Potential Valorization of Organic Waste Streams to Valuable Organic Acids through Microbial Conversion: A South African Case Study

Sesethu Gift Njokweni, Annica Steyn, Marelize Botes, Marinda Viljoen-Bloom and Willem Heber van Zyl *

Abstract: The notion of a “biobased economy” in the context of a developing country such as South Africa (SA) necessitates the development of technologies that utilize sustainable feedstocks, have simple and robust operations, are feasible at small scale and produce a variety of valuable bioproducts, thus fitting the biorefinery concept. This case study focuses on the microbial production of high-value products from selected organic waste streams abundant in the South African agricultural sector using microbes adapted to utilize different parts of biomass waste streams. A ruminant-based carboxylate platform based on mixed or undefined anaerobic co-cultures of rumen microorganisms can convert the carbohydrate polymers in the lignocellulosic part of organic waste streams to carboxylic acids that can be upgraded to biofuels or green chemicals. Furthermore, yeast and fungi can convert the simpler carbohydrates (such as the sugars and malic acid in grape and apple pomace) to ethanol and high-value carboxylic acids, such as lactic, fumaric, succinic and citric acid. This review will discuss the combinational use of the ruminal carboxylate platform and native or recombinant yeasts to valorize biomass waste streams through the production of higher-value organic acids with various applications.

Keywords: biorefinery; carboxylate platform; rumen microorganisms; microbial bioconversion; organic acids; fruit pomace

1. Introduction

The world has been reliant on crude oil and coal as the primary source of energy and materials, driven by a linear economy that primarily focuses on continual growth, consuming resources and discarding waste for more than a millennium. This is particularly true for South Africa, which is currently the world’s most carbon-intensive coal-driven developing country and the largest greenhouse gas producer in Africa [1]. However, with limiting resources and the looming danger of climate change, mankind and particularly South Africa as a water-scarce country, have to consider alternative economic models, including the utilization of renewable sources and limiting waste that ends up in landfills and waterways. Fossil resources are utilized for the industrial production of a large range of value-added products. Many of these products could be replaced by bio-based analogs, which remain secondary in production due to the lower costs and optimized production processes associated with the fossil-based industry [2]. In contrast to fossil resources, bio-based products utilize renewable feedstocks and forms part of the “green chemistry principle” that was introduced by the Environmental Protection Agency (http://www.epa.gov/greenchemistry/basics-green-chemistry (accessed on 21 June 2021)) during the 1990s. Renewable feedstocks include invasive plants, agricultural wastes (including fruit waste streams), forestry residues, and municipal waste streams.

South Africa generates large quantities of agricultural, municipal and fruit waste streams, with a recent bioenergy atlas indicating that approximately 83 MMT of biomass
are annually produced as agricultural residues, forestry residues, organic solid waste, firewood, dedicated energy crops and invasive alien species [3]. Two significant sources are sugarcane bagasse (5.35 MMT) and invasive plant species (11.30 MMT). More than 390 invasive plant species have been identified that cover more than 8% of the country’s total area [4]. The principal invaders are trees and shrubs mainly from the genera Acacia, Hakea and Pinus [5,6]. In the 1880s, Prosopis species (mesquite trees) were introduced as a control mechanism to outcompete these invasions, mainly in the Savannah biome of South Africa (later adopted into the Fynbos biome) [5,6]. The plant was expected to have positive impacts in both the Savannah and Fynbos as it would provide shade, fodder for animals (feeding on the pods) and firewood for locals [4,7]. However, Prosopis has had more negative impacts on the environment and was declared a Category 2 invasive plant under the Conservation of Agricultural Resources Act, 1983 (Act No. 43 of 1983) (CARA). If Prosopis species are not controlled, they will rapidly spread (8% per annum) and can cover up to 56 million Ha in the future. Urgent strategies are thus needed to control the spread of Prosopis species and the mass-scale utilization of their biomass to produce valuable products could contribute to the cost of controlling and eradicating this invasive species [8]. Municipal waste accounted for 28% (liquid and solid) of the total global waste produced in 2016 [9]. In 2017, South Africa generated an estimated 55.6 MMT of general waste, which included 23.1 MMT of municipal solid waste (MSW) [10]. South Africa also generates about 0.5 MMT of paper sludge per annum as part of its large paper and pulp industry [11]. Significant amounts of fruit are produced globally (>742 MMT/year), with South Africa producing more than 7 MMT/year (Table 1). However, significant amounts of fruits wastes (more than 1.3 BMT in 2017) are generated, with as much as 60% being lost or wasted during the production, handling, storage, processing, distribution and consumption of fresh and processed fruits [12].

Table 1. Fruit production (million metric ton) in 2019 [12,13].

| Fruit Crop            | World (MMT) | South Africa (MMT) |
|-----------------------|-------------|--------------------|
| Citrus                | 108.04      | 2.58               |
| Grapes                | 77.14       | 1.99               |
| Apples                | 87.24       | 0.89               |
| Bananas               | 11.68       | 0.41               |
| Pears                 | 23.92       | 0.41               |
| Peaches and nectarines| 25.74       | 0.14               |
| Pineapples            | 28.18       | 0.11               |
| Mangoes and guavas    | 55.85       | 0.11               |
| Watermelons and melons| 12.79       | 0.09               |
| Plums                 | 12.60       | 0.06               |
| Total of all fruits   | 742.83      | 7.06               |

Considering the top three fruit crops produced in South Africa (Table 1), citrus, grape and apple wastes might be worthy biorefinery substrates. Although the building blocks of these fruit wastes may vary based on factors such as climate, harvesting years, fruit cultivars, cultivation and processing methodology, proximate composition analysis of fruit wastes can provide insight on the available carbon in these substrates. For example, grape pomace can contain 19–38% total dietary fibers, 3.68–29.20% pectin and 15–33% total sugar [14–17]. Apple pomace from different varieties can contain 26.8–82.0% total dietary fibers, 3.5–14.32% pectin, 11.5–49.8% fructose, 2.5–22.7% glucose and 0.05–3.28% malic acid [18]. It is also well-known that high concentrations of organic acids (such as citric, malic and tartaric acid) are responsible for the low pH of grape pomace, with a recent study reporting 19.4 g/kg malic acid in Chardonnay grape pomace [19]. Since it is not a first-generation feedstock, fruit waste biomass is a potential biorefinery substrate that doesn’t compete with food sources (such as corn and sugar cane) [20].

In the past four decades, considerable progress has been made towards developing second-generation technologies to produce cellulosic ethanol from cellulosic substrates.
Since the 2010s, several companies (particularly in Europe and the USA) have tried to bring these technologies to commercial fruition, but many were unable to survive financially. Although biomass waste is relatively abundant and cheap, the high capital and operational costs of transport, facility/equipment, pretreatment and exogenous enzyme requirements present major challenges for these advanced technologies to compete with relatively cheap fossil fuels [21,22]. Researchers thus started to consider the microbial production of high-value carboxylic acids (specifically volatile fatty acids) from the carbohydrate polymers in lignocellulosics (coined the carboxylate platform) as an alternative to cellulosic ethanol. Volatile fatty acids (VFAs) are short-chain aliphatic mono-carboxylate compounds with two to six carbon atoms, which include both linear acids such as acetic, propionic, butyric, valeric and hexanoic/caproic acid, and branched-chain acids such as isobutyric and isovaleric acids. This alternative technology could be of particular importance in developing countries in Africa (including South Africa) where the huge capital costs of large-scale cellulosic ethanol plants present financial constraints in addition to the technological challenges. The main advantage of a carboxylate platform that capitalizes on the highly efficient cellulose-digesting systems of mixed anaerobic cultures, is its robustness and minimum input costs for both equipment and running costs [23,24].

According to the International Energy Agency (IEA, Paris, France), sustainable biomass does not compromise food security, has reduced carbon emissions, low water requirements and does not lower the national biodiversity by taking up too much land [25]. These conditions render solid wastes as the clear option for VFA production, but liquid wastes have also been used extensively, especially in reactors with acclimated microbiomes [26]. Among the solid and liquid wastes, agricultural, municipal and industrial wastes have been the most studied and commonly used for VFA production [27,28].

Biorefineries are considered the best approach to utilize and valorize biomass to its maximum extent [2]. The concept of biorefining has been explained as “the sustainable processing of biomass into a spectrum of marketable products and energy” [29]. A biorefinery is thus a facility or group of facilities capable of integrating a variety of technologies to separate biomass resources into their building blocks, which can subsequently be converted into several value-added products [30,31]. In 2004, the U.S. Department of Energy (DOE, Washington, DC, USA) released a list of “Top Value-Added Chemicals from Biomass” that includes carboxylic acids, such as volatile fatty acids, lactic acid and different 4–6 carbon dicarboxylic acids [32]. The importance of organic acids is supported by their respective global market values (Table 2) and numerous applications in various important industries. Compared to a value of about USD 900/MT for bioethanol, the current value of organic acids can vary from about 600 USD/MT for acetic acid to more than USD 2000/MT for C4–C6 carboxylic acids. Organic polymers (such as polylactate) used in plastics reach values of more than USD 3500/MT and the lactic acid derivative, ethyl lactate as high as USD 4400/MT [33,34].

