fixing CO₂ along with sustainable circular bio-economy promotion.

Figures 6

7. Limitations and Future Scope

AF improvement and integration processes have several challenges that need to be addressed before their implementation. Factors such as carbon distribution, biogas supply,

specific integrated reactor design, and culture maintenance need to be explored extensively. Approaches such as decentralized AF operations would reduce transportation costs and utilize resources on-site. Specifically, biohydrogen-targeted processes require certain arrested conditions to eliminate hydrogen/VFA-consuming methanogenic microbiome enrichment. AF amendments with electrode materials (electro-fermentation strategies) neutralize thermochemical barriers and thus help to induce metabolic efficiency for the desired product. However, extensive studies need to be conducted in this emerging field to optimize various factors such as electrode material, reactor design, and potential range. On the other hand, biochar addition into the AF process promotes biogas yields and offers several advantages, such as direct interspecies electron transfer (DIET), microbial colonization, and buffering maintenance. Biochar types and quantities in the AF process require further investigation.

AF helps in treating various organic wastes, and the biogas content can be utilized for electricity production, fuel, domestic applications, and as natural gas substitutes (Sarma et al., 2015). For instance, Europe has 17,376 biogas installations, representing 7.7% of the European Union's primary renewable energy mix, and accounting for 51% of global biogas production in 2015 (Verbeeck et al., 2018). Nevertheless, EU countries are mainly supported through heat or power-associated subsidies. On the other hand, the global biogas market could reach up to USD 50 billion by 2026, necessitating the development of AF processes, including TE analysis (<https://waste-management-world.com>).

SA production integration involving AF requires an effective integrated reactor design, while applications involving biohydrogen necessitate specific high-pressure reactors enabled with safety features. Studies have reported the use of carbonate salts as an alternative to CO₂ for SA production; however, the utilization of biogas CO₂ content reduces production costs and mitigates CO₂ constraints. The current market price of SA derived from fossil or bio is approximately USD

2.94/kg, which needs to be reduced to USD 1/kg in order to establish a sustainable market. Additionally, the selection of raw materials/substrates, culture type, and reactor design are fundamental factors in determining final product costs. SA production costs using corn stover hydrolysate as a carbon source and biotin-supplemented yeast cell hydrolysate as a nitrogen source reduced the SA cost by USD1,120/t (56% cost saving) compared to glucose-based production costs of USD2540/t. On the other hand, 1.7 tons of MCFA production requires nearly 1 ton of acetate and 1 ton of ethanol. Typically, the prices of these substrates are rising rapidly because of the increased demand for bioethanol production. For example, sodium acetate per ton is (at the time of writing) USD 1,600 (cost varies from company to company), and ethanol costs USD2,800/t. Therefore, for the production of 1.7 tons of MCFA, the cost of the substrate (both acetate and ethanol) is USD 4,400. The raw material, bioreactor operation, and downstream processes signify SA production costs. The raw material cost could be reduced via waste utilization, and because of the use of single-microbe culture, the operational costs could not be negotiated. While there remains good scope to reduce costs involved in downstream processes, the separation of SA from the downstream fermentation process consists of three major steps. 1) Separation of microbial cells from the fermentation broth, which is usually performed through centrifugation or membrane filtration. 2) Removal of water and co-products (formate, acetate, lactate, and ethanol) through precipitation, solvent extraction, adsorption, reactive extraction, electrodialysis, zeolite, or membrane separation. 3) SA purification through vacuum evaporation and crystallization (Kumar et al., 2020). For instance, calcium salt-based precipitation is conventionally used for SA separation, wherein the product yield is relatively low and considerable amounts of CaSO_4 (gypsum) are generated as solid waste. Recently, membrane-based separation methods have been considered as a promising and viable technology to achieve purified SA through osmotic pressure drive, forward osmosis, temperature-driven membrane

pervaporation, and pressure-driven crossflow systems. A study conducted by Thuy et al. (2017) reported SA separation (99% purification) using cross-flow nanofiltration, dia-nanofiltration, and microfiltration coupled with crystallization methods. MCFA separation would be easier than that of SA due to its high carbon number and hydrophobic nature. Studies have evaluated using liquid-liquid extraction, membrane-based separation, and ion exchange processes (Q. Wu et al., 2020). Utilization of waste-derived intermediate organic acids (VFAs) as substrates and biologically-produced hydrogen as alternative electron donors reduces production costs (Venkata Mohan et al., 2018). Nevertheless, limitations such as VFA production yields and compositional variations may act as limiting factors for MCFA integration with AF. In addition, the utilization of hydrogen as an alternative electron donor requires extensive research to optimize factors such as hydrogen pressure, safety, and effective utilization. The establishment of fermentative biorefinery leads to the utilization of waste by producing collective bio-based products by promoting a circular bio-economy.

