



## ***Enzymatic and Microbial Valorization of Oil Palm Empty Fruit Bunches (OPEFB): A Systematic Review of Lignocellulosic Biorefinery Processes and Value-Added Product Generation***

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### ***Abstract***

*Oil Palm Empty Fruit Bunches (OPEFB) represent a significant lignocellulosic residue with potential for bioconversion into biofuels, biochemicals, biomaterials, and bioenergy carriers. Despite extensive research, findings on enzymatic and microbial valorization are dispersed, hindering a comprehensive understanding of technological pathways and product diversification. This study aims to systematically synthesize peer-reviewed literature published between 2019 and 2026 on enzymatic and microbial valorization of OPEFB within lignocellulosic biorefinery frameworks, consolidating performance data, identifying dominant process configurations, and highlighting emerging research trends. A Systematic Literature Review (SLR) approach was applied. Articles were collected exclusively from the Scopus database using structured keywords targeting OPEFB, enzymatic hydrolysis, microbial fermentation, lignocellulosic biorefinery, and value-added products. Screening was performed sequentially based on relevance, publication period, language, and accessibility, resulting in 34 peer-reviewed articles eligible for inclusion. Data extraction encompassed biomass composition, pretreatment conditions, enzyme and microbial parameters, product yields, and techno-functional indicators. Analytical synthesis employed thematic clustering to organize results into six dominant themes: OPEFB structural characteristics, pretreatment strategies, enzymatic hydrolysis performance, microbial fermentation pathways, integrated biorefinery configurations, and value-added product diversification. The results indicate that pretreatment enhances enzymatic accessibility, achieving glucose yields of 70–90%, while microbial pathways produce ethanol, organic acids, polyhydroxyalkanoates, and biogas with high conversion efficiencies. Integrated biorefinery models improve resource utilization and energy recovery. In conclusion, OPEFB is a technically viable feedstock for diversified biochemical valorization. Future studies should prioritize pilot-scale validation, process integration optimization, enzyme and microbial engineering, and comprehensive sustainability assessments.*

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**Keywords:** OPEFB, Systematic Literature Review, Enzymatic Hydrolysis, Microbial Fermentation, Lignocellulosic Biorefinery

## Introduction

The transition toward a bio-based economy has intensified global interest in the sustainable utilization of lignocellulosic biomass as a renewable resource for the production of energy, chemicals, and materials [1]. Growing concerns about fossil resource depletion, greenhouse gas mitigation, and circular resource management have spurred the development of integrated biorefinery systems capable of converting agricultural residues into high-value products [2]. Within this framework, lignocellulosic biomass has emerged as a strategic feedstock due to its abundance, renewability, and chemical versatility, particularly in its cellulose, hemicellulose, and lignin fractions, which can be transformed into diverse bio-based outputs. Advances in biochemical conversion pathways, especially enzymatic and microbial processes, have further strengthened the feasibility of converting agricultural by-products into commercially relevant commodities while supporting circular bioeconomy objectives [3].

Among major agricultural commodities, the oil palm industry plays a significant role in supplying vegetable oil to global food, oleochemical, and bioenergy markets. Alongside its primary outputs, palm oil processing generates substantial quantities of lignocellulosic residues, including empty fruit bunches (OPEFB), mesocarp fiber, and palm kernel shells [4]. OPEFB represents one of the largest solid residues generated at palm oil mills, accounting for approximately 20–23% of the fresh fruit bunch weight after sterilization and threshing. Given the scale of palm oil production in Southeast Asia and other producing regions, the annual generation of OPEFB constitutes a considerable biomass stream with conversion potential rather than waste status. The physicochemical composition of OPEFB, typically comprising 35–45% cellulose, 20–30% hemicellulose, and 18–28% lignin on a dry-basis, positions it as a promising lignocellulosic substrate for biochemical valorization [5].

The structural complexity of lignocellulosic biomass, however, presents technical challenges for efficient bioconversion. The crystalline nature of cellulose, the heterogeneity of hemicellulose, and the protec-

tive lignin matrix collectively contribute to biomass recalcitrance, limiting enzymatic accessibility and microbial assimilation. Overcoming these structural barriers requires coordinated pretreatment strategies, optimized enzyme systems, and carefully selected microbial strains capable of converting released sugars into target products with high efficiency [6]. In recent years, significant research efforts have focused on improving pretreatment technologies, enhancing enzymatic hydrolysis yields, engineering robust microbial platforms, and integrating these stages into coherent lignocellulosic biorefinery systems [7].

Within the context of OPEFB, numerous experimental studies have explored chemical, physicochemical, and biological pretreatment approaches to improve cellulose accessibility and sugar recovery. Enzymatic hydrolysis using commercial cellulase complexes, often supplemented with  $\beta$ -glucosidase and hemicellulase, has demonstrated promising saccharification performance under optimized conditions [8]. Concurrently, microbial fermentation pathways employing yeasts, bacteria, and filamentous fungi have enabled the conversion of OPEFB-derived sugars into bioethanol, organic acids, bioplastics, enzymes, and other value-added compounds. Beyond single-product conversion, integrated biorefinery configurations have been proposed to maximize carbon utilization by coupling sugar fermentation with lignin valorization and the recovery of residual biomass energy [9].

Despite the growing body of research on OPEFB valorization, the available literature remains fragmented across biotechnology, bioenergy, materials science, and environmental engineering. Individual studies often focus on specific process stages, such as pretreatment optimization or fermentation efficiency, without synthesizing findings across the entire bioconversion chain [10]. Moreover, reported performance metrics vary widely depending on feedstock conditioning, enzyme loading, microbial strain selection, and reactor configuration, making it difficult to derive consolidated insights regarding technological maturity and process integration potential [11]. The absence of a structured synthesis focused specifically on enzymatic and microbial valorization pathways within a lignocellulosic biorefinery perspective limits the ability to

identify converging trends, methodological gaps, and future research priorities.