Table 2. Global market sizes of important organic acids.

| Organic Acid | Global Market Size and CAGR * | Reference |
|--------------|------------------------------|-----------|
| Citric acid  | USD 3.6 billion by 2020; CAGR of 5.5% | [35] |
| Fumaric acid | USD 660.9 million by 2020; CAGR of 6.1% | [36] |
| Succinic acid| USD 198.5 million in 2020; CAGR of 9.2% | [37] |
| Lactic acid  | USD 2.7 billion in 2020, CAGR of 8.0% | [37] |
| Butyric acid | USD 175 million in 2020; CAGR of 13.2% | [38] |
| Propionic acid| USD 1.53 billion in 2020; CAGR of 2.7% | [37] |
| Acetic acid  | USD 9.3 billion in 2020; CAGR of 5.2% | [37] |
| Valeric acid | USD 15.06 billion in 2020; CAGR of 5.3% | [39] |
| Caproic acid | USD 38 million in 2020; CAGR of 2.9% | [40] |

* CAGR = compound annual growth rate.
Whereas the fiber content in fruit waste could be readily converted to VFAs in the carboxylate platform, the production of high-value lactic or dicarboxylic acids (organic compounds containing two carboxylic acid (\(-\text{COOH}\)) functional groups) from the remaining sugars and organic acids in fruit wastes could potentially increase the economic viability of biorefineries aimed at providing bioethanol as an alternative to current petroleum-based fuels. For example, malic acid is abundant in both grape and apple pomace, which are produced in significant quantities in the South African agricultural industry.

This review will highlight the potential value-addition to different feedstocks (focusing on organic waste streams) readily available in South Africa using alternative technologies. The carboxylate platform is proposed for the conversion of the cellulosics in agricultural wastes, alien species and fruit waste fiber to VFAs. This platform can be complemented by yeast biotechnology for the production of high-value organic acids from the sugars and organic acids in fruit waste. Given the pivotal metabolic role and natural abundance of malic acid, our discussion on the latter will focus on the production of high-value organic acids that can be derived from L-malic acid in fruit pomace, with special emphasis on yeast strains as potential cell factories.

2. The Carboxylate Platform

The carboxylate platform can be characterized as a derivative of anaerobic digestion (AD), which uses mixed anaerobic microbial cultures that originate from various sources to break down organic matter to generate biogas \[41\]. The biological process (Figure 1) involves the bacterial hydrolysis of plant biopolymers into soluble monomers and oligomers such as sugars \[42\]. These soluble intermediates then undergo primary fermentation or acidogenic conversion into carboxylic acids, hydrogen, ethanol, ammonia and \(\text{CO}_2\) \[43\]. These intermediates react further with mixed culture microorganisms in various secondary fermentation reactions to produce a variety of end products. They can undergo an autotrophic homoacetogenic conversion to acetic acid with the generation of additional hydrogen and carbon dioxide \[44\]. Another favored route is the reduction of the carboxylates to produce primary and secondary carboxylates (MixAlco process) \[45\]. The intermediates can also be elongated using reverse-\(\beta\)-oxidation with lactic acid or ethanol as electron donor to produce medium-chain fatty acids such as valerate and caproate respectively \[46\]. Lastly, methanogenic microbes (acetoclastic and hydrotherpic methanogens) convert intermediates into methane and \(\text{H}_2\text{O}\) or \(\text{CO}_2\) \[47\]. The gasses that are produced include 50–70% methane, 25–50% \(\text{CO}_2\) and trace levels of nitrogen, hydrogen and hydrogen sulfide \[48\]. This gas mixture is collectively termed ‘biogas’ and can be burned to produce electricity or compressed for use in motor vehicles. The hydrogen can also be used to produce ‘clean fuel’ or non-\(\text{CO}_2\) fuel \[43\].

Most of the research on the carboxylate platform focuses on biogas, the final product of the secondary fermentation that is mainly composed of methane \[44\]. However, methane has a low energy density and commercial value compared to most intermediates produced within the primary fermentation \[24,43\]. This has motivated the notion of VFA production by methanogenesis inhibition or promoting primary fermentation by operating digesters as a ‘stuck fermenter’ that leads to the accumulation of the intermediates in the primary fermentation, specifically the VFAs \[24\].

2.1. VFA Production

Production of VFAs involves the first three steps of the fermentation summarized above (hydrolysis, acidogenic and acetogenic fermentation), which also produces alcohols and lactic acid \[49\]. Numerous studies have shown that carboxylate platform conversions of glucose to VFAs with mixed microbial cultures predominantly produce acetic, propionic and butyric acid as follows \[49,51–53\]:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2 \tag{1}
\]

\[
1.5\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O} \tag{2}
\]
VFA production and vice versa [54]. The availability of these reducing equivalents for the ensure that NAD is in its oxidized form, i.e., glycolysis will continue efficiently towards NAD is available for mixed culture reactions [55,56]. A low hydrogen partial pressure will hydrogen gas. The hydrogen partial pressure determines whether oxidized or reduced NADH [54]. Hydrogen can be released from NADH by hydrogen dehydrogenase to form converting oxidized nicotinamide adenosine dinucleotide (NAD+) to its reduced form, as they influence the efficiency of VFA production by the mixed culture.

It is therefore essential to investigate both these aspects as they influence the efficiency of VFA production by the mixed culture.

### 2.2. Types of Wastes Suitable for VFA Production

The theoretical estimate of global agricultural waste production is at 4.6 BMT dry matter per annum [57]. These wastes are very complex and differ significantly in composition, with most having a high holocellulose (cellulose and all of the hemicellulosics) content [57]. Most of the holocellulose is covered by lignin, which only leaves 10–25% that could be used for VFA production [58]. These wastes also consist of other non-structural carbohydrates and organic and inorganic polymers, including proteins, lipids and minerals [59]. Protein is easily digestible, but may significantly reduce the pH of the microbiome, while lipids

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \quad (3)
\]

Figure 1. The biological process is initiated by (a) hydrolysis of polymers to monomers and oligomers; (b) these monomers are then converted by mixed cultures via acidogenesis to pyruvate that leads to the production of intermediate carboxylates, ethanol, lactate, hydrogen and carbon dioxide. (c) These products are converted by mixed cultures via various reactions, including autotrophic homoacetogenesis of carbon dioxide and hydrogen to more acetate; (d) reduction of the carboxylates with hydrogen or ethanol to produce various alcohols, e.g., propionate to propanol; (e) chain elongation of the VFA to medium-chain fatty acids by reverse-β-oxidation with ethanol or lactate to produce medium-chain fatty acids; and (f) methanogenesis with either (i) hydrogenotrophic methanogens that produce methane and water from carbon dioxide and hydrogen; or (ii) acetoclastic methanogenesis where acetate is converted to methane and carbon dioxide (adapted from Agler et al. [49] and De Groof et al. [50]).

The process involves glycolysis to convert hexoses and pentoses to pyruvate by converting oxidized nicotinamide adenosine dinucleotide (NAD+) to its reduced form, NADH [54]. Hydrogen can be released from NADH by hydrogen dehydrogenase to form hydrogen gas. The hydrogen partial pressure determines whether oxidized or reduced NAD is available for mixed culture reactions [55,56]. A low hydrogen partial pressure will ensure that NAD is in its oxidized form, i.e., glycolysis will continue efficiently towards VFA production and vice versa [54]. The availability of these reducing equivalents for the mixed culture depends on the composition of the feedstock and the process parameters during the fermentation [24,49]. It is therefore essential to investigate both these aspects as they influence the efficiency of VFA production by the mixed culture.
may increase inhibitory ammonia and hydrogen sulfide levels [60]. In terms of the minerals or ash content of biomass, some endogenous minerals such as calcium are essential for the growth of the microbes, whereas exogenous minerals such as silica may impede enzymatic degradation [61].

Solid organic municipal wastes, brewery spent grains and pulp and paper have been extensively studied for VFA production [27,28]. The liquid forms of waste (slurry suspended in water), include most sludge varieties such as activated sludge and thickened sludge [56]. These wastes vary in composition and normally have a high moisture content (60–90%) and high oxygen-to-carbon ratio [28,43]. This may be undesirable for most acido- genic microbial communities capable of VFA production, as extreme moisture impedes the microbial conversion efficiency [47]. Most of these wastes have a similar range in organic matter content (17.9–20%) but differ in solubility and fermentability [62].

As indicated in Table 3, most of these feedstocks, whether agricultural, municipal or industrial, may require some form of pretreatment to make them less recalcitrant to degradation. This treatment becomes even more necessary for agricultural wastes, which have a higher holocellulose content and cellulose crystallinity that impedes microbial enzymatic degradation [58].

### 2.3. Pretreatment Techniques

There are four main types of feedstock pretreatment techniques: physical, physiochemical, chemical and biological [63]. Physical pretreatment mainly involves mechanical methods, but may also utilize ultrasonic and irradiation strategies to treat wastes for holocellulose accessibility [63]. This method increases the available surface area and porosity of the feedstock that improves microbial enzymatic accessibility [63,64]. The most common form is milling, which breaks down the cellulose and reduces the crystallinity of cellulose [64]. This process produces no chemical inhibitors and non-sophisticated expertise is required. The main issue is the energy requirements of the technique, especially to achieve particle sizes smaller than 6 mm diameter [64]. This is even more prevalent if the moisture level of the feedstock is high as most particles may be stuck on the machine’s rotors and require longer processing [58,63]. Furthermore, the method does not expose a high enough surface area for microbial attack as the lignin-holocellulose complex is not entirely destructed [64]. As a result, physical pretreatment is either used together with some form of chemical (physicochemical pretreatment) or chemicals are used outright (without any physical pretreatment).

Physicochemical pretreatment mainly combines a physical (mechanical) method such as steam explosion at high pressure with the addition of an acid catalyst for softwoods [65]. As an example, Lazuka et al. [66] required just 20 min to achieve the required destruction of the lignin-holocellulose complex (Table 3). This technique has been reported to be more cost-effective than physical pretreatment, which requires longer periods of mechanical milling to reach the desired fiber sizes [63]. However, the incomplete destruction of the lignin-holocellulose matrix results in the build-up of soluble lignin and generates fermentation inhibitors [63,67].

