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Journal of Sustainable Agriculture and Biosystems Engineering
Journal homepage: https://talenta.usu.ac.id/jsabe

Potential of Redox Control in Microbial Fermentation: A Mini Review

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ARTICLE INFO

Article history:
Received 28 July 2025
Revised 03 June 2026
Accepted 15 June 2026
Available online

E-ISSN: 3026-4065

How to cite:
Tarigan J and Raju, "Potential of Redox Control in Microbial Fermentation: A Mini Review," Journal of Sustainable Agriculture and Biosystem Engineering, Vol. 04, No. 1, 2026.

http://doi.org/10.32734/jsabe.v4i01.22270

ABSTRACT

The manipulation of oxidation–reduction potential (ORP) has emerged as a pivotal strategy for optimizing microbial activity and hydrogen yields in anaerobic fermentation processes. This review systematically analyzes open-access research articles published between 2020 and 2025 that investigate the role of ORP in dark fermentation and biohydrogen production. By focusing on 16S rRNA-based microbial profiling, metabolic outcomes, and ORP-controlled operational strategies, we highlight how specific ORP levels selectively enrich hydrogen-producing genera such as *Clostridium* and suppress less efficient or competing microbes like *Veillonellaceae* or *Enterobacteriaceae*. Optimal ORP ranges (e.g., –284 to –322 mV) were consistently associated with increased H_2 productivity (30–70%) and metabolic shifts favoring acetate and butyrate pathways. Chemical agents such as ferricyanide and H_2O_2 were frequently employed to modulate ORP, influencing microbial redox balance and community stability. Additionally, the review discusses the mechanisms linking redox state to electron flow, enzyme activation, and microbial dominance. Despite recent advances, significant research gaps remain in scaling ORP control strategies, standardizing ORP setpoints for various substrates, and integrating real-time ORP monitoring in bioreactors. We conclude that ORP is not merely a passive indicator but a controllable parameter that can guide microbial ecology and improve fermentative hydrogen production. This review provides a foundation for future research on coupling redox management with microbial engineering to design more efficient, resilient, and predictable biohydrogen systems.

Keyword: biohydrogen production, dark fermentation, fermentation, microbial activity, oxidation–reduction potential (ORP)

1. Introduction

Oxidation–Reduction Potential (ORP), also referred to as redox potential, is a thermodynamic parameter that describes the tendency of a chemical or biological system to either donate or accept electrons during oxidation–reduction reactions [1]. In microbial fermentation systems, ORP serves not only as an indicator of oxidative or reductive conditions, but also as a critical factor influencing microbial metabolism, enzymatic activity, intracellular electron transfer, and metabolic pathway regulation [2]. Because microbial fermentation fundamentally relies on electron exchange processes, changes in ORP can significantly alter microbial physiological behavior and product formation patterns. Consequently, ORP has increasingly gained attention as an important operational parameter in anaerobic biotechnology and waste-to-energy conversion systems, particularly in dark fermentation processes for biohydrogen production [3].

Fermentation technology has long been recognized as one of the most important biological processes for converting organic substrates into valuable products such as biofuels, organic acids, alcohols, and biochemicals [4]. The growing global concern regarding fossil fuel depletion, climate change, and greenhouse gas emissions has intensified research efforts toward sustainable and renewable energy technologies [5]. Among various renewable energy options, biohydrogen has emerged as a promising alternative energy carrier because of its high energy content and environmentally friendly combustion products, which mainly produce water vapor without carbon emissions [6]. Compared with conventional fossil fuels, hydrogen possesses superior gravimetric energy density and can potentially contribute to achieving carbon neutrality in future energy systems [7].

Dark fermentation has become one of the most extensively investigated methods for biological hydrogen production due to its relatively low energy requirement, rapid hydrogen generation rate, and ability to utilize a wide range of organic substrates, including food waste, agricultural residues, industrial wastewater, and lignocellulosic biomass [8]. In addition, dark fermentation can operate independently of light energy and is generally considered more practical for large-scale applications than photobiological hydrogen production systems. Nevertheless, the efficiency of dark fermentation remains strongly dependent on microbial activity and environmental conditions, which determine the stability of fermentation pathways and the composition of fermentation end-products.

Several operational parameters are known to affect microbial fermentation performance, including pH, temperature, substrate concentration, hydraulic retention time, nutrient availability, inoculum pretreatment, and mixing intensity [9]. Among these parameters, ORP has recently attracted increasing interest because it directly reflects the electron balance within the fermentation environment [10]. Since anaerobic microorganisms rely heavily on electron transfer reactions to maintain metabolic activity, ORP conditions strongly influence microbial community structure and metabolic pathway selection. In anaerobic fermentation systems, highly reducing conditions generally promote hydrogen-producing pathways, whereas less reductive environments may favor competing pathways such as methanogenesis, solventogenesis, or lactate production.