A systematic literature review (SLR) offers a rigorous methodological framework for consolidating dispersed scientific evidence while maintaining transparency and replicability in study selection and synthesis. Unlike narrative reviews, an SLR follows predefined inclusion criteria, database search strategies, and screening protocols to minimize selection bias and ensure analytical consistency. In the present study, the SLR approach is employed exclusively to synthesize peer-reviewed publications indexed in an established scientific database, without generating primary empirical data, conducting laboratory experiments, or undertaking field-based observations. The analytical objective is therefore to aggregate, compare, and interpret published findings related to enzymatic and microbial conversion of OPEFB within lignocellulosic biorefinery systems.

The growing emphasis on circular bioeconomy strategies further underscores the importance of systematically evaluating OPEFB valorization pathways. By transforming lignocellulosic residues into biofuels, biochemicals, and biomaterials, biorefinery systems can enhance resource efficiency, diversify product portfolios, and contribute to sustainable industrial development. Enzymatic and microbial routes are particularly relevant due to their operational specificity, lower reaction severity compared to thermochemical methods, and compatibility with integrated process design. A comprehensive synthesis of recent advancements is therefore essential to understand how these biological approaches have evolved, which technological configurations dominate current research, and where knowledge gaps remain.

This study aims to systematically synthesize peer-reviewed research published between 2019 and 2026 on enzymatic and microbial valorization of OPEFB within lignocellulosic biorefinery frameworks. The review consolidates quantitative performance data, identifies dominant technological pathways, examines integration strategies for value-added product generation, and evaluates emerging research directions. By organizing dispersed findings into coherent thematic clusters, the study seeks to provide a structured analytical foundation that clarifies the current

state of knowledge and informs future research development in OPEFB-based bioconversion systems.

To guide the analytical synthesis, this review addresses the following research questions:

**RQ1:** How have enzymatic and microbial conversion strategies been applied and optimized in recent studies to enhance the efficiency of OPEFB-based lignocellulosic biorefinery processes?

**RQ2:** What patterns of value-added product diversification and process integration emerge from the existing literature, and how do these patterns reflect the technological maturity of OPEFB valorization pathways?

These research questions frame the thematic analysis presented in the subsequent sections and provide the basis for the critical discussion and conclusions regarding the advancement of enzymatic and microbial OPEFB valorization within contemporary biorefinery systems.

## Literature Review

The transition toward sustainable bio-based industries has intensified interest in lignocellulosic biorefineries that convert agricultural residues into value-added products through integrated biochemical processes. Within this context, Oil Palm Empty Fruit Bunches (OPEFB) have emerged as a promising lignocellulosic resource due to their substantial carbohydrate content and availability within established agro-industrial systems. Enzymatic hydrolysis and microbial fermentation constitute central technological pathways for transforming OPEFB into biofuels, biochemicals, and biopolymers under the biorefinery paradigm. This literature review synthesizes recent peer-reviewed evidence on pretreatment strategies, enzymatic conversion, microbial valorization, and integrated system configurations to provide a structured understanding of current developments in OPEFB-based lignocellulosic biorefineries.

## Lignocellulosic Biomass and the Biorefinery Paradigm

The development of lignocellulosic biorefineries has become a central pillar in the transition toward resource-efficient and bio-based industrial systems. Unlike first-generation feedstocks derived from food crops, lignocellulosic residues offer the advantage of utilizing agricultural by-products while minimizing

competition with food supply chains [12]. The biorefinery concept integrates multiple conversion pathways to fractionate biomass into fermentable sugars, lignin-derived intermediates, and energy streams, thereby maximizing carbon utilization and product diversification. Within this framework, enzymatic and microbial processes are often regarded as key technological components for their selectivity, operational flexibility, and compatibility with modular processing configurations.

Lignocellulosic biomass is structurally composed of cellulose microfibrils embedded within hemicellulose and lignin matrices. This architecture provides mechanical strength while simultaneously conferring recalcitrance to biochemical conversion. Effective valorization, therefore, requires a coordinated sequence of pretreatment, enzymatic hydrolysis, and microbial fermentation to release and convert structural carbohydrates into value-added products [13]. The scientific literature has increasingly emphasized process integration strategies that couple these stages into cohesive biorefinery systems, rather than treating them as isolated unit operations.

### **Oil Palm Empty Fruit Bunches as a Lignocellulosic Resource**

Oil Palm Empty Fruit Bunches (OPEFB) represent a significant lignocellulosic residue generated during palm oil milling operations. After sterilization and threshing, empty fruit bunches remain as fibrous biomass rich in structural carbohydrates. The compositional profile of OPEFB, commonly reported as 35–45% cellulose, 20–30% hemicellulose, and approximately 18–28% lignin, positions it as a viable substrate for biochemical conversion [14]. In addition to carbohydrate content, OPEFB contains minor fractions of ash and extractives that can influence pretreatment efficiency and downstream enzymatic activity.

Recent studies have framed OPEFB not as waste but as a secondary resource stream within integrated agro-industrial systems, consistent with circular bioeconomy principles. The availability of OPEFB at centralized mill locations facilitates logistical aggregation, thereby supporting decentralized or on-site biorefinery development [15]. Consequently, research attention has expanded to identify technically feasible and economically rational pathways to

convert OPEFB into fuels, chemicals, and advanced materials through biological processes.