### Table 3. Summary of typical feedstock types, inoculum and operation parameters used for VFA production.

| Feedstock          | Pretreatment                           | Inoculum                  | Temp. (°C) | Peak VFAs (g/L) | Fermentation Period (Days) | Initial pH | Reference |
|--------------------|----------------------------------------|---------------------------|------------|----------------|---------------------------|------------|-----------|
| Bagasse (~19% lignin) | Ca(OH)₂ at 50 °C for 8 weeks           | Adapted marine wastewater | 55         | 5.63           | 40                        | 7          | [68]      |
| Corn Fiber (~13% lignin) | Dilute H₂SO₄ at 160 °C for 20 min       | Reactor Microbes          | 55         | 11.1           | 419                       | 5.5        | [69]      |
| Wheat Straw (~18% lignin) | Autoclaved at 120 °C for 20 min         | Termite gut (N. ephratae) | 35         | 6.54 (190 mCmol) | 11                       | 6.15       | [66]      |
Table 3. Cont.

| Feedstock                              | Pretreatment                      | Inoculum                              | Temp. (°C) | Peak VFAs (g/L) | Fermentation Period (Days) | Initial pH | Reference |
|----------------------------------------|-----------------------------------|---------------------------------------|------------|-----------------|-----------------------------|------------|-----------|
| Sugarcane trash and 20% chicken manure | Air-lime pretreatment at 50 °C for 4–8 weeks | Marine wastewater                      | 55         | 29.9            | 20                          | 7          | [70]      |
| Mixed Sludge                           | -                                 | Municipal and Industrial wastes        |            |                 |                             |            |           |
| Waste activated sludge                 | -                                 | Adapted marine wastewater              | 55         | 10.67           | 36                          | 7          | [68]      |
| Brewery wastes (spent grain) (~16% lignin) | H$_2$SO$_4$ at 121 °C for 20 min | Anaerobic granular sludge              | 37         | 10              | 3                           | 7          | [72]      |
| Kitchen Waste (~14% lignin)            | Liquid stream treatment           | Reactor microbes                       | 35         | 36              | 32                          | 6          | [73]      |
| Microalgae (Brown alginate neutralized with CaCO$_2$, filtered) | 3% H$_2$SO$_4$ at 120 °C for 250 min | Municipal wastewater microbes          | 35         | 9.8             | 15.5                        | 7          | [74]      |

Chemical methods are used more often due to their efficiency and efficacy for lignocellulose breakdown [67]. Either alkali or acidic chemicals may be used depending on the feedstock (Table 3). Alkali chemicals (bases) are mostly used for low-lignin biomass and increase the surface area for microbial enzymatic degradation by swelling [63]. The bases NaOH, KOH and Ca(OH)$_2$ are mostly used to reduce the polymerization degree and crystallinity of the lignocellulose [63,75]. However, this often requires long reaction times, e.g., Rughoonundun et al. [69] needed 8 weeks to treat bagasse with Ca(OH)$_2$ towards the production of only 5.6 g/L of total VFAs from microbial fermentation (Table 3). This VFA yield compared to the energy input is not cost-effective and the high chemical load of these alkaline chemicals often leads to microbial toxicity and environmental pollution [75]. Acid chemicals, in particular HCl and H$_2$SO$_4$, are often used for high-lignin biomass to break the covalent bonds and Van der Waals forces holding the lignocellulose together [75]. Agler et al. [69] used H$_2$SO$_4$ to break down the lignocellulose in wheat straw (Table 3), which enhanced microbial degradation towards significant VFA production at 11.1 g/L. However, this method is also very environmentally unfriendly and costly due to the corrosion of the machinery. Furthermore, inhibitors such as furfural that impede further processes, may be produced [63].

Recently, there has been a growing interest in biological treatment that uses natural microorganisms to produce hydrolytic and ligninolytic enzymes that depolymerize lignin to open up cell walls [65]. This is followed by the degradation of cellulose and hemicellulose by cellulolytic and hemicellulolytic microorganisms, and finally fermentation towards VFAs [76]. This process is environmentally friendly, requires no chemicals and uses little energy [65]. A variety of microbes have been used, including Actinomycetes on grasses and white-rot fungi such as Phanerochaete chrysosporium on plant biomass [63,65,77]. However, none of these has gained industrial attention due to the slow rate of degradation that may take more than 14 days with partial hemicellulose hydrolysis occurring towards undesired end products such as CO$_2$ [63]. However, the process can be improved if pure microbial cultures or microbial communities with higher rates of degradation are utilized that are also able to ferment hemicellulose to reducing equivalents for the desired end products.

Nonetheless, attempts to adjust feedstock composition for microbial accessibility alone are not enough to drive these fermentations towards the desired products. Process parameters also need to be adjusted to ensure the more efficient degradation and fermentation of the available holocellulose after pretreatment. A short discussion of the most important parameters and how they influence fermentation towards VFAs follows.
2.4. Fermentation Parameters Influencing VFA Production

The operational parameters have an impact on the overall yield and composition of the individual VFA during the fermentation. These parameters are shown in Table 4 and mainly include temperature, pH, retention time, organic loading rate (OLR) and inoculum concentration.

Table 4. Optimal operational conditions for VFA production [78–80].

| Parameter                  | Optimal VFA Production Conditions |
|----------------------------|-----------------------------------|
| Temperature                | 20–40 °C                          |
| pH                         | 5–11                              |
| Retention time             | 0–20 days                         |
| Organic loading rate       | 5–11 gTS/L x d                    |
| Inoculum concentration    | 15–25% v/v                        |

2.4.1. Temperature

Temperature differs with each natural AD habitat, but mostly ranges within mesophilic conditions (20–45 °C) [81]. Lee et al. [27] and Jiang et al. [78] showed that increasing the temperature within the mesophilic ranges, led to higher VFAs yields. This was also validated by Cope et al. [82] who analyzed VFA production patterns in thermophilic sediments. Moreover, the process of chain elongation, in which VFAs are converted to medium-chain fatty acids, can proceed effectively under mesophilic conditions, but appears to be greatly restricted under thermophilic conditions.

2.4.2. pH

Microbial activity is greatly affected by pH during both hydrolysis and acidogenic fermentation as most communities cannot tolerate pH < 3 and >12 [27]. It has been established that the optimal pH for VFA production ranges from pH 5.5 to 7, depending on the type of waste being hydrolyzed [27,79]. However, this is not always the case: Krause et al. [83] reported that pH 8 to 11 was the optimum for sludge, pH 7 was optimal for kitchen wastes and pH 5.2 was optimal for wastewater utilization. Furthermore, it was shown that VFA composition can also be affected as more propionate is produced at lower pH (<5), while more acetate and butyrate are produced at a neutral pH. A variety of studies [56,78,79,84,85] on the effect of pH in VFA production are in agreement that pH has a limited effect on the total amount of VFAs, but plays a significant role in dictating the molar proportions of the VFAs that are produced. This was attributed to a shift in the dominating microbial populations during fermentation, caused mainly by the type of waste biomass being fermented [27]. It is also worth noting that pH plays a role in microbiome manipulation and inhibiting methanogens as too low or too high pH values slow down the enzymatic activity of methanogens [79]. pH manipulation has thus become an important factor in reactor optimization in VFA production fermentations.

2.4.3. Retention Time

Retention time (RT) is an important design and operational parameter that is significant for the economic feasibility of the fermentation process [79]. This measures the time needed for the substrate to remain in the bioreactor before the desired end products are harvested [71]. This is also one of the most manipulated parameters for VFA production as it can directly influence the accumulation of methanogens, which would take up the produced hydrogen and complex it with CO₂ to produce methane. Shorter RT from 0–20 days leads to hydrogen accumulation being mainly used to produce acetate by acetogenic microorganisms, while longer RT results in the accumulation of methanogens that compete for the hydrogen to produce methane [73,79,86]. Therefore, lower RT is required for optimal results for carboxylate platform fermentations promoting VFA production.
2.4.4. Organic Loading Rate

The organic loading rate is a measure of the amount of organic substrate of a certain volume in a reactor that is being anaerobically degraded for a specific period [78]. This is mainly important for continuous bioreactors and is linked to hydraulic retention time, digester volume, flow rate and volatiles solids of influent entering the digester. Jiang et al. [78] showed that VFAs concentration may increase as the organic loading rate increases, but may not result in increased VFAs yield for loadings higher than 16 g/Total Solids (TS)/L × d, with higher VFA yields observed for organic loading rates of 5 to 11 g.TS/L × d. This means that substrate loading needs to be optimized depending on the nature of the substrate used for VFA production as too high loads may lead to reactor failure, while lower loads may lead to less available organic matter for microbial conversion to VFAs.

2.4.5. Inoculum Concentration

The carboxylate platform requires well-adapted microbial consortia to ensure substantial VFA yields. Anaerobic-mixed microbial cultures are more suitable to produce VFAs than pure cultures as their operation is robust, stable, inexpensive and requires no additional energy input for sterilization [24,43]. The VFA production steps detailed earlier are carried out in symbiosis, but are limited by methanogenesis that utilizes accumulated acetate to produce biogas [43]. To prevent the establishment of methanogens, methanogens should be inhibited or an inoculum that produces VFAs within a short retention time should be used [23]. Methanogens can be inhibited by using extreme temperatures (heat shock at >60 °C) or a low operating pH as detailed earlier [23]. However, both these methods are not sustainable as they may be detrimental to the operating microbes [87]. Commercial methanogen inhibitors such as Iodoform, 2-bromoethanesulphonate and Neutral Red have been used, but increase the operational costs [88]. Among the variety of microbial community sources, only a few habitats (i.e., termite hindguts and the rumen), require a short RT to produce sufficient VFAs before methanogens are established [24]. The concentration of the inoculum may affect the extent and rate of VFA production. Mouriño et al. [84] found that at ruminal fluid concentrations lower than 15% or higher than 25%, cellulose digestion was less effective.