8. Conclusions

Fermentative transformation of organic waste is a low-cost economic process that possibly emits CO₂ and discharges secondary waste into the environment. Approaches like combined bioprocessing support the concept of “complete carbon turnover” and produce commercially important renewable chemicals, namely SA and MCFA. Combining AF biogas with SA production and digestate for MCFA synthesis mines every constituent of waste, including CO₂. The progressive integration of SA production with AF utilizes biogas CO₂ as a carbonate source and offers purified methane. Coupling with the biohydrogen process has dual benefits in terms of CO₂ fixation and increased SA yields, wherein hydrogen acts as an alternative electron donor to promote NADH/NAD⁺ availability. Apart from SA, MCFA production using VFAs as a substrate

along with fermentative-derived low-cost ethanol or hydrogen as an electron donor facilitates sustainable AF residue management. These renewable products attain a considerable position to replace fossil-derived commodities and facilitate complete carbon flux, simultaneously supporting bio-based economic growth in a sustainable framework.

Authorship Credit Statement

A. Naresh Kumar: Visualization, methodology, conceptualization, writing - original draft; **Omprakash Sarkar:** Data curation, Writing - part of the original draft; **K. Chandrasekhar:** Writing – part of the original draft; **Tirath Raj:** Writing -part of the original draft; **Vivek Nrishetty:** Writing – part of the original draft; **S. Venkata Mohan:** Conceptualization, methodology, writing - review & editing, proofreading; **Ashok Pandey:** Methodology, validation, writing - review & editing, proofreading; **Sunita Varjani:** Writing – part of the original draft; **Sunil Kumar:** Writing – part of the original draft; **Pooja Sharma:** Writing – part of the original draft; **Byong-Hun Jeon:** Writing – part of the original draft; proofreading **Min Jang:** Writing – part of the original draft, proofreading; **Sang-Hyoun Kim:** Resources, project administration, research facility, writing -review & editing, and funding acquisition.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science and ICT) (No. NRF-2019H1D3A1A01102655 and 2020R1A2B5B02001757).

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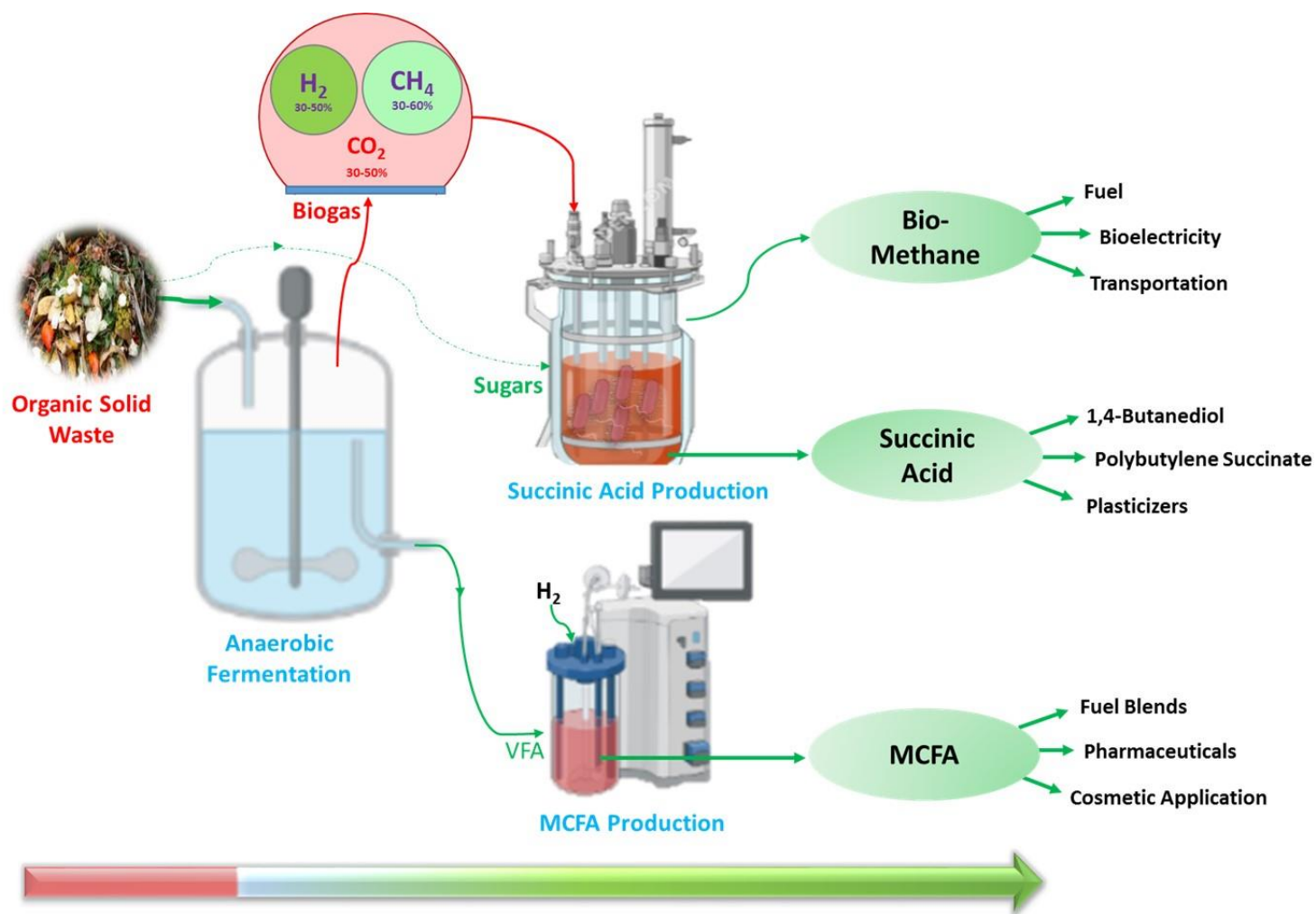