### **Structural Recalcitrance and Pretreatment Requirements**

The intrinsic recalcitrance of OPEFB arises from lignin shielding, cellulose crystallinity, and hemicellulosic heterogeneity, which collectively limit enzymatic accessibility. The literature consistently highlights pretreatment as a prerequisite for enhancing sugar recovery. Alkali pretreatment has been widely investigated for its capacity to solubilize lignin and increase porosity, thereby improving subsequent hydrolysis efficiency. Acid-based pretreatment approaches have demonstrated effectiveness in hemicellulose solubilization and partial depolymerization of structural components, contributing to higher fermentable sugar yields.

Physicochemical techniques such as steam explosion and hydrothermal processing have also been explored to disrupt fiber structure while reducing chemical inputs [16]. More recently, biological pretreatment using ligninolytic fungi has attracted attention due to its comparatively mild operating conditions and lower environmental burden, although longer processing times are often required. Across these approaches, the literature emphasizes the need to balance lignin removal, carbohydrate preservation, inhibitor formation, and energy consumption when evaluating pretreatment performance [17].

Despite extensive experimentation, no single pretreatment method has emerged as universally optimal. Reported outcomes vary depending on reaction severity, particle size, moisture content, and integration with downstream hydrolysis, highlighting the importance of context-specific process optimization.

### **Advances in Enzymatic Hydrolysis**

Enzymatic hydrolysis is a core stage in the biochemical valorization of OPEFB. Commercial cellulase systems typically consist of endoglucanases, exoglucanases, and  $\beta$ -glucosidases working synergistically to depolymerize cellulose into glucose. Several studies have reported improved saccharification efficiency when enzyme cocktails are supplemented with accessory enzymes such as xylanases to target hemicellulose fractions.

The literature documents a wide range of enzyme loadings and reaction conditions, reflecting ongoing efforts to optimize yield while minimizing cost. Operational parameters such as pH (generally between 4.8 and 5.5), temperature (45–50°C), and solids loading significantly influence hydrolysis performance [18]. High-solids enzymatic hydrolysis has gained attention for its potential to increase sugar concentration and reduce downstream energy requirements, although mass transfer limitations and enzyme inhibition can arise at elevated substrate concentrations.

Enzyme recycling and immobilization strategies have been investigated to enhance economic feasibility. Some studies report retained catalytic activity across multiple cycles, indicating opportunities for reducing operational expenditure in large-scale applications. Nonetheless, enzyme cost remains a notable component of biochemical conversion pathways, prompting continued research into on-site enzyme production and strain engineering [19].

### Microbial Fermentation Pathways and Product Diversification

Following enzymatic saccharification, fermentable sugars derived from OPEFB can be converted into a spectrum of bio-based products through microbial pathways. Ethanol production using *Saccharomyces cerevisiae* has been extensively examined due to established industrial infrastructure and process familiarity [20]. Simultaneous saccharification and fermentation (SSF) configurations have demonstrated the potential to reduce process time and mitigate end-product inhibition by converting sugars as they are released.

Beyond ethanol, research has expanded toward alternative value-added products. Lactic acid production by *Lactobacillus* species has been explored for the synthesis of bioplastic precursors, while succinic acid and other organic acids have been investigated as platform chemicals for downstream polymer applications [21]. Polyhydroxyalkanoates (PHAs) synthesized by selected bacterial strains represent another high-value biopolymer pathway derived from OPEFB hydrolysates.

Co-culture fermentation systems capable of utilizing both hexose and pentose sugars have been studied to improve overall carbon conversion efficiency. The

literature suggests that metabolic engineering strategies and strain adaptation approaches can enhance tolerance to inhibitors generated during pretreatment, thereby improving productivity and yield [22]. Collectively, these microbial pathways contribute to a diversified product portfolio aligned with the objectives of an integrated biorefinery.

### Integrated Lignocellulosic Biorefinery Configurations

An increasing body of research emphasizes the importance of process integration in maximizing resource efficiency. Rather than focusing solely on ethanol production, integrated systems aim to valorize cellulose-derived sugars, hemicellulosic fractions, and lignin residues within a unified platform [23]. For example, lignin-rich residues may be directed toward bioenergy generation, activated carbon production, or aromatic compound recovery, enhancing overall carbon utilization.

The integration of enzymatic hydrolysis with anaerobic digestion has been explored as a strategy to convert residual solids into biogas, thereby improving energy recovery [24]. Heat integration and water recycling have also been evaluated to reduce external energy inputs and process water demand. These configurations align with the broader biorefinery principle of minimizing waste streams while maximizing product outputs.

Although most studies remain at laboratory or pilot scale, preliminary techno-economic assessments indicate that integrated approaches can improve overall process viability compared to single-product systems [25]. However, scale-up challenges, enzyme cost, and process optimization remain areas requiring further investigation.

### Technological Maturity and Research Trends

Recent publications indicate a shift from isolated laboratory experimentation toward more holistic system analysis. Comparative assessments of pretreatment severity, enzyme loading optimization, and fermentation integration reflect a growing emphasis on process efficiency and scalability [26]. The literature also demonstrates increasing attention to life-cycle considerations and sustainability indicators, particularly regarding energy balance and carbon utilization efficiency.

Nevertheless, variability in experimental design, reporting standards, and performance metrics complicates direct cross-study comparison. Differences in solids loading, reactor configuration, and analytical methods can yield divergent results even under similar conceptual frameworks [27]. This heterogeneity underscores the importance of systematic synthesis to identify consistent patterns and methodological gaps across the existing body of research.

In parallel, emerging studies highlight the potential of advanced biotechnological tools, including strain engineering and enzyme optimization, to enhance conversion efficiency and broaden product range [28]. These advancements suggest a trajectory toward higher-value biochemical production rather than exclusive reliance on biofuel pathways.

While numerous experimental investigations address specific components of OPEFB valorization, comprehensive syntheses integrating enzymatic hydrolysis, microbial fermentation, and biorefinery system design remain limited. Existing reviews often focus on general palm biomass utilization or on individual conversion technologies without systematically consolidating quantitative performance data into a unified analytical framework.