The process conditions described above along with feedstock composition and pretreatment all play an important role in VFA production. To improve the efficiency of the process, they need to be optimized and manipulated in unison. Additionally, the mixed culture should be acclimated to produce VFAs either naturally, by manipulation of parameters or through long-term enrichment. Examples of such communities are shown in Table 3 where most are enriched and adapted with the feedstock that will be used for the main fermentation.

2.5. Microbial Communities Adapted for VFA Production

Anaerobic digestion towards VFAs is driven by a complex microbiome that under-goes interspecies hydrogen transfer and shares reducing power in a form of syntropy that allows them to break down a variety of biomass [89]. In most fermentations for VFA production, this microbiome is more than 99% dominated by bacterial species. Furthermore, most of the process steps from hydrolysis to fermentation are dominated by the phylum Firmicutes and species from the Clostridia and Bacilli classes [90–92]. The hydrolysis of feedstock has been found to involve species from families such as Clostridiaceae, Streptococcaceae, whereas acidogenesis that produce VFAs, alcohols and hydrogen is dominated by genera such as Clostridium, Bacteroides, Butyricibrio, Eubacterium, Lactobacillus and Bifidobacterium [81,89,93]. Among these genera, clostridia have the most dominant role in both VFAs and ethanol production regardless of their habitat [93,94]. These microbial communities utilize all types of biomass due to their syntropic association and efficient three-step biological pathway, as described earlier.
Most of the carboxylate platform systems based on adapted communities from habitats such as sewage digesters [95], bioreactors [96] wastewaters [97], termites [66] and extreme sediments [82], have been extensively reviewed. The main characteristics in each of these communities include (i) a naturally acclimated ‘core microbiome’ similar to the one described above dominated by Firmicutes, Bacteroidetes and Proteobacteria that allows feedstock flexibility for VFA production; (ii) a short RT; and (iii) operational characteristics such a slightly acidic pH, a temperature that ranges from either mesophilic to thermophilic, and a high salt tolerance [82]. The latter was found to be especially important when VFAs are produced as they are buffered and present in their salt form. To avoid product toxicity, each community requires a decent tolerance, especially under batch conditions. Most of these communities still had to undergo further acclimation to meet all these requirements, especially lowering the RT. The only community that was naturally capable of short RT, was termite hindguts (Table 3) that produced 6.54 g/L in 11 days, while communities that seem to be capable of high salinity tolerance came from the extreme sediments [82].

However, another community that is naturally capable of all these requirements with minimal manipulation is that of the mammalian rumen. Its utilization has been underappreciated compared to the other carboxylate platform systems, despite having shorter RT, feedstock flexibility and significant salt tolerance [49]. The main reason for its underutilization has been the incomplete conversion of the plant biomass [24]. However, this does not outweigh the potential economic benefits of using such a cost-effective and naturally evolved system to produce VFAs. The remainder of this review will briefly focus on the (cow) rumen community and its potential role in driving the carboxylate platform towards hydrocarbon biofuels.

2.6. The Rumen-Modeled Carboxylate Platform

Ruminants have evolved to be established cellulosic biomass converters to produce milk, meat and wool [98,99], using VFAs as their main source of energy and building blocks [24]. VFAs are the major products of ruminal fermentation at rates that can be inhibitory to the microflora due to a reduced pH, but the host animal regulates the pH in a range of pH 5.5 to 7 that prevents immediate inhibition [24]. The fermentations are thermodynamically driven, favoring the production of acetic, propionic and butyric acid with less energy required than longer chain acids, producing sufficient excess energy for the microbial cell to produce ATP [100]. Numerous studies [4,23,49,101] have found that the three dominant VFAs, namely acetic acid, propionic acid and butyric acid, are produced at a ratio of 6:2:1, although the ratios vary with the feedstock type.

Rumen microbial communities carry out all three biological pathways of AD within the same bioreactor (rumen), making them a natural example of consolidated bioprocessing [23]. Therefore, modeling or mimicking the rumen in vitro to produce VFAs presents a potentially cost-effective and sustainable platform. Weimer et al. [23,24] detailed some aspects of ruminants that have made them naturally adapted to carry out efficient fermentations, including the core microbiome, fermentation conditions and feedstock flexibility.

2.7. Characteristics of Rumen That Favor VFA Production

The microbiome of the rumen consists of a variety of bacteria, methanogenic archaea, protozoa and chytrid fungi, which are almost all strict anaerobes [102,103]. This microbiome is present in all ruminants, differing only in the role and dominance of different species [18]. Numerous phylogenetic studies utilizing rumen on a variety of feedstock have revealed that >80% of the process is dominated by a ‘core microbiome’ consisting of mainly the phyla Firmicutes and Bacteroidetes, with Proteobacteria being the third most abundant [85,93,104,105]. Molecular studies have also shown that this core microbiome displays resilience and overlap of function among multiple species that enhance the stability and digestive function across a wide range of cellulosic biomass [103]. The core microbiome is stable and maintains dominance regardless of source, location or feedstock type being fermented [103].
The composition of the microbiome is stable and may shift based on the specific feedstock and the fermentation conditions [106]. This is due mainly to functional redundancy or overlap of microbial species within the consortium that has a larger effect on the proportions and type of VFAs produced during the fermentations [103]. This overlap results from rumen microbes that have limited access points within feedstock despite being able to degrade a variety of complex polymers and thus resulting in incomplete degradation [107]. Considering that 99% of species coverage in the rumen is made up of more than 72,218 bacterial sequences, it is clear that many species will degrade similar sites, leading to competition and overlap [108]. However, this overlap mainly occurs after long periods or RT as the initial microbes that degrade the feedstock first belong to the core microbiome [109]. These sentiments have been further highlighted in recent studies on long-term (2.5 years) successive transfers of rumen inocula [106] and storage (3 months) [110] that resulted in shifts of the microbial communities.

The initial stability of the core microbiome is enhanced by a stable environment in ruminants that consists of a constant temperature at 39 °C, oxidation-reduction potential at about −140 mV and a slightly acidic to nearly neutral pH [111]. Maintaining these conditions in vitro enables the ‘core microbiome’ to be retained after transfer to reactors [112]. These conditions allow the rumen to degrade various cellulose biomass with complex composition rapidly and efficiently in short RT both in vivo and in vitro.

The degradability of waste biomass by ruminal microbes is measured using the disappearance of Neutral Detergent Fiber (NDF) [113]. The insoluble fiber in substrates that is not readily degradable, comprises cellulose, hemicellulose, lignin and some protein fractions [113]. This method has been used extensively for ruminal degradability analysis due to its strong correlation with total organic matter degradability [114]. The disappearance (Neutral Detergent Fiber disappearance, NDFd) is expressed in percentage and differs for each substrate. A considerable amount of digestibility is achieved in RT less than 96 h (Table 5) with only mechanical treatment in each of the in vitro experiments. The improved accessibility to the holocellulose results in more rapid fermentation of cellulose for VFA production.

**Table 5.** In vitro digestibility of various wastes by the rumen microbes. Wastes detailed have only undergone mechanical pretreatment by milling and without additional chemicals.

| Feedstock                  | Period (h) | NDFd % | Reference     |
|----------------------------|------------|--------|---------------|
| Brewers’ grains            | -          | 96     | 37            | [114]         |
| Alfalfa hay                | -          | 96     | 45            | [114]         |
| Citrus Pulp                | -          | 48     | 76            | [115,116]     |
| Apple pomace               | -          | 96     | 75            | [115]         |
| Grape pomace               | -          | 96     | 55            | [115,117]     |
| Ryegrass                   | -          | 96     | 79            | [114]         |
| Oat                        | -          | 96     | 80            | [114]         |
| White clover               | -          | 96     | 77            | [114]         |
| Reed canary grass          | -          | 48     | 58            | [118]         |
|                           | Leaves     | 96     | 36            | [119]         |
| Prosopis juliflora         | Stems      | 96     | 31            | [119]         |
|                           | Branches   | 96     | 20            | [119]         |

The Firmicutes *Ruminococcus albus* and *Ruminococcus flavefaciens* and the Fibro-bacteres *Fibrobacter succinogenes* are the primary NDF degradation microorganisms in the core microbiome [100,120]. *Butyrivibrio fibrisolvens*, *Clostridum longisporum* and *Clostridium locheadii* represent the main secondary NDF-degrading microbes. The deg-radiative activities of both these primary and secondary fermenters are diverse and their coordinated effort results in the production of various hydrolytic products in the form of monosaccharides and disaccharides, which can be used by these species for growth [100,120].

The cellulolytic rate of the primary NDF degradation bacterial species is second only to that of the well-established thermophilic anaerobe *Clostridium thermocellum* (0.16 h⁻¹).
at ranges of 0.05 to 0.08 h\(^{-1}\), with \textit{Ruminococcus flavefaciens} displaying the fastest rate in Sigmacell 20 microcrystalline cellulose studies \cite{86, 120, 121}. Weimer \cite{122} tested the performance of rumen microbial communities on various pure substrates that included cellulose, xylan, hemicellulose, protein sources, starch and DNA in vitro. This experiment verified the flexibility of ruminants as significant yields of VFAs were produced from cellulose and other substrates. However, most of the substrates were in their purified form as opposed to complex biomass. To make substantial conclusions on the flexibility of rumen microbes to degrade abundant biomass, it is also important to investigate the degradation of complex wastes.

