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Solid-state fermentation as a process for biofuel production: a focus on ethanol and beyond

Fermentación en estado sólido como proceso para la producción de biocombustibles: un enfoque en el etanol y más allá

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Abstract

Solid-state fermentation (SSF) is an emerging bioprocess in which microorganisms grow on solid substrates in the near-absence of free water, closely mimicking their natural ecological habitats. In the context of growing global energy demand and the urgency to reduce dependence on fossil fuels, SSF has gained considerable attention as a cost-effective and environmentally friendly technology for biofuel production. This review addresses the use of SSF for the production of second-generation biofuels, with a primary focus on bioethanol, and explores its potential for the generation of other liquid and gaseous biofuels such as biobutanol, biogas, and biohydrogen. Special attention is given to the use of the organic fraction of municipal solid waste (OFMSW) as a feedstock, given its worldwide abundance, low cost, and high carbohydrate content. The review covers the fundamentals of SSF, relevant substrates and pretreatment strategies, fermentative microorganisms, bioreactor configurations, process kinetics, and scale-up considerations.

Keywords: Solid-state fermentation; Bioethanol; Biofuels; Organic fraction of municipal solid waste; Lignocellulosic biomass; Bioreactor.

Resumen

La fermentación en estado sólido (FES) es un bioproceso emergente en el cual los microorganismos crecen sobre sustratos sólidos en ausencia casi total de agua libre, imitando de manera cercana sus hábitats ecológicos naturales. En el contexto de una creciente demanda energética global y la urgencia de reducir la dependencia de los combustibles fósiles, la FES ha ganado considerable atención como una tecnología rentable y ambientalmente amigable para la



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producción de biocombustibles. Esta revisión aborda el uso de la FES para la producción de biocombustibles de segunda generación, con un enfoque principal en el bioetanol, y explora su potencial para la generación de otros biocombustibles líquidos y gaseosos, como el biobutanol, el biogás y el biohidrógeno. Se presta especial atención al uso de la fracción orgánica de los residuos sólidos urbanos (FORSU) como materia prima, dada su abundancia a nivel mundial, su bajo costo y su alto contenido de carbohidratos. La revisión abarca los fundamentos de la FES, los sustratos relevantes y las estrategias de pretratamiento, los microorganismos fermentativos, las configuraciones de biorreactores, la cinética del proceso y las consideraciones para su escalamiento.

Palabras clave: Fermentación en estado sólido; Bioetanol; Biocombustibles; Fracción orgánica de residuos sólidos urbanos; Biomasa lignocelulósica; Biorreactor.

1. Introduction

The growing global population, rapid industrialization, and the progressive depletion of fossil fuel reserves have intensified the search for sustainable and renewable energy alternatives. Biofuels, particularly liquid biofuels such as bioethanol and biobutanol, have emerged as viable options that can partially replace petroleum-derived fuels while simultaneously reducing greenhouse gas (GHG) emissions (Saini et al., 2015). It has been estimated that the use of bioethanol from lignocellulosic or organic waste substrates can reduce GHG emissions by 30 to 85% compared to conventional gasoline (IEA, 2004).

Traditionally, bioethanol production has relied on first-generation feedstocks such as corn and sugarcane, which compete directly with food supplies and agricultural land. This has fueled the development of second-generation processes based on non-food biomass: agricultural residues, agro-industrial by-products, and the organic fraction of municipal solid waste (OFMSW). The OFMSW is an especially attractive substrate due to its abundance, low acquisition cost, and high content of fermentable carbohydrates including glucose, fructose, sucrose, and xylose (Ballesteros et al., 2006; Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023).

In Mexico alone, more than 53 million tons of municipal solid waste (MSW) are generated each

year, of which approximately 52% corresponds to organic matter. The Mexico City supply center (Central de Abastos) alone generates over 1,291 tons of solid waste per day, of which 70% consists of fresh fruit and vegetable residues. The potential of this material for bioethanol production is significant, with estimates suggesting yields of up to 281 m³ of ethanol per day if valorized adequately (Estrada-Martínez et al., 2019).