The redox environment is considered one of the most influential ecological factors governing microbial community composition and microbial interactions in anaerobic bioreactors [11]. Hydrogen-producing fermentative bacteria such as *Clostridium* spp. commonly dominate under highly reducing conditions, often below −300 mV, where hydrogenase enzymes can function optimally for proton reduction and hydrogen evolution [12]. Under such conditions, acetate and butyrate fermentation pathways are typically favored, resulting in higher hydrogen yields and more efficient substrate conversion. Conversely, under mildly reducing or unstable ORP conditions, methanogenic archaea and hydrogen-consuming microorganisms may become dominant, reducing hydrogen productivity through hydrogen consumption and methane formation [13]. The competition among microbial groups within different ORP environments demonstrates the importance of redox regulation in controlling microbial metabolism and product selectivity.

In addition to influencing microbial community dynamics, ORP also affects intracellular biochemical processes. Variations in ORP can alter the $NADH/NAD^+$ ratio, electron carrier availability, ATP generation efficiency, and enzymatic activity associated with fermentation metabolism. Changes in cellular redox balance may subsequently modify carbon flux distribution toward different metabolic products, including hydrogen, ethanol, volatile fatty acids, methane, and solvents. Therefore, ORP regulation provides opportunities not only for improving hydrogen production efficiency but also for directing fermentation pathways toward desired end-products.

Despite its significant physiological and operational importance, ORP has not yet been widely implemented as a primary control parameter in industrial-scale fermentation systems [14]. In most fermentation studies and industrial applications, operational optimization primarily focuses on parameters such as pH, temperature, organic loading rate, and substrate composition, whereas ORP is often monitored only as a secondary or observational parameter. This limited application may partly result from the complexity of microbial redox interactions and the lack of comprehensive understanding regarding optimal ORP ranges for different fermentation systems.

Nevertheless, recent studies have increasingly demonstrated the advantages of active ORP regulation in improving anaerobic fermentation performance. Various approaches have been explored to manipulate ORP conditions, including the addition of reducing agents, oxidizing agents, electrode-assisted systems, and electro-fermentation technologies. Maintaining ORP within specific reductive ranges has been shown to enhance hydrogen production, stabilize microbial activity, suppress methanogenesis, and improve overall process efficiency by selectively enriching hydrogen-producing microbial communities. In some studies, ORP regulation has also been associated with shorter lag phases, improved substrate degradation efficiency, and enhanced tolerance against environmental stress conditions.

Beyond biohydrogen production, ORP has also been investigated in other fermentation-related fields, including wastewater treatment, anaerobic digestion, food fermentation, and biochemical production systems. In wastewater treatment processes, ORP monitoring is frequently used to evaluate anaerobic digestion performance and to control nitrification–denitrification pathways. In food and beverage fermentation, ORP influences microbial succession, flavor compound formation, and product quality stability. These findings indicate that ORP has broad applicability as a universal monitoring and control parameter in microbial biotechnology.

The increasing integration of fermentation technology within circular economy and waste-to-energy frameworks further emphasizes the importance of ORP research. Organic wastes generated from agriculture, households, food industries, and agro-industrial processes contain substantial amounts of biodegradable carbon that can potentially be converted into renewable energy and value-added bioproducts through microbial fermentation. Optimizing ORP conditions may improve the efficiency of waste conversion processes while simultaneously reducing environmental pollution and greenhouse gas emissions. Therefore, understanding the relationship between ORP, microbial ecology, and metabolic regulation is essential for developing sustainable and economically viable bioenergy technologies.

Although numerous studies have investigated ORP in microbial fermentation systems, the existing literature remains fragmented and highly diverse in terms of experimental conditions, substrates, microbial consortia, and reactor configurations. Many studies focus only on reactor performance or hydrogen yield without comprehensively examining the interactions among ORP, microbial community dynamics, and metabolic pathways. Furthermore, inconsistencies in ORP measurement methods and operational conditions make it difficult to establish generalized conclusions regarding optimal ORP ranges for specific fermentation systems.

A comprehensive and systematic synthesis of current research is therefore needed to better understand the role of ORP in microbial fermentation processes and to evaluate its potential as a controllable operational parameter for sustainable bioenergy production. Such understanding is important not only for improving hydrogen production efficiency but also for advancing the broader application of fermentation technologies in waste valorization and renewable energy development.

Although several reviews have discussed ORP regulation and biohydrogen production, most previous studies focused primarily on reactor performance, hydrogen yield, and operational optimization. Limited attention has been given to the role of ORP as a microbial selection factor that shapes microbial community structure and metabolic pathway regulation. Furthermore, recent advances in microbial community profiling and molecular techniques have generated new evidence regarding ORP-driven microbial dominance, yet findings published between 2020 and 2025 remain fragmented across different fermentation systems. Therefore, a focused synthesis of recent studies is needed to integrate current knowledge regarding ORP regulation, microbial ecology, and fermentation performance.