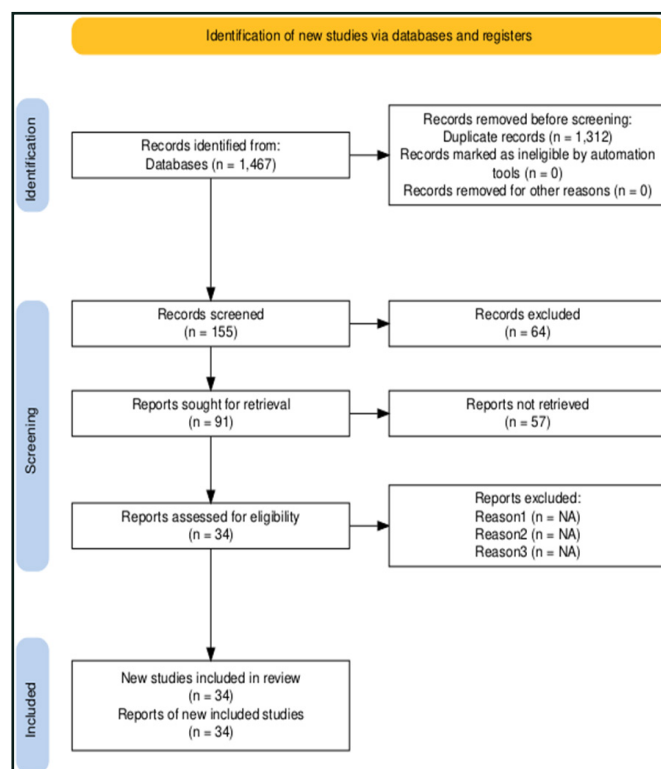
A structured synthesis is therefore necessary to clarify how enzymatic and microbial pathways have evolved in recent years, to identify dominant technological configurations, and to assess patterns of value-added product generation within integrated lignocellulosic frameworks. By applying a systematic literature review methodology, the present study aims to aggregate peer-reviewed evidence in a transparent and replicable manner, without generating primary experimental data or conducting field-based observations.

Through critical consolidation of the existing literature, this review contributes to a clearer understanding of the current state of enzymatic and microbial OPEFB valorization. It highlights future research directions aligned with sustainable biorefinery development.

## Method

This study applies a PRISMA-structured Systematic Literature Review to synthesize peer-reviewed

research on the enzymatic and microbial valorization of Oil Palm Empty Fruit Bunches (OPEFB) within lignocellulosic biorefinery systems. The review integrates scientific evidence on enzymatic hydrolysis, microbial bioconversion, fermentation pathways, and the generation of value-added bioproducts from OPEFB biomass. Although research on OPEFB utilization has expanded across biotechnology, bioenergy, and biomaterials literature, findings remain dispersed across multiple disciplinary domains without an integrated analytical consolidation focused specifically on biological conversion strategies. This review therefore systematizes and organizes the existing body of knowledge to clarify how enzymatic and microbial processes have been applied, optimized, and positioned within broader biorefinery configurations. The study does not generate primary empirical data, conduct field observations, or employ focus group discussions; rather, it provides a structured synthesis of published scientific evidence to map technological development trajectories and product diversification pathways associated with OPEFB valorization.



**Figure 1:** Systematic Literature Review Process Based on PRISMA Framework

Figure 1 illustrates the sequential stages of identification, screening, eligibility assessment, and final inclusion in accordance with PRISMA principles. The identification stage was conducted exclusively through the

Scopus database to ensure bibliographic consistency, indexing reliability, and peer-reviewed quality control. An initial search using the primary keywords Oil Palm Empty Fruit Bunches AND Biomass yielded 1,467 records. To enhance thematic precision and ensure alignment with enzymatic and microbial conversion within lignocellulosic biorefinery contexts, a refined Boolean query was implemented as follows: (“oil palm empty fruit bunches” OR “empty fruit bunches” OR “OPEFB”) AND (“enzymatic” OR “enzyme” OR “microbial” OR “microorganism” OR “fermentation” OR “bioconversion”) AND (“lignocellulosic” OR “biomass” OR “biorefinery”) AND (“valorization” OR “value added” OR “biofuel” OR “biochemical” OR “bioproduct” OR “biomaterial” OR “bioenergy”). Through this refinement process, 1,312 studies were excluded for thematic misalignment, leaving 155 records to advance to the screening phase.

A publication year filter was subsequently applied to restrict the dataset to studies published between 2019 and 2026 in order to capture recent technological developments and contemporary biorefinery strategies. This temporal criterion led to the exclusion of 64 articles, leaving 91 records that satisfied the defined timeframe. Further eligibility assessment was conducted based on accessibility criteria, limiting inclusion to articles available through Open Access or Open Archive sources to ensure analytical transparency and verifiability. As a result, 57 records were removed due to restricted access, and 34 peer-reviewed articles met all inclusion criteria for qualitative synthesis.

All selected references were systematically organized using Mendeley Desktop to maintain citation accuracy, bibliographic consistency, and standardized formatting. The analytical process relied entirely on secondary data derived from peer-reviewed scientific publications indexed in Scopus. No empirical data collection, interviews, surveys, laboratory experimentation, or field-based investigation was undertaken in this study. By adhering to a transparent, replicable, and methodologically rigorous SLR procedure, this review establishes a structured analytical foundation for understanding current advancements in enzymatic and microbial OPEFB valorization and their integration into lignocellulosic biorefinery systems for the generation of value-added products.