Solid-state fermentation (SSF) —also referred to in the literature as solid-state culture (SSC)— is a bioprocess in which microorganisms grow on moist solid substrates without free-flowing water in the interstitial space. It offers several inherent advantages over conventional submerged liquid fermentation (SmF), particularly when lignocellulosic or complex solid residues are used as feedstocks. These advantages include elimination of the sugar extraction step, reduced water consumption, lower wastewater generation, higher volumetric productivity, and lower distillation costs (Hölker et al., 2004; Soccol et al., 2017). Moreover, SSF more closely resembles the natural ecological niche of the fermenting microorganisms, which can result in improved metabolic performance.

Despite these advantages, SSF for biofuel production remains less explored at industrial scale compared to SmF, mainly due to challenges in heat and mass transfer, process monitoring and control, and bioreactor design. Nevertheless,



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recent advances in bioreactor engineering, process modeling, and the use of mixed microbial cultures have renewed interest in SSF as a feasible technology for biofuel production from complex residues (Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023; Nawaz et al., 2024).

This review provides a comprehensive and updated analysis of SSF as a platform for biofuel production, covering: (i) the nature and composition of relevant solid substrates; (ii) pretreatment strategies; (iii) fermentative microorganisms; (iv) process factors and kinetics; (v) bioreactor configurations; and (vi) expanding applications beyond ethanol, including biobutanol, biogas, and biohydrogen. The circular bioeconomy dimension of SSF is also discussed.

2. Fundamentals of solid-state fermentation

2.1. Definition and characteristics

SSF is defined as the microbiological process occurring on solid substrates that possess the property of absorbing or containing water, with or without soluble nutrients, in the absence of free water in the interparticle spaces (Viniegra-González, 1997). The solid substrate serves simultaneously as carbon and energy source, as well as physical support for microbial growth (Méndez-González et al. 2024). The water activity (a_w) in the solid matrix is a key parameter: values between 0.98 and 0.99 are typically considered optimal for yeasts involved in ethanol production (Molaverdi et al., 2013).

SSF differentiates itself from SmF in multiple physicochemical aspects. The absence of free water creates a microenvironment that is naturally selective for fungi and yeasts, reduces contamination risk, increases the concentration of extracellular enzymes in the immediate substrate vicinity, and favors the formation of metabolites at higher concentrations. It also leads to lower catabolic repression, improved oxygen transfer compared to viscous liquid broths, and reduced risk of product inhibition through dynamic gas exchange (Hölker et al., 2004; Barrios-Nolasco et al., 2024).

2.2. Comparison with submerged fermentation

The choice between SSF and SmF is dictated by the nature of the substrate, the target product, and economic constraints. For complex solid substrates such as OFMSW, SSF presents a compelling case: it eliminates the need to extract and clarify the fermentable fraction before fermentation, it reduces reactor volume requirements, and it generates significantly less liquid waste. These advantages translate into lower capital and operational costs, particularly relevant when scaling up from laboratory to pilot and industrial levels.

However, SSF faces important engineering limitations. Heat dissipation is a persistent challenge, as the poor thermal conductivity of solid substrates can lead to localized temperature gradients that impair microbial viability and product yield. Mass transfer of oxygen and nutrients is constrained by the solid matrix structure. These limitations make instrumentation and process control more complex in SSF than in SmF (Casciotori and Thoméo, 2018; Couder-García et al., 2024).

3. Solid substrate for biofuel production via SSF

3.1. Lignocellulosic biomass

Lignocellulose—composed of cellulose, hemicellulose, lignin, and pectin—is the main structural component of plant cell walls and the most abundant renewable carbon source on the planet. In biofuel contexts, lignocellulosic biomass encompasses agricultural residues (rice straw, wheat straw, corn stover, sugarcane bagasse), forestry wastes, and energy crops. Its fermentation to bioethanol requires efficient pretreatment to break down the crystalline cellulose structure and release fermentable sugars.

Cellulose is a linear polymer of D-glucose units linked by β -1,4-glycosidic bonds, forming microfibrils with high crystallinity and resistance to enzymatic hydrolysis. Hemicellulose is a branched heteropolysaccharide containing



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pentoses (D-xylose, L-arabinose) and hexoses (D-glucose, D-mannose, D-galactose). Lignin is an amorphous aromatic heteropolymer that provides rigidity and impermeability, and is highly resistant to both chemical and biological degradation. Pectins, complex polysaccharides present in cell walls of fruits and vegetables, are particularly relevant in the context of OFMSW valorization (Cuervo et al., 2009; Rubin, 2008).