Figure 1: Schematic representation of anaerobic fermentative integrated succinic acid and MCFA production

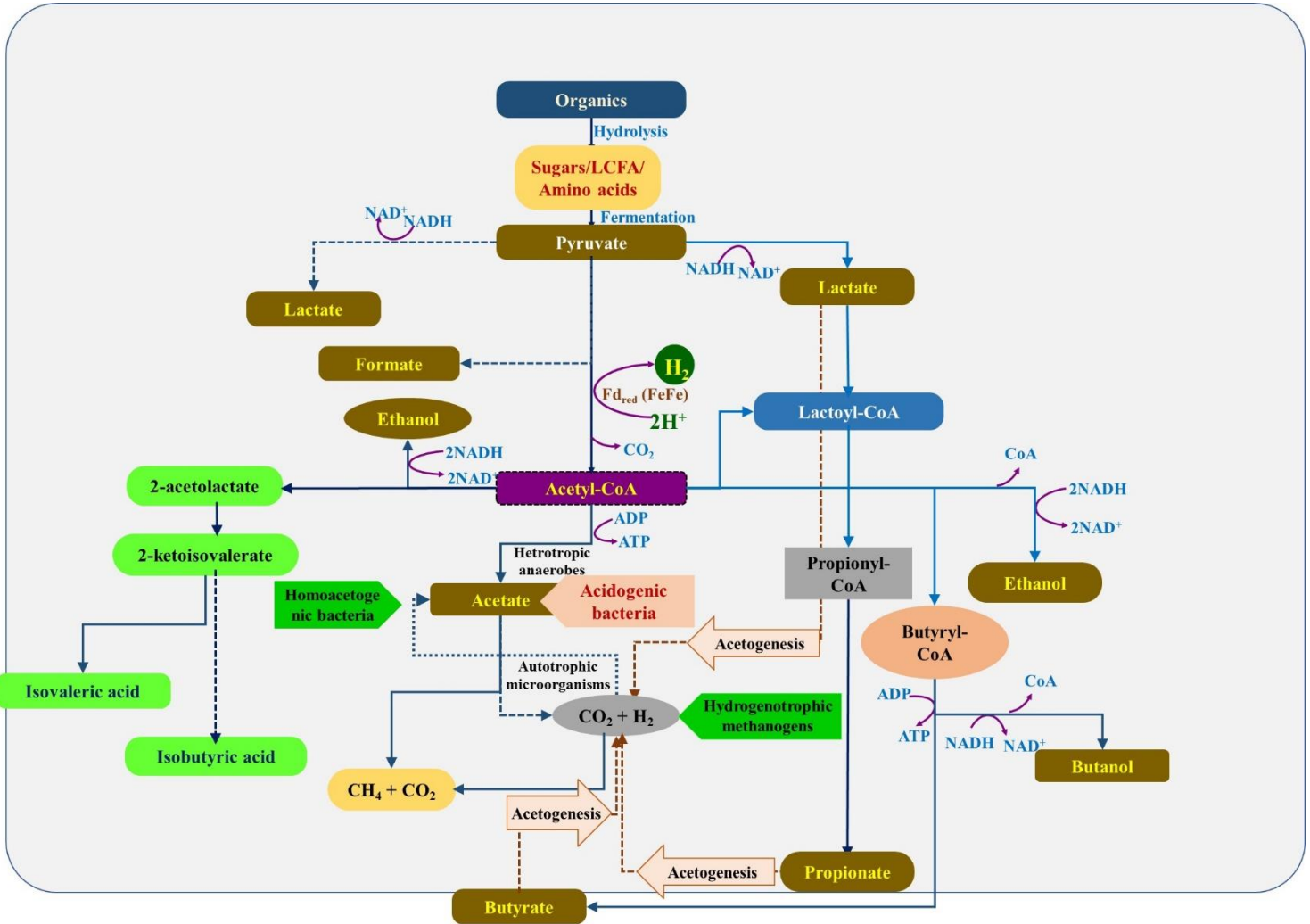


Figure 2: Schematic representation