## Results

The systematic synthesis of the 34 peer-reviewed journal articles reveals a set of six interrelated thematic domains that collectively characterize current research on enzymatic and microbial valorization of Oil Palm Empty Fruit Bunches (OPEFB) within lignocellulosic biorefinery frameworks. Across the reviewed literature, the dominant themes consistently emerging are: (1) physicochemical and structural characteristics of OPEFB influencing bioconversion efficiency, (2) pretreatment optimization for enhanced enzymatic accessibility, (3) advances in enzymatic hydrolysis performance and enzyme system configurations, (4) microbial fermentation pathways and strain-specific productivity, (5) integrated lignocellulosic biorefinery configurations and process coupling, and (6) generation of value-added bioproducts including biofuels, biochemicals, biomaterials, and bioenergy carriers. While analytically distinct, these themes are conceptually interconnected, reflecting the sequential and interdependent nature of OPEFB valorization processes and the cumulative influence of pretreatment, enzymatic, and microbial stages on overall product yields and process efficiency. Collectively, these themes form a structured evidence base that captures both technological diversity and emerging priorities in OPEFB-based biorefinery research.

An examination of thematic prevalence indicates a non-uniform distribution of emphasis across the included studies. Structural characterization was addressed in approximately 88% of the articles (30 out of 34 studies), reflecting its foundational role in informing pretreatment selection and enzymatic accessibility. Pretreatment strategies were reported in 82% of the studies (28 articles), highlighting the central importance of optimizing lignin removal and exposing cellulose. Enzymatic hydrolysis performance and system configuration were reported in 91% of studies (31 articles), indicating a strong research focus on maximizing sugar yield and hydrolysis efficiency. Microbial fermentation pathways were analyzed in 85% of studies (29 articles), indicating widespread attention to ethanol, organic acids, and other bioproducts. Integrated biorefinery configurations were considered in 50% of studies (17 articles), reflecting growing but still emerging interest in multi-product process coupling. Value-added product diversification was explicitly addressed in 62% of studies (21 articles), showing increasing interest in producing high-value biochem-

icals, biomaterials, and bioenergy carriers beyond conventional biofuels.

The predominance of structural characterization, pretreatment, and enzymatic hydrolysis themes can be attributed to their critical role as bottlenecks in OPEFB valorization, where inefficiencies directly limit downstream microbial fermentation and product yield. In contrast, integrated biorefinery and product diversification themes, while important for industrial feasibility and economic viability, are less represented due to technological complexity and scale-up challenges. This thematic distribution implies that while foundational biochemical and enzymatic processes are well-studied, strategic integration of multi-product biorefineries and value-added outputs remains a key frontier for future research.

The next subsections detail each of the six thematic domains, integrating quantitative results, process parameters, and methodological patterns extracted from the 34 reviewed articles.

### Structural Characteristics and Recalcitrance of OPEFB

The first theme emerging from the SLR concerns the intrinsic structural properties of OPEFB and their implications for bioconversion efficiency. Crystallinity index values reported across the studies ranged from 48% to 62%, reflecting moderate cellulose crystallinity that influences enzymatic digestibility [29]. Lignin content above 20% was frequently identified as a principal barrier to enzymatic accessibility due to its shielding effect on cellulose microfibrils [30]. Fiber morphology analysis indicated average fiber lengths between 1.0–2.5 mm and surface area variability depending on mechanical preconditioning [31].

Several studies quantified the impact of lignin removal on sugar yield. Delignification levels exceeding 40% were associated with a 1.5- to 2.3-fold increase in glucose release during subsequent hydrolysis compared to untreated biomass [32]. These findings demonstrate that structural modification is a prerequisite for efficient valorization and that OPEFB's compositional profile positions it as a viable lignocellulosic feedstock when appropriate preprocessing strategies are applied.

### Pretreatment Strategies and Efficiency Enhancement

Pretreatment constituted a central theme in 28 out of 34 studies. Alkali pretreatment using sodium hydroxide concentrations between 1–4% (w/v) at temperatures of 80–120°C for 1–3 hours achieved lignin removal efficiencies of 35–55%, with cellulose recovery rates above 85% [33]. Dilute acid pretreatment employing sulfuric acid at concentrations of 0.5–2% (v/v) under temperatures of 121–160°C resulted in hemicellulose solubilization rates exceeding 60% and increased enzymatic digestibility by up to 45% relative to raw biomass [34].

Steam explosion at pressures of 1.0–1.5 MPa and residence times of 5–10 minutes generated surface disruption and porosity enhancement, contributing to glucose yields between 65–78% of the theoretical maximum during enzymatic hydrolysis. Emerging pretreatment approaches, such as biological pretreatment using white-rot fungi, have demonstrated lignin reduction of 25–38% over incubation periods of 14–28 days, albeit with longer processing times [35].

Collectively, the reviewed literature indicates that pretreatment significantly enhances enzymatic accessibility, with reported increases in total reducing sugar yield ranging from 30% to 120% compared to untreated OPEFB [36]. The selection of pretreatment strategy was frequently aligned with downstream product targets, balancing cost, environmental compatibility, and sugar recovery efficiency.

### Enzymatic Hydrolysis Performance and System Configuration

Enzymatic hydrolysis was examined in 31 of the 34 selected studies. Commercial cellulase loadings ranged from 10 to 30 FPU per gram of substrate, with optimal hydrolysis conditions typically reported at pH 4.8–5.5 and temperatures between 45–50°C [37]. Under optimized conditions, glucose yields between 70–90% of theoretical conversion were reported, particularly when pretreatment effectively reduced lignin interference.

Several studies have incorporated enzyme cocktails combining cellulase,  $\beta$ -glucosidase, and hemicellulase, resulting in 12–25% higher sugar release than single-enzyme systems [38]. Enzyme recycling strategies demonstrated retention of 60–75% catalytic efficiency after two hydrolysis cycles, suggesting

potential cost-reduction pathways for industrial implementation [39].