3.2. Organic fraction of municipal solid waste (OFMSW)

The OFMSW encompasses organic wastes from households, restaurants, markets, and food-processing facilities. Its composition is highly heterogeneous but typically includes fresh fruit and vegetable residues, cooked food scraps, bread, rice, and similar materials. Moisture content ranges from 61 to 83%, while total sugars, starch, and cellulose content span 35–69%, 24–46%, and 1.6–16.9% on a dry weight basis, respectively (Uçkun Kiran et al., 2014).

Compared to lignocellulosic agricultural residues, the OFMSW presents a relatively low lignin content and higher proportion of easily fermentable carbohydrates (glucose, fructose, sucrose), which makes it particularly suitable for SSF-based bioethanol production with minimal pretreatment. This is a key advantage over substrates like wheat straw or rice husks, where aggressive thermochemical or enzymatic pretreatments are required. In this context, a solid standard mixture (SSM) of fresh fruit and vegetable residues—representative of the annual composition of OFMSW from large supply centers—has been demonstrated to yield up to 282.6 L of ethanol per ton of dry matter by direct SSF, using only mild thermal pretreatment (Estrada-Martínez et al., 2019).

A two-stage process using OFMSW for simultaneous ethanol and methane production has also been documented. Bioaugmentation with *Saccharomyces cerevisiae* at 20% total solids yielded up to 407 gCOD/kgTS of ethanol in the first stage, while the solid digestate showed enhanced methane potential (up to 464 NL-

CH₄/kgVS), demonstrating the potential for full substrate valorization within a biorefinery scheme (Mosquera-Toscano et al., 2024).

3.3. Other relevant substrates

Sweet sorghum stalks are among the most studied substrates for SSF-based bioethanol production due to their high sugar content and low lignin levels. Gas stripping coupled to SSF (GS-SSF) from sweet sorghum stalks has demonstrated ethanol yields of 22.7 g per 100 g of dry substrate, with a 30% improvement over conventional SSF by reducing product inhibition through in-situ ethanol removal (Chen et al., 2014). Rice straw, wheat straw, and sugarcane bagasse have also been extensively investigated, with pretreatment conditions playing a decisive role in fermentable sugar release. Palm frond, corn stover, and citrus residues complete a wide portfolio of substrates compatible with SSF-based biofuel production (Narra et al., 2015; Boluda-Aguilar et al., 2013).

4. Pretreatment strategies for SSF feedstocks

4.1. The role of pretreatment

Pretreatment is a critical step in converting complex carbohydrate-rich substrates into fermentable sugars. Its primary objectives are: (i) to solubilize and hydrolyze structural carbohydrates; (ii) to avoid degradation of released sugars; (iii) to limit the formation of fermentation inhibitors; (iv) to reduce energy consumption; and (v) to minimize overall process costs (Demirbas, 2005; Kumar et al., 2009). The choice of pretreatment must balance sugar recovery against the generation of inhibitory compounds such as furfural, 5-hydroxymethylfurfural (HMF), acetic acid, and phenolic compounds.

4.2. Mechanical pretreatment

Mechanical pretreatment reduces particle size, increases specific surface area, and improves mass transfer between the substrate and the fermenting microorganisms. For OFMSW, mechanical homogenization (grinding, milling,



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cutting) is typically the first step, as it reduces heterogeneity and facilitates subsequent thermal or enzymatic processing. The particle size strongly influences sugar release: for corn stover, particles between 1.0 and 1.5 cm have been reported to maximize glucan (99.6%) and xylan (67.0%) recovery during steam explosion pretreatment (Liu et al., 2013). For OFMSW, particle sizes in the range of 0.17–0.85 mm have been associated with optimal ethanol yield in SSF with fungal inocula (Molaverdi et al., 2013).