2.8. VFAs Conversion Route Suitable for Rumen Fermentation towards Hydrocarbon Fuel

VFAs cannot be directly used as a fuel due to limiting characteristics such as water solubility, a high oxygen:carbon ratio and short carbon chains with low density that decreases the recoverability of these carboxylates \cite{123}. However, because of their high gross energy, they are favorable building blocks for the production of more energy-dense fuel/chemical sources \cite{23}. They can either be upgraded directly from the fermentation broth to hydrocarbons as substrates for electrochemical conversion to hydrocarbon fuel or upgraded into more energy-dense forms for other routes that require extraction and purification (refer to Figure 1). Various conversion strategies have been tested and it is widely agreed that the most efficient is the conversion to hydrocarbon fuels by Kolbe electrolysis or conversion to primary or secondary alcohols \cite{49}. Analysis of these routes by Holtzapple et al. \cite{54} has shown that the production of primary alcohols (MixAlco platform) yields the most kg octane per kg cellulose used. However, this process mainly uses lime for pretreatment (Table 3) and the conversion of VFAs to alcohols and ketones \cite{23, 124} and the cost of production is negatively impacted by the cost of chemical waste removal \cite{124}. However, in the same study, it was shown that the Kolbe electrolysis route towards hydrocarbons is the most efficient with enthalpy efficiency and yields, compared to the production via primary or secondary alcohols, or derived from ethanol or through the thermochemical platforms \cite{54}. Briefly, the process proceeds by electrochemical decarboxylation of two mixtures (RCOO) of VFAs leading to the production of alkane (R-R) and CO\(_2\):

\[
\text{Anodic reaction: } \text{RCOO}^- + \text{R'}\text{COO}^- \rightarrow \text{R-R'} + 2 \text{CO}_2 + 2 \text{e}^- \\
\text{Cathodic reaction: } 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{H}_2
\]

The use of the primary alcohol route requires completely destructed biomass and further chemical conversion of the VFAs to alcohols with lime may be less ideal for the rumen microbiome compared to the Kolbe electrolysis route. This is because the Kolbe route simply requires VFAs either in their present form within broth after fermentation or biologically elongated to the more energy-dense form as MCFA \cite{54, 125}. Elongation of VFAs to MCFA (C\(_6\)-C\(_8\)) is achieved using microbial communities dominated by microbial species capable of reverse-\(\beta\)-oxidation, as prominent in \textit{Clostridium} species \cite{126}. These microbes use reverse-\(\beta\)-oxidation to release reducing equivalents to detoxify their growing environment \cite{50}. This is conducted by converting acetate and ethanol to butyrate and thereafter to caproate and longer MCFA. This natural process can be mimicked in vitro in a consolidated single bioreactor or conducted via a two-stage bioprocess where the ruminal microbiome can be used to produce the acetate and augmentation with the \textit{C. kluyveri} to elongate the resultant acetate to longer MCFA. The electron donor (ethanol) can be added into the fermentation broth or ethanol-producing microorganisms can be used for its production. The latter process presents a more economical process, but there are limited studies to validate this. Prominent ethanol-producing yeasts, such as \textit{Saccharomyces cerevisiae}, and \textit{Clostridium thermocellum} have the potential to improve the process economics of such a platform, but there is currently no published literature on such a process. However, even fermentations that result in short VFA chain lengths with low carbon chains can be
utilized efficiently with the Kolbe electrolysis method, although the preference would be to elongate to the more energy-dense forms which are MCFA [127].

2.9. Main Challenges Associated with a Rumen Carboxylate Platform and Incentives

The ruminal carboxylate platform is compatible with other processes that use AD and has the potential to outweigh their process economics as it has higher loadings of biomass, higher microbial densities, higher biopolymer fermentation rates and higher yields of VFAs with low methane production [24]. Despite these favorable conditions, a few challenges need to be addressed to increase the feasibility of utilizing rumen habitats for hydrocarbon biofuel production. These include valorizing abundant wastes to increase the economic value-addition. Additionally, optimization and manipulation of process parameters to prevent methanogenesis could result in higher yields.

2.9.1. Increasing the Cost Differential between Input Biomass and Product

One of the main drawbacks of biofuel production is the marginal value addition due to feedstock costs and product value [123]. The carboxylate platform also requires additional sources of revenue to render it more economically feasible. Most biofuel production processes have targeted the market of animal feeds, where the digestate or fermentation co-products are sold as a protein source for animals [123]. This can improve or dispel the concerns of incomplete digestion by rumen microbes as the remaining undigested residues can be turned into a source of revenue. Although finding potential ways of increasing the yield should still have higher preference, rumen microbes themselves are a source of protein with a stable and particularly nutritious amino acid balance [128]. Although present at low volumes after fermentation, they can still be purified and added as supplements to cattle feed.

The successful use of feedstock residues as an additional protein source will depend on seasonal availability, nutritional balance and competitive pricing [23]. Seasonal wastes such as fruit wastes, known to have substantial amounts of protein, need to be accessed for biofuel production with their residues used for animal feed. Table 5 showed that citrus pulp can be degraded up to 76% in just 48 h, hence it may be worthy to investigate other fruit wastes as potential sources of energy for VFA production. Recently, Njokweni et al. [118] conducted in vitro rumen fermentations to investigate the degradability and VFA production from citrus, apple and grape pomace wastes. This study showed that both citrus (77% NDF degradability, 136 mM VFA concentration after 96 h fermentations and 12.16 mmol alkyl/g) and apple pomace (75% NDF degradability, 126 mM VFA concentration and 11.31 mmol alkyl/g) can be valorized to produce significant amounts of VFAs that can serve as substrates for hydrocarbon production. When the Kolbe electrolysis process is utilized, the VFAs produced from the fermentation can act as direct determinants of the hydrocarbons that will be produced [127].

A second potential process to add additional revenue is to investigate the hydrolysis and fermentation of invasive alien species, for example P. juliflora, that disrupt local biodiversity in SA. There is a need to find ways to valorize Prosopis, including investigating its potential as a sustainable raw material for ethanol and VFA production. Recently, P. juliflora was investigated for its potential as feedstock for VFA production from in vitro ruminal fermentations without prior pretreatment or pH control [119]. As expected, the digestibility (Table 5) of this recalcitrant shrub without pretreatment was low, but the amounts of VFAs produced were significant, namely 8.07 g/L for its leaves, 6.71 g/L for stems and 6.51 g/L for its branches without pH control. These VFAs concentrations are high enough for potential use as substrates for electrochemical conversion via the Kolbe process as detailed earlier. It is even made more attractive by the fact that no expensive alkali/acidic chemicals were required for pH control and pretreatment, which can reduce the cost of production. Thus, using rumen fluid to break down invasive species and using the digestate as a potential protein source for animal feeds, may provide additional value for the carboxylate platform.
2.9.2. Towards Implementing a Cost-Effective Carboxylate Platform Industry

Incentives towards implementing the carboxylate platform lie in its potential for cost reduction in its bioconversion towards VFAs and hydrocarbons. The potential operational benefits include non-aseptic operation coupled with feedstock flexibility, minimal requirements, physical pretreatment, short fermentation periods while yielding comparable VFAs to other platforms with longer fermentation periods. Furthermore, the primary products of the carboxylate platform can be converted further with various mixed cultures or pure cultures to produce a variety of bioproducts in secondary fermentations, including chain elongation to MCFA, carboxylate reduction to alcohols, acetogenesis to acetate, capturing the hydrogen produced to utilize as clean gas or allowing methanogens to establish themselves and convert the intermediates to biogas (Figure 1). The potential production of multiple products from biomass via the different routes of secondary fermentation is in line with the principle of a biorefinery concept, which renders the platform particularly attractive to researchers. This motivates research on finding alternative strategies for full degradation of feedstock by rumen such as those listed above as the potential towards hydrocarbon fuel generation with this platform is high.

3. Production of Organic Acids

3.1. Use of Microbial Hosts to Produce Industrial Important Organic Acids

As intermediates of the tricarboxylic acid (TCA) cycle, organic acids play an important metabolic role in all living cells to produce reduced NADH and FADH$_2$ as well as other cellular components. These organic acids have functional groups that render them potential building blocks for the commercial production of other high-value chemicals [129], such as acetic, lactic, citric, fumaric, succinic and malic acid. Citric, fumaric, succinic and malic acid are (mitochondrial) TCA cycle intermediates, whereas lactic and acetic acids are usually produced in the cytosol from malic acid and pyruvic acid, respectively. A simplified representation of the TCA cycle (Figure 2) indicates the C6 and C5 inputs via the Embden–Meyerhof–Parnas (EMP) and pentose phosphate pathway (PPP), respectively, as well as other pathways related to organic acid intermediates of interest (highlighted in the text boxes). These pathways include malolactic fermentation, glyoxylic acid cycle (GAC), malate-pyruvate interconversion and oxaloacetate-pyruvate conversion.