of anaerobic fermentative products

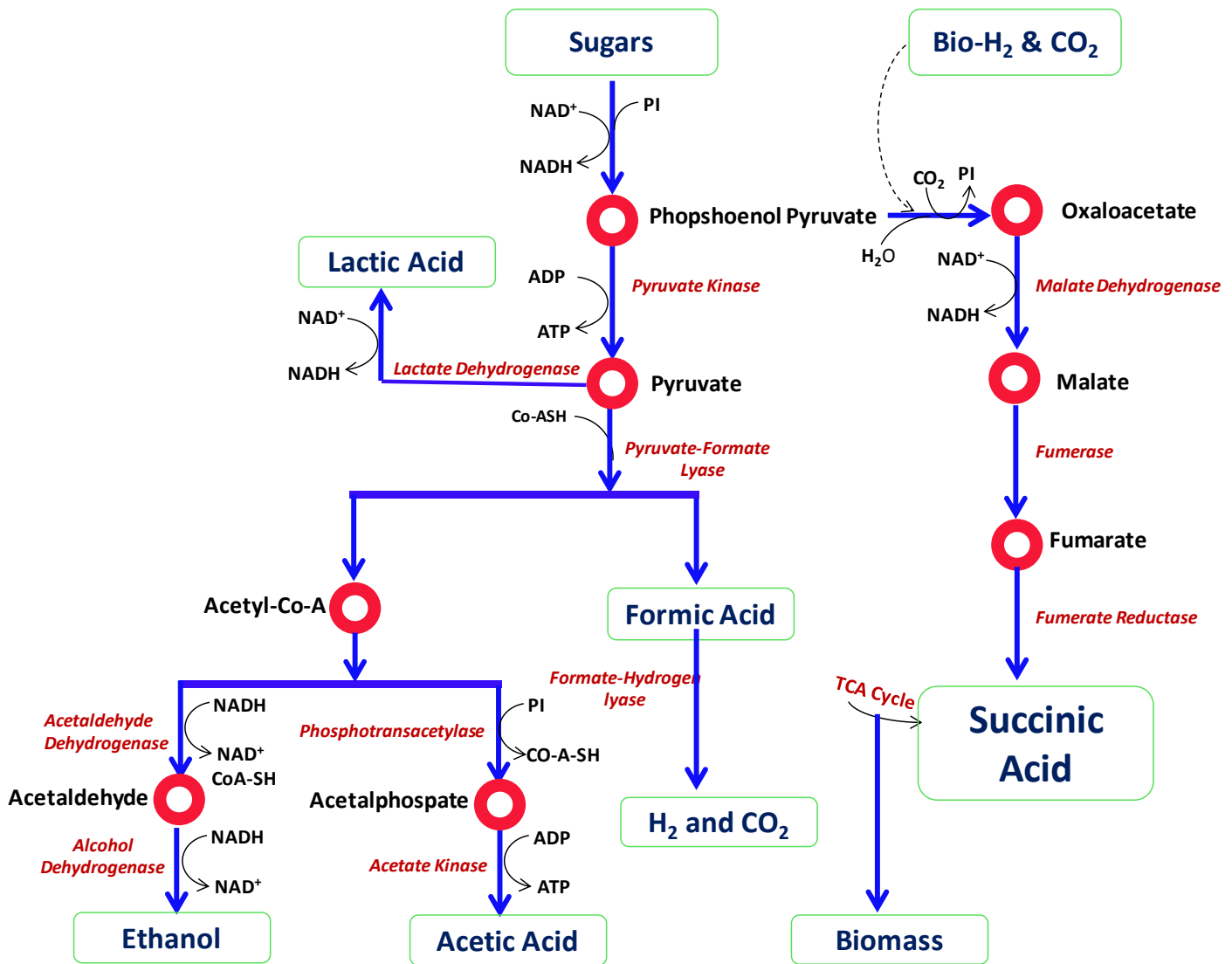


Figure 3: Fermentative (bacteria/yeast) integrated succinic acid production using CO₂ and biohydrogen