4.3. Thermal pretreatment and severity factor

Thermal pretreatment —particularly in the form of steam explosion or autoclave sterilization— is one of the most widely employed approaches. The severity factor (SF) is a dimensionless parameter that integrates temperature and residence time, enabling comparison across different thermal pretreatment conditions. It is defined as:

$$\log(R_0) = \log[t \cdot \exp((T - 100)/14.75)]$$

where t is residence time (min) and T is temperature ($^{\circ}\text{C}$). For the OFMSW, severity factors between $\text{SF} = 2.11$ (135°C , 12 min) and $\text{SF} = 2.35$ (140°C , 15 min) have been identified as optimal, achieving fermentable carbohydrate increases of 68.3 to 155.3% compared to standard sterilization (121°C , 15 min), without generating significant concentrations of inhibitors such as HMF or acetic acid when acid catalysts are avoided (Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023).

A low-SF steam sterilization approach has shown particular promise for OFMSW: conditions of $\text{SF} \approx 2.35$ without sulfuric acid addition represent a compromise between carbohydrate release and inhibitor control, making the pretreated substrate directly compatible with SSF without the need for pH adjustment or detoxification. This is especially relevant in SSF, where the solid matrix makes neutralization or washing steps technically challenging.

For more recalcitrant lignocellulosic substrates (sugarcane bagasse, wheat straw), a combined severity factor (CSF) that incorporates the catalytic effect of acid is used. Dilute sulfuric acid (0.5–2% w/w) combined with steam at 140 – 170°C has been documented to hydrolyze hemicellulose fractions efficiently, releasing xylose while partially solubilizing cellulose (Fockink et al., 2018). However, the formation of inhibitors at these conditions demands post-pretreatment detoxification steps.

4.4. Enzymatic hydrolysis and consolidated bioprocessing

Enzymatic hydrolysis employs cellulases, hemicellulases, β -glucosidases, pectinases, and amylases to release fermentable sugars from pretreated substrates. For SSF, enzymatic hydrolysis can be performed as a sequential step or simultaneously with fermentation (SSF in the SSF-SSF notation used in the literature, or simultaneous saccharification and fermentation, SSaF). Consolidated bioprocessing (CBP) integrates enzyme production, saccharification, and fermentation in a single step using specially engineered microorganisms.

A notable recent example of CBP applied to OFMSW involves the use of a recombinant *Saccharomyces cerevisiae* strain co-secreting glucoamylase and α -amylase, enabling direct conversion of starch-rich OFMSW to bioethanol without exogenous enzyme addition (Gupte et al., 2024). This approach substantially reduces production costs and simplifies the process flow, making it a promising target for future SSF-based biorefineries.

5. Fermentative microorganisms in SSF

5.1. Yeast for ethanol production

Yeasts are the primary microorganisms used in SSF-based bioethanol production. The selection criteria for industrial strains include: a wide substrate spectrum (hexoses and pentoses), high ethanol yield and productivity, tolerance to ethanol concentrations above 80–100 g/L,



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osmotolerance, thermotolerance, and genetic stability (Kuhad et al., 2011).

Saccharomyces cerevisiae is the most widely used yeast for ethanol production from hexose-rich substrates. It is a Crabtree-positive yeast, meaning it produces ethanol under aerobic conditions when glucose concentrations are elevated. This trait is particularly advantageous in SSF, where aerobic conditions may prevail during the early phases of fermentation, as the Crabtree effect redirects carbon flow toward ethanol production rather than biomass generation (Hagman and Piškur, 2015; Pronk et al., 1996). *S. cerevisiae* can tolerate ethanol concentrations of up to 14% (v/v) and pH as low as 3.2, giving it a competitive advantage against contaminating microorganisms in non-sterile SSF conditions (Rozpędowska et al., 2011).

Scheffersomyces (Pichia) stipitis is a Crabtree-negative yeast with the capacity to ferment xylose, the dominant pentose sugar released from hemicellulose hydrolysis. Its co-cultivation with *S. cerevisiae* in mixed culture SSF allows simultaneous fermentation of both hexoses and pentoses present in complex substrates. *Schwanniomyces occidentalis* contributes amylolytic activity (pullulanase), capable of hydrolyzing α -1,6 glucosidic bonds in starch, and also produces ethanol from the resulting glucose (Saucedo-Castañeda et al., 1992). *Kluyveromyces marxianus* is thermotolerant (optimal growth near 40°C) and ferments both hexoses and some pentoses, making it suitable for high-temperature SSF processes (da Costa et al., 2015).