Malic acid, an important intermediate of the TCA and the GAC [130,131], could potentially serve as a substrate for bioconversion to other high-value organic acids associated with the TCA cycle. Malic acid (also known as hydroxybutanoic or hydroxysuccinic acid) is present in fruits, plants and animals [132] with the L-stereoisomer representing the biologically active form used in the food and beverage industry. Malic acid is abundant in both grape and apple pomace, which are produced in significant quantities in the South African agricultural industry. Malic acid is also found in vegetables such as Asparagus and Brassica species [133] and other plants such as Cichorium intybus, hops (Humulus lupulus) and Taraxacum obovatum [134]. Given the pivotal metabolic role and natural abundance of malic acid, our discussion will focus on the production of high-value organic acids that can be derived from L-malic acid, with special emphasis on yeast strains as potential cell factories.

Although yeast species are not known as natural producers of organic acids, the TCA cycle is operational in their mitochondrion under aerobic conditions. Yeasts are well-established robust industrial microorganisms with good pH tolerance that enable fermentations at low pH, which reduces the need for neutralization and simplifies separation/purification processes. These characteristics render yeast more attractive than bacteria and filamentous fungi as potential hosts to produce organic acids in a waste-based biorefinery. More detail on the current production processes for several high-value organic acids derived from malic acid and their potential applications will be discussed in the following sections.
More detail on the current production processes for several high-value organic acids derived from malic acid in filamentous fungi as potential hosts to produce organic acids in a waste-based biorefinery. These characteristics render yeast more attractive than bacteria. Isomerization with mineral acids, peroxy compounds or thiourea as catalysts [135]. The high-yielding petrochemical synthesis of fumaric acid remains the most widely used production method, but requires high temperature and results in by-products that reduce the fumaric acid yields [135,137].

There have been promising reports on the production of fumaric acid via microbial fermentation with natural (wild) or mutagenized strains, whereas others turned to metabolically engineered strains. Mucoralean fungi of the genus Rhizopus are considered the main natural producers of fumaric acid and include species such as R. arrhizus, R. formosa, R. nigricans and R. oryzae [138–146]. Whereas R. arrhizus, with a high production value of 121.0 g/L (but a yield of only 0.37 g/g glucose), was once considered the most suitable fungal strain for fumaric acid production, R. oryzae has become the frontrunner due to a higher yield (0.91 g/g), productivity (4.25 g/L/h) and lower nutritional requirements [147–149]. Other Mucoralean fungi (Cirnella...)

**Figure 2:** Metabolic pathways for the conversion of hexoses and pentoses to organic acids in *S. cerevisiae*. A simplified illustration of the TCA cycle showing the interconnectedness of various metabolic pathways in eukaryotic mitochondria. Steps in the Embden–Meyerhof–Parnas (EMP) pathway, pentose phosphate pathway (PPP) and TCA cycle are indicated by solid lines, whereas alternative bioconversions are indicated by dashed lines, i.e., recombinant malolactic fermentation (MLF) pathway and glyoxylic acid cycle (GAC). Grey dashed arrows indicate a pathway often deleted or interrupted (such as PDC and alcohol dehydrogenase to produce lactic acid) or an alternative pathway utilized (such as ME for the conversion of malate to pyruvate). The relevant enzymes are indicated as (CS (citrate synthase), FRD (fumarate reductase), FUM (fumarase), IDH (isocitrate dehydrogenase), LDH (lactate dehydrogenase), MDH (malate dehydrogenase), ME (malic enzyme), MLE (malolactic enzyme), MS (malate synthase), OAD (oxaloacetate decarboxylase), OGDH (α-ketoglutarate dehydrogenase), (KGDH) α-ketoglutarate dehydrogenase, PC (pyruvate carboxylase), PDC (pyruvate decarboxylase) and SDH (succinate dehydrogenase).

### 3.1.1. Fumaric Acid

Fumaric acid (one enzymatic reaction away from malic acid in the TCA cycle) (Figure 2) is used in the agricultural, chemicals, food and beverage as well as pharmaceutical industries [135]. Some of the primary applications include the production of alkyd-, paper- and unsaturated-polyester resins and plasticizers [136], whereas the unique flavor and non-toxicity of fumaric acid allow for its use as an acidulant in food, beverages and animal feed [135]. Commercial fumaric acid is generally synthesized via maleic acid isomerization with mineral acids, peroxy compounds or thiourea as catalysts [135]. The high-yielding petrochemical synthesis of fumaric acid remains the most widely used production method, but requires high temperature and results in by-products that reduce the fumaric acid yields [135,137].

There have been promising reports on the production of fumaric acid via microbial fermentation with natural (wild) or mutagenized strains, whereas others turned to metabolically engineered strains. Mucoralean fungi of the genus *Rhizopus* are considered the main natural producers of fumaric acid and include species such as *R. arrhizus*, *R. formosa*, *R. nigricans* and *R. oryzae* [138–146]. Whereas *R. arrhizus*, with a high production value of 121.0 g/L (but a yield of only 0.37 g/g glucose), was once considered the most suitable fungal strain for fumaric acid production, *R. oryzae* has become the frontrunner due to a higher yield (0.91 g/g), productivity (4.25 g/L/h) and lower nutritional requirements [147–149]. Other Mucoralean fungi (*Cimella*...
and Cunninghamella species), non-Mucoralean fungi (Aspergillus glaucus, Caldariomycels fumago and Penicillium griseofulvum) and bacteria (Bacillus macerans, Erwinia chrysanthemi, Thermoanaerobacter ethanolicus and Zymomonas mobilis) have also been reported as fumaric acid producers [150–152]. For a more detailed discussion on fungal and bacterial candidates and relevant adaptations towards fumaric acid production, please refer to a review by Guo et al. [135].

Although yeasts are not generally known as organic acid producers, strains of Brettanomyces, Candida utilis, Pachysolen tannophilus and Scheffersomyces stipitis produce fumaric acid [153,154]. With their higher resistance to acidic environments and non-hyphal nature, yeast strains could be competitive alternative microbial hosts to produce organic acids such as fumaric acid. Various yeast strains have been adapted for fumaric acid production using different genetic modifications and expression systems, including S. cerevisiae, Candida glabrata (previously known as Torulopsis glabrata) and S. stipitis (Table 6). The expression of hopefully both heterologous enzymes and transporters are often involved in the genetic modifications of the yeast candidates.

To produce fumaric acid from malic acid using microbial bioconversion, the simplest route would be via the reductive TCA pathway with the help of fumarase. As indicated in Table 6, the native fumarase gene of S. cerevisiae is often deleted in recombinant organic acid-producing strains given its irreversible conversion of fumarate to malate [155]. A positive aspect of a reversible fumarase conversion of malate to fumarate is the energy-neutrality of the step. The expression of a Schizosaccharomyces pombe transporter (encoded by mae1) is another popular modification to allow for the export of fumaric acid.

3.1.2. Succinic Acid

Succinic acid (two enzymatic reactions away from malic acid in the TCA cycle, Figure 2) has application in the agricultural, food and pharmaceutical industries in addition to its use as an ion chelator and surfactant [156]. It serves as a precursor for various valuable industrial chemicals and the synthesis of biodegradable and/or bio-based polymers such as polyamides (Nylon x,4 and polybutylene succinate) [157], which have important applications in the plastics industry. Succinic acid is mainly produced via the petrochemical route with catalytic hydrogenation of maleic anhydride or maleic acid [158,159].

The microbial hosts that naturally produce succinic acid are often capnophilic in nature [160] and most of the bacteria that produce significant titers, such as Actinobacillus succinogenes, Anaerobiospirillum succiniciproducens, Bacteroides fragilis and Mannheimia succiniciproducens, originated from the rumen of ruminants (see previous reviews [156,161] for detailed descriptions). Fungal candidates previously assessed for succinic acid production include Pichia kudriavzevii, S. cerevisiae and Yarrowia lipolytica [155,161–167].

There are three biosynthetic pathways for microbial succinic acid production, namely the reductive TCA pathway, the oxidative TCA pathway and the GAC pathway. Based on these options, various metabolic engineering strategies can be explored to produce succinic acid from suitable substrates and various yeast strains have been genetically modified for succinic acid production, with Y. lipolytica strains yielding some of the best results (Table 6).

Under the normal functioning of the aerobic oxidative TCA pathway, succinate cannot accumulate unless the succinate dehydrogenase enzyme is inactivated to inhibit the conversion of succinate to fumarate. Genes involved in the production of ethanol are often also deleted to increase succinate yield. To convert malic acid to succinic acid in yeast such as S. cerevisiae, the simplest method would be via the reductive TCA pathway, aided by a fumarase that allows for the reversible conversion of malate to fumarate. The subsequent conversion of fumarate to succinate involves an NADH-dependent step, which some researchers have tried to balance out by deleting another NADH-dependent step (glycerol-3-phosphate dehydrogenase 1 involved with glycerol production) [155].
### Table 6. Yeast strains modified for organic acid production (adapted from [133,159,162,168].)