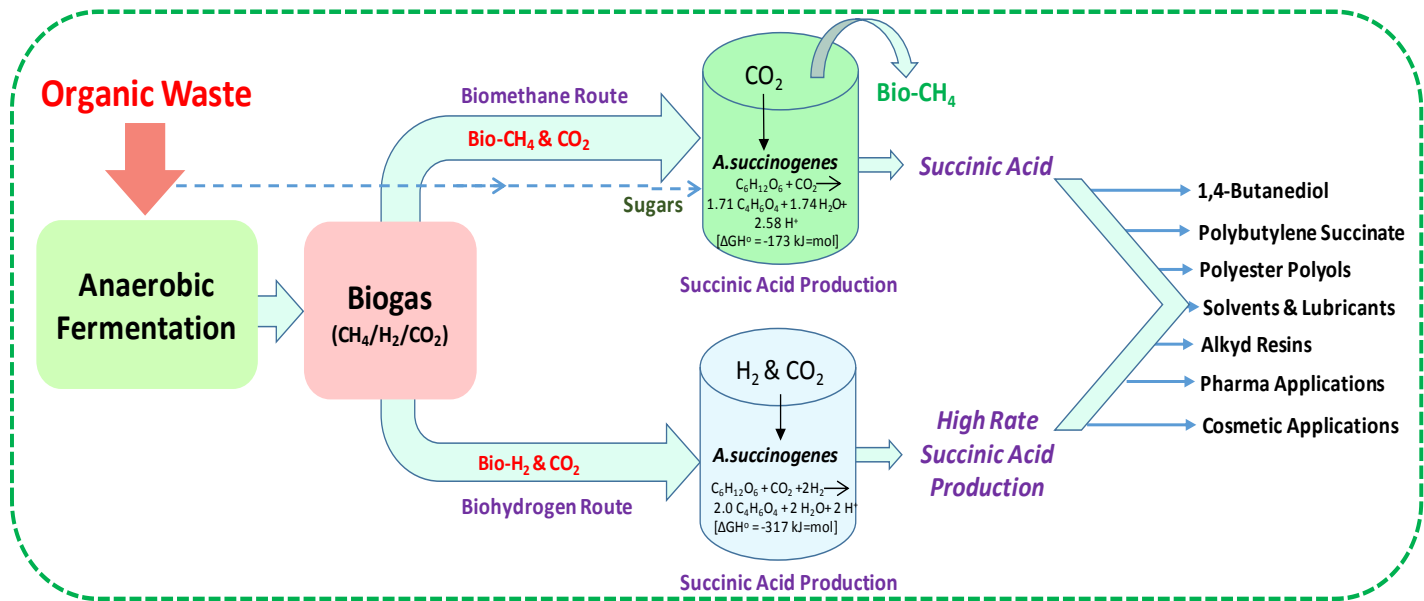


Figure 4: Biogas up-gradation and CO₂ fixation for succinic acid production