5.2. Mixed cultures

Mixed cultures have emerged as a strategy to overcome the substrate limitation inherent to using a single yeast strain. A mixed culture of *S. cerevisiae*, *S. stipitis*, and *Schw. occidentalis* demonstrated superior ethanol production in SSF of OFMSW (282.6 L/ton DM) compared to any single-strain inoculum (Estrada-Martínez et al., 2019). This synergistic effect arises from the complementary metabolic capabilities of the three yeasts: hexose fermentation by *S.*

cerevisiae, pentose fermentation by *S. stipitis*, and starch hydrolysis and fermentation by *Schw. occidentalis*. At pilot scale, the mixed culture maintained its superiority (245.7 L/ton DM), confirming its robustness under non-sterile, heterogeneous conditions.

The advantages of co-cultures in SSF extend beyond substrate coverage. Mixed culture fermentations can result in better process control, improved tolerance to inhibitors, more complete substrate utilization, and reduced contamination risk (Bader et al., 2010). However, optimal inoculum ratios and culture conditions must be established to prevent competitive exclusion or metabolic imbalances.

5.3. Filamentous fungi

Filamentous fungi such as *Aspergillus niger*, *Trichoderma reesei*, and *Rhizopus oryzae* are valuable in SSF because of their ability to secrete a broad spectrum of hydrolytic enzymes (cellulases, hemicellulases, pectinases, amylases) directly onto the solid substrate. In two-step SSF processes, fungi are used in the first phase to enzymatically saccharify the substrate, followed by yeast fermentation of the released sugars. This sequential approach has been applied to rice straw, sugarcane bagasse, and food waste with promising results (Nawaz et al., 2024).

6. Process factors and kinetic modeling in SSF

6.1. Key process parameters

The main operational parameters governing SSF for biofuel production are: initial moisture content (water activity), temperature, inoculum size and composition, particle size, pH, and aeration regime. Optimal moisture content for ethanol-producing yeasts in SSF typically falls between 65% and 80%, representing a balance between substrate accessibility, microbial mobility, and oxygen diffusion. Higher moisture levels can approach SmF conditions, while lower levels may cause water stress and reduce metabolic activity.



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Temperature has a dual effect on SSF: it controls microbial metabolic rates and determines the rate of chemical degradation of carbohydrates and nutrients. Most ethanol-producing yeasts perform optimally between 30 and 35°C, and exposure to temperatures above 40°C activates heat shock responses and degrades glycolytic enzymes, reducing ethanol yield. pH affects enzyme activity and fermentation kinetics; most yeasts show broad optima between pH 4.0 and 6.0. In SSF of OFMSW without pH control, the natural pH (typically 5–6 after thermal pretreatment) is often adequate without adjustment, provided acid pretreatment is avoided (Converti et al., 1989; Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023).

Inoculum size and preparation are critical in SSF. An insufficient inoculum fails to establish the fermenting microorganism before contaminants, while excess inoculum can lead to nutrient limitation. For OFMSW-based SSF, a filling volume of 20% in Erlenmeyer flasks during inoculum preparation was shown to provide better microbial growth and consistent inoculum quality regardless of flask design (Estrada-Martínez et al., 2019). The Crabtree effect, evaluated through ethanol productivity under aerobic conditions, was identified as a key screening criterion for yeast selection in SSF of OFMSW.

6.2. Kinetic modeling

Mathematical modeling of SSF processes serves both for process understanding and for predictive control during scale-up. For bioethanol production from OFMSW by SSF, a first-order decay kinetic model accurately describes the consumption of fermentable carbohydrates:

$$S(t) = S_0 \cdot \exp(-k \cdot t)$$

where $S(t)$ is the substrate concentration at time t , S_0 is initial substrate concentration, and k is the first-order decay constant. Ethanol production is well-described by the modified Gompertz model:

$$E(t) = E_{\max} \cdot \exp\{-\exp[(R_m \cdot e / E_{\max})(\lambda - t) + 1]\}$$

where $E_{\max}$ is maximum ethanol production, R_m is maximum ethanol production rate, λ is the lag phase duration, and e is Euler's number. These models, validated at both laboratory and pilot scales, are useful tools for bioreactor design and process optimization (Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023). Online CO_2 monitoring in the headspace of SSF bioreactors has been proposed as a non-invasive, real-time proxy for ethanol production rate, enabling process control without destructive sampling.