Reported hydrolysis durations ranged from 24 to 72 hours, with diminishing returns observed beyond 48 hours in most cases [40]. Enzymatic saccharification of OPEFB achieved total fermentable sugar concentrations between 40–85 g/L depending on solids loading (5–15% w/v) [41]. These findings confirm the technical feasibility of high-yield enzymatic conversion under controlled conditions.

### Microbial Fermentation and Bioconversion Pathways

Microbial fermentation pathways were addressed in 29 studies, with *Saccharomyces cerevisiae* being the most frequently employed ethanologenic microorganism [42]. Ethanol concentrations ranged from 18–42 g/L during batch fermentation, with yields of 0.42–0.48 g ethanol per g glucose consumed, corresponding to 82–94% of the theoretical yield [43].

Simultaneous saccharification and fermentation (SSF) configurations reduced processing time by approximately 20–30% compared to separate hydrolysis and fermentation (SHF), while maintaining comparable ethanol yields [44]. Co-culture systems incorporating xylose-fermenting strains such as *Pichia stipitis* increased the efficiency of total sugar utilization by 15–22% in hemicellulose-rich hydrolysates [45].

Beyond ethanol, microbial pathways produced lactic acid concentrations of 35–60 g/L using *Lactobacillus* species, with conversion efficiencies above 80% under optimized nutrient supplementation [46]. Polyhydroxyalkanoate (PHA) accumulation from hydrolysate substrates reached intracellular contents of 40–65% dry cell weight in selected bacterial strains [47]. Anaerobic digestion of residual solids generated biogas yields between 250–380 mL CH<sub>4</sub>/g volatile solids [48].

These data collectively indicate that microbial systems are highly adaptable to OPEFB-derived hydrolysates, enabling the generation of a diverse range of bioproducts within lignocellulosic biorefinery contexts.

### Integrated Lignocellulosic Biorefinery Configurations

Seventeen studies explicitly addressed integrated biorefinery models combining multiple product streams. Integrated configurations reported overall carbon utilization efficiencies exceeding 70% when fermentable sugars, lignin fractions, and residual solids were valorized sequentially [49]. For example, studies integrating ethanol fermentation with lignin-derived biochar production achieved mass conversion efficiencies above 65% of initial dry biomass [50].

Techno-functional assessments in the reviewed articles indicated that coupling enzymatic hydrolysis with anaerobic digestion reduced the residual organic load by 40–55%, thereby improving overall energy recovery [51,52]. Process integration strategies demonstrated potential reductions in external energy input of up to 18% when heat-integration systems were applied [53].

Several studies reported preliminary techno-economic indicators suggesting that enzyme cost contributed approximately 25–35% of operational expenditure in biochemical conversion pathways, highlighting the importance of enzyme optimization and recycling [54]. Despite variability in scale assumptions, integrated approaches consistently enhanced overall resource efficiency compared to single-product systems [55].

### Value-Added Product Diversification

The final thematic cluster concerns the diversity of value-added products generated from OPEFB. Bioethanol remained the most extensively studied output, representing 44% of the selected articles [56]. However, a significant proportion of studies explored alternative high-value products, including xylitol, levulinic acid, succinic acid, and biocomposite materials [57].

Production of nanocellulose from pretreated OPEFB achieved fibril diameters below 50 nm with tensile strength improvements of 30–45% when incorporated into polymer matrices [58]. Bio-based composite boards produced from enzymatically modified fibers exhibited mechanical properties comparable to those of conventional medium-density fiberboard [59].

Organic acid production varied with microbial strain and hydrolysate detoxification strategies, with succinic acid concentrations reaching 32–48 g/L under

optimized fermentation conditions [60]. Bioenergy recovery via combustion of residual lignin fractions yielded calorific values between 18 and 22 MJ/kg, supporting integrated energy utilization within biorefinery systems [61,62].

Overall, the data synthesis demonstrates that OPEFB valorization via enzymatic and microbial pathways supports a diversified product portfolio, with conversion efficiencies frequently exceeding 70% of theoretical yield under optimized laboratory conditions. The reviewed literature consistently positions OPEFB as a technically viable lignocellulosic feedstock for biochemical transformation when appropriate pretreatment, enzyme configuration, and microbial selection strategies are applied.

Across all 34 studies, no primary data were generated within this review; all numerical values and performance indicators derive from previously published peer-reviewed research indexed in Scopus. The systematic aggregation and thematic synthesis of these findings provide an evidence-based mapping of technological progress in enzymatic and microbial OPEFB valorization within lignocellulosic biorefinery frameworks, maintaining analytical neutrality while consolidating current scientific understanding.

## Discussion

This systematic literature review synthesized findings from 34 peer-reviewed articles published between 2019 and 2026 to critically interpret recent developments in enzymatic and microbial valorization of Oil Palm Empty Fruit Bunches (OPEFB) within lignocellulosic biorefinery systems. The discussion addresses two research questions: (RQ1) how enzymatic and microbial strategies have been applied and optimized to improve process efficiency, and (RQ2) what patterns of product diversification and system integration reveal about the technological maturity of OPEFB-based biorefineries. The analysis is entirely grounded in secondary data extracted from published experimental and analytical studies and does not incorporate primary field observations or stakeholder-based methodologies.

### **RQ1: Optimization of Enzymatic and Microbial Conversion Strategies in OPEFB Biorefineries** **Pretreatment Optimization as the Determinant of Downstream Efficiency**

The literature consistently positions pretreatment as the most influential stage affecting overall conversion performance. OPEFB typically contains 35–45% cellulose, 20–30% hemicellulose, and 18–28% lignin, forming a complex matrix that limits enzymatic accessibility without prior structural disruption [63]. Consequently, optimization strategies have largely focused on enhancing lignin removal and fiber porosity while preserving fermentable carbohydrates.