| Microbial Strain | Genetic Modifications | Substrate; Culturing Method | Titer (g/L) | Yield (g/g) | Reference |
|------------------|-----------------------|-----------------------------|-------------|-------------|-----------|
| **Fumaric acid** |                       |                             |             |             |           |
| S. cerevisiae    | Expression of RoMDH-SDH1, RoPYC-KGD2-SUCGL2 and SFCL1-SpMAE1; Deletion of thi2, fun1, ura3, leu2, try1 and his3 | Glucose; shake flasks | 33.1 | 0.33 | [169] |
| C. glabrata      | Expression of ADB1-RoPYC-AsPCK-SyMAE1 and ADB2-RoMDH-5x-FDH1-ADB3-RoFUM; Deletion of ura3 and arg8; Scaffold (1:1:2) | Glucose; batch fermentation | 21.6 | 0.22 | [170] |
| T. glabrata       | Expression of kgd2, SUCGL2, sdh1, Smae1, sfc1 and ASL; Deletion of ura3 and arg8 | Glucose; shake flasks | 15.8 | 0.15 | [171] |
| PMCFfg            | Expression of ASL, ADSL and Smae1; Deletion of ura3 and arg8 | Glucose; shake flasks | 8.8 | 0.15 | [172] |
| S. cerevisiae    | Expression of Ymae1; Deletion of ura3, leu2, Psfum1 and Psfum2 | Glucose; shake flasks | 5.6 | 0.11 | [173] |
| **Succinic acid** |                       |                             |             |             |           |
| S. cerevisiae PMCFfg | Expression of pyeC, mdh3, funC, frdS1; deletion of his3, fun1, gpd1, pdc1, pdc5 and pdc6 | Glucose; batch fermentation | 13.0 | 0.13 | [155] |
| S. cerevisiae AH22ura3 | Deletion of sdh1, sdh2, idh1 and idp1 | Glucose; anaerobic batch fermentation | 3.6 | 0.07 | [163] |
| P. kudriavzevii 13 723 | Expression of pyeC, fun1, mae, mdh and frd1; deletion of ura and pdc | Glucose | 48.2 | 0.45 | [164] |
| Y. lipolytica Y-3314 | Expression of sdc1, sdh2 and suc2 | Glycoler; aerobic batch fermentation | 45.4 | 0.36 | [165] |
| P. kudriavzevii 13 171 | Expression of pyeC, fun1, mae, mdh and frd1; deletion of cyb2a | Glucose | 23.0 | n/a | [162] |
| Y. lipolytica Y-3314 | Deletion of sdc1, sdc2 and deletion of ach | Glycoler; fed-batch fermentation | 198.2 | n/a | [166] |
| Y. lipolytica Y-3314 | Expression of pck, ssc2; deletion of ach | Glycoler; fed-batch fermentation | 110.7 | 0.53 | [167] |
| **Citric acid**   |                       |                             |             |             |           |
| Y. lipolytica Wratislavia 1.31 | Acetate-negative mutant was obtained after wild strain Y. lipolytica A-101 was exposed to UV irradiation | Crude glycerol (86% wt/wt); fed-batch fermentation | 155.20 | 0.55 | [176] |
| S. lipolytica NTG9 | A citrate nonutilizing strain (NTG9) was obtained after mutagenesis of S. lipolytica ATCC 20228 with nitrosoguanidine | Canola oil; MultiGen fermentor | 152.30 | 113.4 | [177] |
| Y. lipolytica NG40/UV5 | Mutagenesis with UV irradiation and N-methyl-N-nitro-N-nitrosoguanidine | Rapessed oil; 10-L ANKDM-2M fermentor | 140.0 | 1.50 | [178] |
| Y. lipolytica 1.31 | Acetate-negative mutant was obtained after mutagenesis | Glycoler; stirred tank bioreactor | 124.5 | 0.62 | [179] |
| **Lactic acid**   |                       |                             |             |             |           |
| S. cerevisiae strain | Bos Taurus L-LDH Integrated (6 copies) | Cane juice-based media; microaerobic batch fermentation | 122 | n/a | [180,181] |
| C. utilis Cupd1 | Bos Taurus L-LDH (optimized) Integrated (2 copies) | Glucose; shake flasks | 103.3 | 0.95 | [182] |
| S. pombe VKPM Y-3127 | S. pombe VKPM Y-285 transformed with R. oryzae IdhA gene | Glucose | 80–100 | n/a | [183] |
| K. marxianus YKX071 | YKX056, pKX055, PfLDH, ΔKmDLD1, BmLDH, ScJEN1, KmPFK | Corncob residue; fed batch fermentation | 103 | n/a | [184] |
| K. marxianus CD607 | L. helveticus L-LDH integrated into PDC1 locus | Glucose; shake flasks | 94–99 | 0.9–0.98 | [185] |
| C. boitissi KY2199 | L-lactate dehydrogenase-encoding gene | Glucose; aerobic batch fermentation | 85.9 | 1.01 | [186] |

#### 3.1.3. Citric Acid

Citric acid (2-hydroxy propane 1, 2, 3-tricarboxylic acid) with its non-toxic nature, GRAS status and pleasant sour taste is considered to be one of the most important natural organic acids (Table 2) [187]. Like malic acid, it can be found in a variety of fruits such as berries, grapes, lemons, limes, oranges and tangerines. Its extensive use as an acidulant,
antioxidant, buffer, emulsifier and preservative in food industries as well as its application in chemical, medical and pharmaceutical industries, add to its massive global market value. Production of this organic acid can be done via physical or chemical methods, which are complex, expensive and not environmentally friendly [188–190]. Efficient and continuous production methods are required to meet the high demand for this organic acid that far exceeds its current supply.

Microorganisms with the ability to produce citric acid, some of which are being utilized in industry, include bacteria such as Arthrobacter paraffinens, Bacillus licheniformis, B. subtilis and Corynebacterium species; fungi such as Aspergillus aculeatus, A. awamori, A. carbonarius, A. flavus, A. foetidus, A. fumigatus, A. niger, A. phoenicis, Mucor piriformis, Penicillium janthinellum, P. restrictum, Talaromyces species, Trichoderma viride and Ustulina vulgaris; as well as yeasts such as Candida citroformans, C. guilliermondii, C. intermedia, C. lipolytica, C. oleophila, C. tropicalis, Hansenula anomala, Saccharomyces lipolytica and Y. lipolytica [187,191,192].

As citric acid and malic acid both form part of the TCA cycle, many microbes already have the main metabolic pathway for the conversion of malic acid to citric acid—although optimization may be required. Sawant et al. [187] reviewed the bacterial and fungal candidates and their relevant adaptations towards citric acid production. A summary of noteworthy yeast strains developed for citric acid production is provided in Table 6. In particular, acetate-negative mutant Yarrowia strains have been shown to have lower selectivity towards undesirable isocitric acid as a by-product [179]. The main microbial candidate, A. niger, for which several commercial strains have been developed [193–197], is commonly used for commercial citric acid production via submerged fermentation on carbohydrate substrates such as molasses. However, the high probability of cation contamination requires tight control of process parameters [178,187,198]; the sensitivity to trace metals and other impurities have thus limited the utilization of inexpensive carbon and nitrogen sources [129].

3.1.4. Lactic Acid

Lactic acid (2-hydroxypropanoic acid) is an organic acid widely distributed in nature and has been used by mankind for decades [168]. It enjoys GRAS status and has been applied in the chemical, cosmetic, food and pharmaceutical industries [199]. It is also an important role player in the production of bioplastics such as polylactide polymers (polylactic acid, PLA) [200], which are more easily degradable than conventional plastics.

Lactic acid has a chiral carbon atom with two enantiomeric forms and can be produced either via chemical synthesis (generally producing a racemic mixture of DL-lactic acid) or fermentative routes [199]. Although metabolic engineering of yeasts for the production of lactic acid has been employed since the 1990s [201], nearly all industrially produced lactic acid is obtained via glucose fermentation by lactic acid bacteria (LAB) [202–204]. The latter requires the addition of complex nutrients to the fermentation media, such as corn steep liquor and yeast extract. These supplements are expensive and cause problems with the downstream purification of lactic acid [205]. In addition, LAB has a limited pH tolerance that results in high amounts of salts or gypsum being produced during the recovery of lactic acid.

The yeast S. cerevisiae is considered an attractive alternative to LAB due to its GRAS status, pH tolerance, robustness, simple nutrient requirements and long history as an industrial workhorse. However, as previously mentioned, it does not produce organic acids in large quantities [206]. Researchers have therefore also investigated non-conventional yeasts as hosts to produce lactic acid from various substrates with different levels of success (reviewed by Sauer et al. [200]). A summary of yeast strains that have been modified and tested for lactic acid production is provided in Table 6. In-depth reviews of non-yeast strains and the use of low-cost renewable materials for lactic acid fermentations have previously been published [200,207].

Microbial L-lactic acid can be produced from L-malic acid via different enzymatic pathways [208]. The malolactic fermentation (MLF, Figure 2) pathway is the most direct
method and involves the conversion of L-malate (dicarboxylic acid) to L-lactate (monocarboxylic acid) and carbon dioxide (CO$_2$). This bioconversion utilized by LAB is catalyzed by the malolactic enzyme (MLE), which is made up of two identical subunits and requires the presence of Mn$^{2+}$ and nicotinamide adenine dinucleotide (NAD$^+$) [209].

In 2012, Schümann and co-workers [210] suggested two approaches to acquire microbial strains capable of degrading malic acid for application in the wine industry. The first is based on wild malic acid-degrading yeast strains that can simultaneously perform alcoholic and malolactic fermentations, i.e., malo-alcoholic fermentations (MAF), whereas the other approach involves the utilization of LAB or genetically modified yeast strains. These approaches could be further investigated for malic acid conversion as part of a fruit waste biorefinery, in particular for grape and apple waste that is rich in malic acid.

As seen in Table 6, most studies on the microbial production of lactic acid in yeast involved a modification in the lactate dehydrogenase (LDH) genes with glucose as substrate. However, Kong et al. [183] recently developed an effective platform for the production of L-lactic acid (of high optical purity) from the carbohydrate polymers of lignocellulosics and inexpensive nitrogen sources using a yeast host. Taking into account the most promising genetic modifications to date, the conversion to pyruvate and subsequent conversion to lactic acid via genetically modified LDHs could be considered instead of using the most direct (malolactic) pathway to produce lactic acid from malic acid.