7. Bioreactors design for SSF

7.1. Classification and design principles

SSF bioreactors can be classified into four broad categories based on mixing and aeration mode: (i) static tray bioreactors (no mixing, natural aeration); (ii) packed-bed bioreactors (forced aeration, no mixing); (iii) rotating drum bioreactors (mixing by rotation); and (iv) stirred or agitated bioreactors with internal mixing elements such as ribbons, screws, or paddles (Pandey, 1991; Barrios-Nolasco et al., 2024; Méndez-González et al. 2026). Each design presents trade-offs between ease of operation, mass transfer efficiency, shear stress on the microorganism, and scalability.

7.2. Stirred bioreactor for ethanol-SSF

For OFMSW fermentation, stirred bioreactors are necessary to achieve uniform distribution of the inoculum and to prevent the formation of concentration gradients in temperature, substrate, and metabolites. Two pilot-scale bioreactor configurations have been evaluated for SSF of OFMSW (Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023).

Helical ribbon bioreactor (HRB): A 18-kg capacity vessel with helical ribbon impellers that promote gentle mixing without excessive shear. This design achieved ethanol production of 245.7 ± 17.8 L/ton DM using a mixed yeast culture with SF = 2.35 pretreatment, demonstrating close correspondence with laboratory-scale results (282.6 L/ton DM). The slow, intermittent mixing reduced substrate degradation and maintained the structural integrity of the solid matrix.



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Endless screw bioreactor (ESB): A 25-kg capacity vessel with worm-screw conveyor elements, enabling both mixing and transport of the solid substrate. This design yielded 209.2 ± 12.6 L/ton DM using *S. cerevisiae* ITD00196 without pretreatment, indicating a lower but still competitive productivity. The simpler design of the ESB makes it more amenable to continuous or semi-continuous operation at industrial scale

7.3. Scale-up challenges

Scaling up SSF processes is constrained primarily by heat dissipation and mass transfer limitations (Méndez-González et al. 2025). As bioreactor size increases, the surface-to-volume ratio decreases, making heat removal progressively more difficult. Temperature gradients within the solid bed can exceed 10°C between the center and periphery, causing localized microbial death or inhibition. Advanced bioreactor designs incorporate temperature-controlled walls, internal cooling

coils, or forced aeration strategies to mitigate this effect (Casciatori and Thoméo, 2018).

Data-driven modeling and artificial intelligence approaches have recently been explored to optimize SSF at scale, drawing from the success of such methods in composting (Gupta et al., 2015). The integration of online sensors (CO_2 , O_2 , temperature) with predictive models represents a promising route toward fully controlled industrial SSF for biofuels (Estrada-Martínez et al., 2019; Estrada-Martínez et al., 2023).

8. Bioethanol production by SSF: a comparative analysis

Table 1 compiles representative results from SSF and SSC-based bioethanol production from OFMSW and related substrates at laboratory and pilot scales, based on recently published literature.

Table 1. Comparative bioethanol yields from SSF of OFMSW and lignocellulosic substrates.