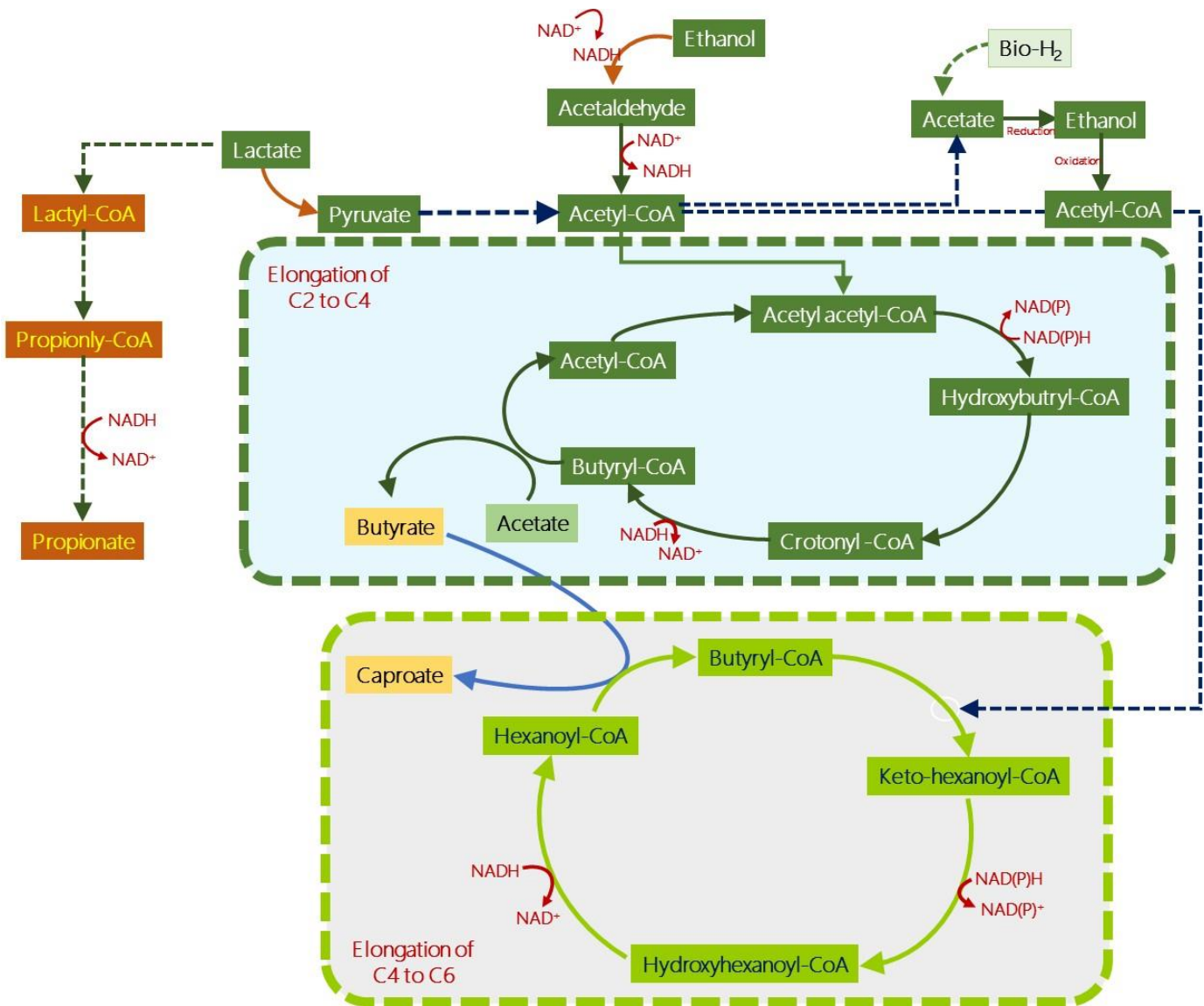


Figure 5: Medium-chain fatty acids production mechanism using bio-hydrogen, lactate, and ethanol as electron donors