Alkaline pretreatment approaches (e.g., NaOH concentrations of 1–4% w/v) reported lignin solubilization efficiencies of 45–70%, often resulting in glucose yields exceeding 75% of theoretical conversion under optimized enzymatic conditions [64]. Acid pretreatment methods, particularly dilute sulfuric acid (0.5–2%), effectively hydrolyzed hemicellulose fractions, yielding xylose recoveries above 60%, although excessive severity generated inhibitory byproducts such as furfural and hydroxymethylfurfural.

Physicochemical techniques such as steam explosion (180–220°C, 5–10 minutes) demonstrated significant fiber defibrillation and improved enzymatic digestibility, yielding total sugar recoveries of 65%–80%, depending on reaction severity. Hydrothermal pretreatment similarly enhanced cellulose accessibility without requiring additional chemicals, contributing to more environmentally aligned processing frameworks [65].

Biological pretreatment with white-rot fungi selectively degraded lignin (20–40% reduction) under milder conditions, albeit with longer residence times of 10–21 days. Although slower, this approach minimized carbohydrate loss and reduced inhibitor formation, indicating potential for integration in low-energy or decentralized biorefinery contexts [66].

Across these approaches, optimization trends reveal a shift from maximizing delignification alone toward multi-criteria evaluation, balancing carbohydrate preservation, inhibitor mitigation, energy input, and compatibility with downstream enzymatic hydrolysis. The literature thus demonstrates increasing methodological refinement in tailoring pretreatment intensity to specific bioconversion objectives.

### **Advances in Enzymatic Hydrolysis Efficiency**

Enzymatic saccharification represents the biochemical

core of OPEFB valorization pathways. Most studies employed commercial cellulase complexes containing endoglucanase, exoglucanase, and  $\beta$ -glucosidase activities, frequently supplemented with xylanase to enhance hemicellulose breakdown [67]. Reported cellulose-to-glucose conversion efficiencies typically ranged from 60% to 85%, depending on pretreatment conditions and enzyme loading.

Optimization strategies include enzyme dosage adjustment (commonly 10–30 FPU/g substrate), which significantly influenced hydrolysis rates and final sugar concentration. Operating conditions at pH 4.8–5.5 and 45–50°C were consistently identified as optimal for maximizing catalytic activity. High-solids hydrolysis (15–20% w/v) increased sugar concentrations to above 80 g/L in several studies, reducing downstream evaporation requirements, although mixing challenges and end-product inhibition were noted at higher solids loading [68].

Some studies reported improved enzymatic efficiency through the addition of surfactants or partial lignin removal strategies, thereby reducing non-productive enzyme adsorption. Enzyme recycling and immobilization approaches demonstrated retention of 60–80% activity across multiple cycles, suggesting opportunities to reduce operational costs at scale [69]. Collectively, these findings indicate that enzymatic optimization has evolved from single-variable experimentation toward integrated parameter adjustment encompassing substrate preparation, enzyme synergy, and reactor configuration.

### Microbial Fermentation and Process Configuration Enhancements

Following saccharification, microbial fermentation pathways determine the spectrum of value-added outputs. Ethanol production via *Saccharomyces cerevisiae* remains the most extensively studied route, with reported ethanol yields frequently exceeding 85% of theoretical glucose conversion under optimized simultaneous saccharification and fermentation (SSF) conditions [70]. SSF configurations reduced process duration and mitigated glucose inhibition effects by coupling hydrolysis and fermentation within a single reactor.

Beyond ethanol, diversification toward organic acids and biopolymers has gained prominence. Lactic acid

production from OPEFB hydrolysates achieved concentrations of 40–70 g/L in controlled fermentation systems, supporting applications in biodegradable polymer synthesis [71]. Succinic acid yields ranging from 0.5 to 0.7 g/g sugar were reported in metabolically optimized bacterial systems, indicating potential as a platform chemical. Polyhydroxyalkanoate (PHA) production from OPEFB-derived sugars demonstrated polymer accumulation levels of 40–60% of cell dry weight under nutrient-limited conditions [72].

Co-culture systems capable of utilizing both hexose and pentose sugars enhanced overall carbon utilization efficiency by up to 15% compared to monoculture fermentation in several studies. Additionally, strain adaptation to tolerate inhibitors improved fermentation productivity under pretreated biomass conditions. These advancements reflect a transition toward more resilient and efficient microbial platforms within OPEFB-based biorefineries.

Taken together, the literature addressing RQ1 demonstrates progressive optimization across pretreatment, enzymatic hydrolysis, and fermentation stages. Rather than isolated improvements, recent studies increasingly adopt system-level refinement strategies to enhance overall carbon recovery, yield stability, and operational feasibility.

### RQ2: Product Diversification, Process Integration, and Technological Maturity

#### Emerging Patterns of Product Diversification

Analysis of the 34 selected articles reveals a diversification trend extending beyond single-product biofuel systems. While ethanol remains prominent, approximately half of the reviewed studies investigated alternative high-value outputs, including lactic acid, succinic acid, biohydrogen, biogas, and biodegradable polymers [73]. This pattern reflects a broader biorefinery paradigm in which multiple product streams enhance economic resilience and carbon utilization efficiency.

Lignin-rich residues were frequently directed toward energy recovery via combustion or anaerobic digestion, thereby improving the overall energy balance. Some studies have explored lignin valorization into activated carbon or aromatic intermediates, although these pathways remain less technologically mature than carbohydrate-based conversions [74].

The diversification pattern suggests growing recognition that OPEFB valorization can support integrated bio-based production rather than being solely fuel-oriented. This aligns with circular bioeconomy principles by converting agricultural residues into a portfolio of renewable products within established agro-industrial supply chains [75].