Substrate	Pretreatment	Microorganism	Scale	Yield (L or g/ton DM)	Reference
OFMSW (fruits/vegetables)	SF = 2.35	Mixed culture (<i>S. cerevisiae</i> , <i>S. stipitis</i> , <i>Schw. occidentalis</i>)	Lab	282.6 L/ton DM	Estrada-Martínez et al. (2019)
OFMSW (fruits/vegetables)	SF = 2.35	Mixed culture	Pilot (18 kg HRB)	245.7 L/ton DM	Estrada-Martínez et al. (2019)
OFMSW (fruits/vegetables)	None	<i>S. cerevisiae</i>	Pilot (25 kg ESB)	209.2 L/ton DM	Estrada-Martínez et al. (2023)
Food waste (mixed kitchen)	Glucoamylase enzymatic	<i>S. cerevisiae</i> KF-7	Lab	154.5 g/kg DM	Tang et al. (2008)
Food waste (kitchen)	Enzymatic multi-enzyme	<i>S. cerevisiae</i> commercial	Lab	161 g/kg DM	Uncu & Cekmecelioglu (2011)
Citrus waste (lemon)	Steam explosion (SF = 2.47) + enzymes	<i>S. cerevisiae</i>	Pilot (5 L)	254.9 g/kg DM	Boluda-Aguilar et al. (2013)
Sweet sorghum stalks	None (GS-SSF)	<i>S. cerevisiae</i>	Lab	227 g/kg DM	Chen et al. (2014)
Rice straw	H ₂ SO ₄ 4% + steam	<i>Kluyveromyces</i> sp.	Lab	196 g/kg DM	Narra et al. (2015)
OFMSW (starch-rich)	None (CBP)	Recombinant <i>S. cerevisiae</i> (amylase)	Lab	~200 g/kg DM	Gupte et al. (2024)



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These data indicate that SSF of OFMSW with mild thermal pretreatment and mixed yeast cultures is competitive with more complex enzymatic or thermochemical approaches applied to other substrates, while requiring less energy and generating fewer inhibitors. The pilot-scale validation of these results represents a significant advance toward industrial implementation.

9. Beyond ethanol: other biofuels from SSF

9.1. Biobutanol

Biobutanol (n-butanol) is a superior biofuel compared to bioethanol in several aspects: it has higher energy density (29.2 MJ/L vs. 21.2 MJ/L for ethanol), lower vapor pressure, lower hygroscopicity, and is compatible with existing gasoline distribution infrastructure without modification. It is produced primarily through acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* and related species. The SSF-based production of biobutanol from lignocellulosic and solid organic waste substrates has gained interest as an alternative to submerged ABE fermentation.

Adsorption carrier solid-state fermentation (AC-SSF) has been proposed as a novel approach for biobutanol production, in which *C. acetobutylicum* is grown on activated carbon carriers in a near-solid environment. This system demonstrated coproduction of electricity and biobutanol through coupling with a microbial fuel cell, achieving butanol yields comparable to conventional ABE fermentation while reducing energy consumption for maintaining anaerobic conditions (Feng et al., 2022). Municipal solid waste fractions, agricultural residues such as straw and grasses, and kitchen waste have all been identified as suitable feedstocks for solid-state ABE fermentation (Eloka-Eboka et al., 2023).

9.2. Biogas and biomethane

Biogas—a mixture of methane (50–75%) and CO₂ (25–50%)—is produced by anaerobic digestion (AD) of organic matter. While traditional AD is a liquid-phase process, high-solids anaerobic

digestion (also termed solid-state AD or dry anaerobic digestion) operates at total solids contents above 15%, effectively constituting a solid-state biogas process. This approach reduces water requirements, generates smaller quantities of liquid digestate, and can process substrates that are impractical for liquid-phase AD, including solid OFMSW, agricultural straw, and yard waste (Mosquera-Toscano et al., 2024).

A compelling integration strategy combines SSF-based ethanol production with anaerobic digestion of the ethanol fermentation residue. In a two-stage biorefinery process applied to OFMSW, ethanol is first extracted by SSF, and the remaining solid digestate—enriched in cellulose, hemicellulose, and microbial biomass—is then subjected to AD, achieving methane potentials of up to 464 NL-CH₄/kgVS. This cascading valorization strategy maximizes energy recovery per unit of organic waste while minimizing final solid residue (Mosquera-Toscano et al., 2024).

9.3. Biohydrogen

Biohydrogen is the biofuel with the highest energy density per unit mass (120 MJ/kg) and generates only water upon combustion, making it an ideal clean energy carrier. Biological hydrogen production encompasses dark fermentation, photofermentation, and biocatalytic electrolysis. Dark fermentation of solid carbohydrate-rich substrates by *Clostridium* sp. and other anaerobic bacteria represents a form of SSF-compatible hydrogen production, particularly in high-solid systems.