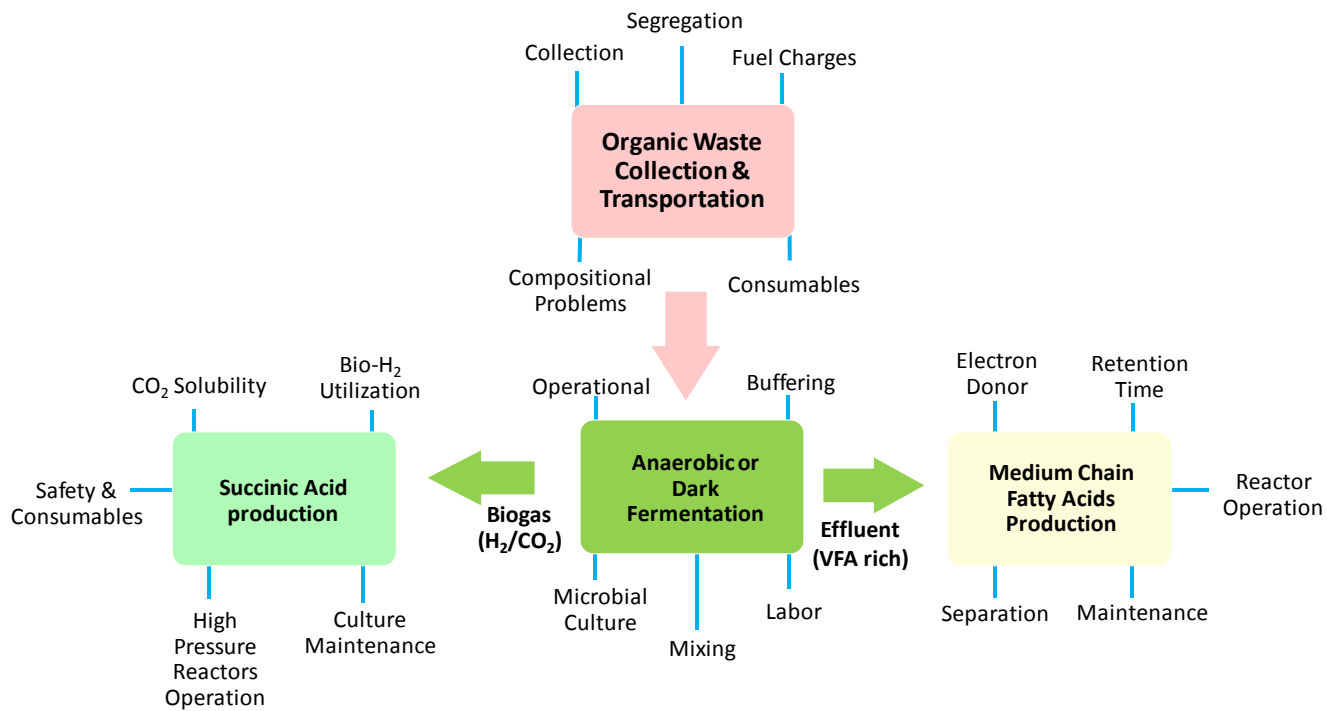


Figure 6: Factors influencing the techno-economic analysis of fermentative combined bioprocess approach

Table 1: Succinic acid production using biogas and CO₂ along with sugars

CO ₂ Loading Rate	Conditions	Substrate	SA Yield	Reference
0 bar	<i>Citrobacter amalonaticus</i> , pH:7, (1.6 L High-pressure gas fermenter)	Glucose 30 g/L	1.36 g/L	Amulya et al., 2019
0.6 bar			2.07 g/L	
0.8 bar			6.68 g/L	
1 bar			12.07 g/L	
2 bar			14.86 g/L	
0.1 vvm	<i>A. succinogenes</i> DSM 22257, pH:6.8 (3.5-L Labfors Bioreactor)	Glucose 40 g/L	26.6 g/L	Tan et al., 2018
0.3 vvm			29.9 g/L	
0.5 vvm			30.7 g/L	
0.7 vvm			30.6 g/L	
0.9 vvm			30.4 g/L	
40 kPa (Biogas: 60% CH ₄ , 40% CO ₂)	<i>Actinobacillus succinogenes</i> 130Z, pH:6.75 (3 L Sartorius BIOSTAT)	Glucose 30-32 g/L	12.85 g/L	Gunnarsson et al., 2014
56 kPa (Biogas: 60% CH ₄ , 40% CO ₂)			14.39 g/L	
101.325 kPa (100% CO ₂)			17.53 g/L	
140 kPa (100% CO ₂)			19.28 g/L	
25.33 kPa	<i>A. succinogenes</i> ATCC 55618, pH: 7.1-7.5 (5 L BioFlo 110 Bioreactor)	Glucose 100 g/L	8.84 g/L	Zou et al., 2011
50.66 kPa			10.21 g/L	
75.99 kPa			10.44 g/L	
101.33 kPa			10.97 g/L	
1.3 to 1.4 bar (Biogas: 40% CH ₄ , 60% CO ₂)	<i>B. succiniciproducens</i> (3 L Sartorius BIOSTAT)	Glucose 15 g/L and Xylose 2 g/L	3.8 ± 0.8 g/L	Babaei et al., 2019

2.96 vvm (CO ₂ saturation: 54%)	<i>Actinobacillus succinogenes</i> 130Z, pH: 6.8, Continuous fermentations	Glucose 25 g/L	12.2 g/L	Herselman et al., 2017
6.0 vvm CO ₂ (CO ₂ saturation: 67%)			12.2 g/L	
8.0 vvm CO ₂ (CO ₂ saturation: 77%)			11.3 g/L	

Table 2: Combined bioprocess approaches for collective biobased products synthesis