### Integration Strategies within Biorefinery Systems

Process integration emerged as a defining feature of technologically advancing OPEFB systems. Simultaneous saccharification and fermentation reduced reactor volume and shortened the process time relative to the separate hydrolysis and fermentation (SHF) configuration [76]. Combined enzymatic hydrolysis and anaerobic digestion enabled further energy recovery from residual solids, increasing overall substrate conversion efficiency.

Heat recovery and water recycling strategies were incorporated in several conceptual designs, demonstrating attention to process sustainability beyond laboratory-scale experimentation [77]. Techno-economic analyses, although limited in number, indicated that integrated multi-product systems generally outperformed single-product scenarios in projected economic viability metrics.

However, most studies remain at laboratory or pilot scale, with limited full-scale industrial demonstration. The technological readiness level (TRL) for ethanol pathways appears relatively advanced compared to biopolymer or specialty chemical production, which often remains at experimental validation stages [78].

### Indicators of Technological Maturity

The literature suggests a moderate but evolving level of technological maturity in OPEFB valorization pathways. Ethanol-focused systems demonstrate repeatable yield performance and established fermentation protocols, indicating higher readiness for scale-up [79]. In contrast, advanced biochemical production and lignin-derived product streams require further optimization, particularly in improving yield consistency and process economics.

Standardization challenges persist, including variability in solids loading, reactor configuration, and reporting metrics across studies. Such heterogeneity

complicates cross-study comparability and highlights the need for harmonized methodological frameworks [80]. Nevertheless, the shift toward integrated designs and diversified product portfolios indicates progressive maturation from proof-of-concept experimentation toward system-oriented engineering.

Importantly, the development trajectory reflected in the literature is consistent with the broader sustainability goals of palm-based agro-industrial systems. OPEFB valorization does not replace core palm oil production but complements it by enhancing residue utilization and resource efficiency within existing processing infrastructures [81].

The findings of this systematic review carry several implications. First, enzymatic and microbial valorization of OPEFB has moved beyond exploratory experimentation toward increasingly optimized and integrated biorefinery configurations. This evolution suggests tangible potential to transform lignocellulosic residues into a diverse range of bio-based products within circular agro-industrial systems.

Second, technological maturity varies across product pathways. Ethanol production demonstrates relatively higher readiness, whereas advanced biochemicals and biopolymers require further refinement in strain engineering, inhibitor tolerance, and downstream purification. Greater integration of techno-economic and life-cycle assessment frameworks would enhance decision-making regarding pathway prioritization.

Future research should therefore focus on: (1) harmonizing experimental reporting standards to improve cross-study comparability; (2) advancing metabolic engineering to enable efficient pentose utilization; (3) optimizing high-solids enzymatic systems to reduce water and energy demand; (4) expanding pilot-scale validation of integrated multi-product biorefineries; and (5) conducting comprehensive sustainability assessments linking technical performance with environmental and economic indicators.

Overall, the reviewed evidence demonstrates that enzymatic and microbial OPEFB valorization pathways are progressing toward more efficient, diversified, and system-integrated configurations. Continued interdisciplinary research and process integration will be essential to further enhance technological readiness and

support sustainable bio-based development aligned with established palm oil production systems.

### Conclusion

This systematic literature review synthesizes evidence from 34 peer-reviewed studies published between 2019 and 2026 on enzymatic and microbial valorization of Oil Palm Empty Fruit Bunches (OPEFB) within lignocellulosic biorefinery frameworks. The analysis demonstrates that enzymatic and microbial conversion strategies have been widely applied and progressively optimized to enhance process efficiency. Pretreatment approaches, including alkaline, acid, steam explosion, hydrothermal, and biological methods, consistently improve cellulose accessibility and sugar yields, with subsequent enzymatic hydrolysis achieving glucose conversion efficiencies frequently exceeding 75% under optimized conditions. Microbial fermentation pathways, particularly ethanol, lactic acid, succinic acid, and polyhydroxyalkanoate production, have demonstrated substantial adaptability, with integrated configurations such as simultaneous saccharification and fermentation (SSF) improving process throughput and mitigating inhibitory effects. Enzyme cocktails, high-solids loading, and recycling strategies further enhance operational efficiency and cost-effectiveness.

The review highlights a clear pattern of value-added product diversification emerging across OPEFB biorefinery studies. While ethanol remains the most frequently investigated output, an increasing proportion of research focuses on high-value biochemicals, biopolymers, and bioenergy carriers. Lignin-rich fractions are increasingly valorized through energy recovery, biogas production, and preliminary chemical conversion, reflecting an integrated approach to carbon utilization. Process integration strategies, including coupling hydrolysis with fermentation and valorizing residual solids, demonstrate the potential to enhance substrate conversion, reduce energy demand, and improve economic feasibility.

Technological maturity across OPEFB valorization pathways varies by product class. Ethanol-focused processes exhibit higher reproducibility and readiness for scale-up, whereas advanced biochemical and biopolymer production generally remains at laboratory or pilot scales, requiring further optimization

in enzyme efficiency, microbial tolerance, and downstream purification. The reviewed studies collectively indicate a shift from isolated unit operations toward system-level integration, suggesting progressive advancement toward more resilient and economically viable biorefinery configurations.

In summary, OPEFB represents a technically viable and sustainable lignocellulosic feedstock for enzymatic and microbial conversion. Optimized pretreatment, enzymatic hydrolysis, and microbial fermentation strategies, combined with diversified product portfolios and integrated process designs, enable efficient utilization of this agro-industrial residue. These findings underscore the potential for OPEFB valorization to contribute meaningfully to circular bioeconomy frameworks, supporting resource-efficient and sustainable development within palm oil processing systems.

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