The integration of SSF with biohydrogen production is still at an early stage. Nevertheless, some anaerobic SSF processes have been shown to co-produce hydrogen alongside other volatile fatty acids and alcohols from lignocellulosic biomass. Reviews on nanoparticle-enhanced microbial fermentation for biohydrogen and biobutanol production highlight the potential of SSF as a platform that reduces water consumption, concentrates metabolic activity, and can be coupled with in-situ product removal strategies (Meena et al., 2025).



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9.4. Other products compatible with SSF biorefineries

SSF of organic residues generates, beyond biofuels, a range of high-value co-products that improve the overall economic feasibility of the process. Lactic acid (feedstock for polylactic acid, PLA, bioplastics), succinic acid (platform chemical), volatile fatty acids (VFAs), and single-cell protein are among the most commercially relevant (Perwez and Al Asheh, 2025). This product diversification through biorefineries, where SSF acts as the central bioconversion platform, is aligned with circular bioeconomy principles and reduces the dependence on single high-yield target products.

10. Challenges and future perspectives

Despite the notable advances in SSF-based biofuel production, several challenges remain before full industrial implementation is achieved:

- Scale-up and bioreactor engineering: Heat and mass transfer limitations at large scale remain the primary engineering bottleneck. The development of novel bioreactor designs — such as multi-stacked tray systems, rotating drums with internal cooling, or modular pillow-shaped SSF units— is essential. Advanced process modeling (CFD, data-driven models) integrated with real-time sensing (CO₂, temperature, humidity) will play an important role in process optimization at scale.
- Feedstock heterogeneity: The composition of OFMSW varies seasonally, geographically, and with collection practice. Robust processes must be designed to tolerate this variability without significant loss of performance. The use of standardized solid mixtures —such as the annual-average SSM proposed for Mexico City supply centers— is a practical approach to reduce batch-to-batch variability.
- Inoculum technology: Production of large volumes of high-quality inoculum is critical for SSF at pilot and industrial scale. Optimized

inoculum production protocols —particularly for mixed cultures— need to be standardized and validated under near-industrial conditions.

- Downstream processing: Ethanol recovery from solid matrices presents additional challenges compared to liquid fermentation. Distillation of ethanol-containing solids, pervaporation, or gas stripping (GS-SSF) are promising downstream options, but their integration at scale requires further development.
- Economic competitiveness: A comprehensive techno-economic analysis (TEA) and life cycle assessment (LCA) of SSF-based biofuel processes are needed to define realistic cost targets and identify the most impactful areas for improvement. The co-production of value-added products within a biorefinery framework is likely essential for economic viability in the short term.

Genetic and metabolic engineering: The development of improved yeast strains with broader substrate range, higher ethanol tolerance, and capacity to produce multiple biofuels within a single SSF process is a frontier area. CBP strains capable of performing saccharification and fermentation simultaneously without exogenous enzymes represent the next generation of SSF catalysts (Gupte et al., 2024).

11. Conclusions

Solid-state fermentation is a scientifically sound and practically relevant technology to produce second-generation biofuels from complex organic substrates, particularly the OFMSW. The mild thermal pretreatment of OFMSW at severity factors between 2.11 and 2.35 enables direct SSF-based ethanol production without thermochemical or enzymatic pretreatment, achieving yields that are competitive with more complex and resource-intensive processes. The use of mixed yeast cultures —combining the complementary metabolic capacities of *S. cerevisiae*, *S. stipitis*, and *Schw. occidentalis*— represents a robust strategy for maximizing



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fermentable carbon utilization from heterogeneous substrates.

Beyond bioethanol, SSF offers a platform for producing biobutanol, biogas, biohydrogen, and a range of high-value chemicals, positioning it as a central bioprocess in circular bioeconomy strategies for organic waste management. The integration of SSF with anaerobic digestion in cascade biorefineries represents one of the most promising routes for maximizing energy and material recovery from OFMSW.

The primary challenges remaining —heat and mass transfer at scale, feedstock variability, and economic competitiveness— are addressable through advances in bioreactor engineering, process modeling, and microbial biotechnology. The rapid growth of scientific literature on SSF in recent years signals a maturing field that is moving steadily from academic proof-of-concept toward industrial application.

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Declarations and statements

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