Substrate	Bioprocess	Primary Product	Secondary Product	Reference
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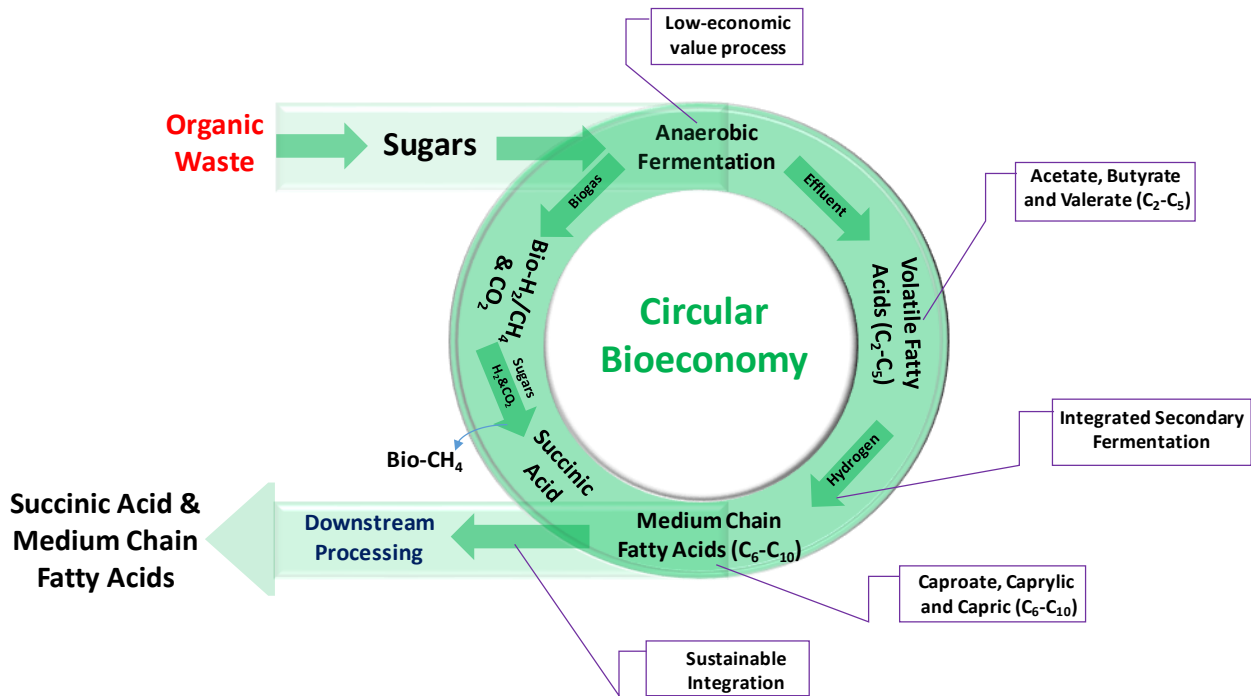
Glucose	Anaerobic Fermentation Combined process	Succinic Acid (0.635 g g ⁻¹)	Methane (95.4 % (v v ⁻¹))	Gunnarsson et al., 2014
Deoiled Algal Biomass	Fermentation and Bio-Anoxygenesis	Biopolymers (0.43 ± 0.20 g PHB/g DCW)	Bio-Ethanol (0.145 ± 0.008 g/g DAB)	Naresh Kumar et al., 2020
Cocoa pods	Anaerobic Digestion and Slow Pyrolysis	Methane (25±2.3 wt %)	Biochar (75.60± 2.31 wt % at 500°C)	Ghysels et al., 2020
Food Waste	Solid-state fermentation (SSF) and dark fermentation	Glucose (0.434 g glucose/g food waste)	Biohydrogen (52.4 mL H ₂ /g food waste)	Han et al., 2016
Food Waste	Anaerobic Fermentation and Chain Elongation	Acidified Food Waste (Rich in SCFA and Ethanol)	n- Caproate (5.5 g/L/d, total: 25.7 g/L)	Roghair et al., 2018
Greek-yogurt waste	Anaerobic Fermentation and Chain Elongation	Lactic Acid (1.54 g L ⁻¹ hr ⁻¹) Thermophilic reactor (50°C)	n- Caproate (0.07 g L ⁻¹ hr ⁻¹)	Xu et al., 2018
Sewage Sludge	Microbial Electrohydrolysis and Anaerobic Fermentation	Organic Solubilization (27.5%) Externally Applied Potential (-0.8V)	Volatile fatty acids (VFA: 4.65 g/L) and Bio-H ₂ (21%)	Kumar et al., 2019
Organic Municipal Solid Waste	Anaerobic Fermentation and medium-chain fatty acid production	Acidified Organic Waste (Rich in SCFA and Ethanol)	1.9 g MCFA I ⁻¹ d ⁻¹ (0.5 mol e eq I ⁻¹ d ⁻¹)	Grootscholten et al., 2014

	(Two-stage)			
Glucose	Integrated ethanol fermentation and succinic acid production	Bioethanol (50.1 g/L)	Succinic Acid (0.83 mol succinate/mol glucose)	Zhang et al., 2017

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



Graphical abstract

Highlights

- Biogas CO₂ utilization for succinic acid production is comprehensively elaborated
- Bio-H₂ utilization for medium-chain and succinic acid production are deliberated
- Discussed model valorize the fermentation process and contribute to bioeconomy growth
- Progressive integration with anaerobic fermentation enables the concept of zero waste