3.1.5. Acetic Acid

Acetic acid, a two-carbon volatile organic acid and traditional food preservative, is regarded as an important platform chemical that is mainly produced via synthetic production methods relying on petroleum-derived acetaldehyde, butane, ethylene or methanol [193]. Although not included on the DOE list, the production of vinyl acetate monomers (VAM) from acetic acid serves as a driving force in the global demand for this organic acid [35]. Acetic acid is also used in the food industry as an acidity regulator and condiment (vinegar), the production of industrial cellulose acetate and as a descaling agent in households. It is estimated that only 10% of the global acetic acid production is achieved via biological routes [211,212] with prokaryotes as the main microbial players. Acetic acid bacteria can oxidize ethanol under aerobic conditions to produce acetic acid, whilst acetogens can convert hexoses into three molecules of acetic acid under anaerobic conditions [213]. Previous studies especially focused on Acetobacter species and a recent review [212] on biotechnical acetic acid production provides more detail on the relevant pathways.

Yeasts as acetic acid production hosts have not gained much attention and the presence of acetic acid is often unwanted in yeast-based industries (such as winemaking). However, as part of a biorefinery concept, the production of this organic acid could be attractive due to its growing market [214]. A recent study using malic acid-degrading yeasts indicated that acetic acid could be acquired as a by-product when cultivated in media containing malic acid and glucose/xylose [215] or fruit wastes such as apple and grape pomace. Other studies have suggested the use of renewable substrates such as apple pomace to produce bioethanol that could then be converted to higher levels of acetic acid [216,217]. To produce acetic acid from malic acid, the conversion of the latter to pyruvate (via the malic enzyme) would potentially allow for acetic acid formation (Figure 2).

3.2. Natural versus Genetically Modified Yeasts

From the discussion above, it is clear that yeast strains currently used for the production of organic acids for industrial applications represent only a small fraction of the biodiversity found in nature [218,219]. It is known that the natural fungal diversity is vast and mostly unexplored, with unknown fungal species and strains that may have important industrial-related characteristics that could be utilized and/or transferred to industrial strains to create novel yeasts with beneficial characteristics [220]. Indigenous yeast species/stains underwent continuous adaptation and evolution in their specific environments and have valuable phenotypes for specific industries. A recent study by
Steyn et al. [213] included the isolation of native yeasts from South African fruit wastes rich in malic acid and subsequent screenings indicated that these native yeast strains were innately better adapted for extracellular malic acid degradation. Niches identical or similar to a fermentation environment should thus be explored for the isolation of “wild” yeasts with potential commercial applications.

The production of specific chemical compounds via the biotransformation of selected substrates (such as fruit waste) requires the optimization of strains and their enzymatic abilities to increase production levels, whether that be through random mutagenesis, directed evolution or specific genetic modifications. The modification of a biochemical pathway, metabolic engineering and protoplast fusion have successfully been employed for the overproduction of enzymes, biofuels and organic acids [221]. Since most of the enzymes involved in these biotransformations are located inside the cell, new or better organic acid transporters could aid the import and export of relevant compounds. For example, the expression of the C4-dicarboxylic acid transporter from Schizosaccharomyces pombe (encoded by mae1) effectively exported L-malic acid, fumaric acid and succinic acid from recombinant S. cerevisiae cells [222].

Known yeast strains that could be involved in the commercial production of citric, fumaric, lactic and succinic acid are summarized in Table 6. When considering the metabolic routes linked to malic acid, we can highlight potential strains and genetic modifications that could be useful in the conversion of malic acid in fruit wastes to these high-value organic acids. It is, however, important to remember that metabolic changes might have implications that have to be carefully considered (such as redox imbalances and impacts on yeast growth). Most of the strains highlighted in Table 6 were evaluated on simple substrates like glucose and glycerol, but it would be interesting to generate data for more complex substrates as part of a larger biorefinery concept. A recent study considering fruit waste streams of South Africa indicated that two natural yeast strains of Pichia kudriavzevii and S. cerevisiae could produce acetic acid and ethanol when fermented in apple or grape pomace [215]. In the debate of natural vs. genetically modified yeast strains, the ideal (but not so simple) answer would be to exploit the natural abilities of wild yeasts and to “perfect” those strains with the relevant genetic modifications. However, as the latter becomes more complicated when the unknown genetic background of native strains come into play, this debate will probably continue for the foreseeable future.

3.3. Challenges and Incentives

A common challenge in the production of organic acids via microbial fermentation is the economic competitiveness of the established traditional petrochemical process, despite their negative environmental impacts. The immediate environmental advantage of such alternative bio-based compounds is the reduced utilization of fossil fuels and thereby a reduction in greenhouse gas emissions [223]. However, as pointed out in a review by Fiorentino et al. [2], thorough assessments of economic, energetic and environmental feasibilities of biomass value chains are still lacking whilst the technical aspects of biobased chemistry have received much attention. In order to measure the sustainability of a bio-based chemical vs. its fossil-based equivalent, a holistic approach is necessary in which green chemistry metrics and life cycle analyses (LCAs) should be considered. Additionally, analysis of the four sustainability assessment metrics proposed by Sheldon and Sanders [224], i.e., the material and overall energy efficiencies, capital and variable costs as well as land use criteria, could help highlight potential concerns.

The production of specific organic acids via yeast strains may also offer yet unknown challenges. In the case of fumaric acid, a hurdle to overcome is the stronger and main catalysis of fumaric acid conversion to L-malic acid (as opposed to the reverse reaction) that also hinders the cytosolic accumulation of fumaric acid in larger quantities [225]. This is due to the natural high affinity of yeasts fumarase, with the cytosolic enzyme in S. cerevisiae exhibiting a 17-fold higher affinity towards fumaric acid than L-malic acid [226]. Whilst succinic acid can be produced at high yields by bacteria utilizing glucose, yeasts
such as S. cerevisiae offer other attractive opportunities such as low pH robustness and researchers have shifted attention to these eukaryotes with their clear genetic backgrounds. Unfortunately, the productivity, titer and yield of metabolically engineered S. cerevisiae strains have proven to be lower than for other succinic acid-producing microbes [161] and often require multiple deletions. The production of lactic acid from L-malic acid via the MLF pathway results in the production of CO$_2$ (and thus carbon loss), whereas yeast production of citric acid may result in the unwanted by-product isocitric acid [177].

The utilization of agro-industrial wastes (such as malic acid-containing fruit wastes) for microbial fermentation and thereby the bioproduction of high-value bioproducts, is an economically important solution to minimize various environmental problems [227]. The management of fruit wastes poses a severe problem globally and is mainly treated via the traditional methods of composting, incineration, landfilling and land spreading, as well as being incorporated into low-quality animal feed [227–229]. These methods can have a variety of negative effects, such as the high cost to treat and transport wastes to landfills, the production of greenhouse gases, emitting foul smells due to microbial activity, contaminating the underground water table and creating breeding opportunities for human disease vectors [227]. For a more detailed discussion on the potential benefits of enabling circular economies based on waste biorefineries (especially in developing countries), refer to the review by Nizami et al. [230].

Aside from economic incentives given the large global market for organic acids and the environmental benefits of valorizing organic wastes, bio-based organic acids discussed in this review, e.g., fumaric acid, could have additional environmental applications such as the reduction of greenhouse gas emissions from livestock by limiting methane production when incorporated into their feed [231]. In comparison to the other C$_4$ dicarboxylic acids, the extremely low water-solubility of fumaric acid may simplify downstream separation [232]. A primary challenge of biologically synthesized organic acids has often been the downstream recovery of these products from aqueous streams or fermentation broths. Studies throughout the years have considered various electrodialysis methods to recover organic acids from aqueous solutions as well as methodology involving adsorption, chromatography, crystallization, distillation, ion-exchange, liquid extraction, membrane separation and precipitation. In their recent comprehensive review on the recovery of organic acids from aqueous solution, Kumar et al. [233] deemed reactive extraction as a promising downstream processing technique to intensify organic acid recovery from aqueous streams or fermentation broths and extensively discuss this energy-saving process that allows for production scale flexibility as well as a high degree of selectivity and separation.

4. Conclusions

As a developing country, South Africa needs to explore biobased technologies that allow the utilization of biomass and waste streams through simple and robust processes that are not too capital-intensive and that could work at a small scale to produce a variety of valuable bioproducts. The in vitro utilization of the rumen microbiome in the carboxylate platform could be a valuable alternative small-scale platform to supplement bioethanol production in SA. The stability, resilience, overlap of function, robustness and flexibility of the microbiome makes it an attractive community for AD bioprocesses. Furthermore, this community produces all the enzymes required for hydrolysis and fermentation, making it a natural form of consolidated bioprocessing. Though no techno-economic analysis has yet been conducted for a rumen-based carboxylate platform, it can be estimated by comparing the production costs with other microbial community profiles, such as sewage microbial communities that have been used extensively for biogas production in SA. It is predicted that the use of physical instead of chemical pretreatment, natural acclimation to short RT, mesophilic temperature utilization, initial pH adjustment with buffer only and overall diverse end-product yields would reduce costs and improve the overall economic feasibility of the platform. Several yeast microbial hosts have shown promise for the conversion of sugars and malic acid present in fruit wastes to produce valuable dicarboxylate acids, thus
advancing the biorefinery concept. However, for such biorefineries to become economically feasible, industrial-scale fermentation processes with sufficient yields in organic acids will have to be developed and integrated with existing biobased processes. Combining the carboxylate platform and organic acid production could offer a unique opportunity for the conversion of fruit and mixed biomass wastes to higher-value organic acids.

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