2. Methodology

This study employed a systematic literature review approach to examine the role of Oxidation–Reduction Potential (ORP) in microbial activities during fermentation processes, particularly in relation to biohydrogen production and anaerobic fermentation systems. The review was conducted through a structured and stepwise procedure to ensure a comprehensive, transparent, and reproducible selection of relevant scientific literature.

Relevant literature was retrieved from the Elsevier database ScienceDirect. ScienceDirect was selected because the objective of this review was to focus on peer-reviewed experimental studies related to ORP-controlled fermentation systems. The database provides extensive coverage of biotechnology, renewable energy, and fermentation research while ensuring consistency in article retrieval and screening. To improve the specificity and accuracy of the literature search, Boolean operators were applied using several combinations of keywords associated with ORP and microbial fermentation processes. The primary search terms included “ORP”, “oxidation–reduction potential”, “microbial activity”, and “fermentation”. These keywords were combined using Boolean operators such as AND and OR to broaden or narrow the search scope appropriately. Examples of search queries included: (“ORP” AND “microbial activity” AND “fermentation”) and (“oxidation-reduction potential” AND “biohydrogen production”). The literature search covered publications published between 2020 and July 2025 in order to focus on recent scientific developments and current technological trends in ORP-controlled fermentation systems.

The inclusion criteria applied in this study were: (1) original research articles published between 2020 and July 2025; (2) studies investigating the relationship between ORP and microbial activity in fermentation systems; (3) articles discussing ORP regulation, microbial community dynamics, fermentation performance, or biohydrogen production; (4) studies available in full-text format; and (5) publications written in English. Meanwhile, the exclusion criteria consisted of: (1) review papers, book chapters, conference abstracts, and articles lacking detailed methodologies; (2) studies unrelated to fermentation processes, such as ORP applications in soils, aquaculture, corrosion studies, or electrochemical sensor development without fermentation context; (3) duplicate publications; and (4) articles with insufficient experimental or analytical information relevant to the objectives of this review.

The article selection process was conducted through several stages, including identification, screening, eligibility assessment, and final inclusion. Initially, a total of 1,088 documents were identified from the database search. The titles and abstracts of these articles were subsequently screened to evaluate their relevance to the research topic. Articles that clearly did not meet the inclusion criteria were excluded during the preliminary screening stage. The remaining studies then underwent full-text assessment to determine their eligibility based on the established criteria. After a comprehensive evaluation process, five articles were ultimately selected for qualitative synthesis and detailed analysis.

The selected articles were analyzed descriptively to identify the role of ORP in microbial fermentation systems, including its influence on microbial activity, metabolic pathways, fermentation efficiency, hydrogen production performance, and microbial community dynamics. Information extracted from the selected studies included substrate type, fermentation conditions, ORP range, microbial characteristics, and observed fermentation outcomes. The findings from the selected studies were subsequently synthesized to evaluate current research trends, identify knowledge gaps, and assess the potential of ORP as a controllable operational parameter in sustainable fermentation and waste-to-energy applications.

3. Result and Discussion

3. 1 Overview of Selected Studies

The five selected studies consistently demonstrate that Oxidation–Reduction Potential (ORP) is a critical operational parameter influencing microbial activity, metabolic pathways, and fermentation performance across various fermentation systems. Although the studies investigated different substrates, fermentation objectives, and microbial inocula, all findings indicate that ORP strongly affects microbial metabolism and determines the direction of product formation during fermentation processes.

The first study [15] investigated continuous hydrogen production from glycerol using acidogenic sludge under controlled ORP conditions. The researchers reported that increasingly reductive conditions, reaching approximately −540 mV, significantly enhanced hydrogen production and promoted the enrichment of hydrogen-producing Clostridium species. The study suggested that ORP regulation could improve both microbial activity and fermentation stability through selective microbial enrichment.

The second study [16] focused on wine fermentation and evaluated the effects of controlled oxygenation on fermentation kinetics and product quality. Increased ORP values accelerated alcoholic fermentation and improved oxidative stability while reducing browning susceptibility. The study demonstrated that ORP modification may influence not only fermentation performance but also volatile compound formation and sensory characteristics.

Table 1. Overview of Reviewed Studies

Fermentation Type	Substrate and Microorganisms	Controlled ORP	Key Findings	Main Insights
Dark fermentation [15]	Glycerol; sludge (dominant *Clostridium*)	~–540 mV (via H_2O_2 & ferricyanide)	H_2 yield increased by 50–70%	Oxidizing ORP altered microbial diversity and metabolic pathways
Alcoholic fermentation (wine) [16]	Grape must; native yeast	+169 mV (Ox); +150 mV (no-Ox)	Faster fermentation, improved sensory and oxidative stability	Oxygen addition regulated redox potential and volatile profile
Dark fermentation [17]	Glucose; *Clostridium* (heat-treated)	–220 to –410 mV; optimal at –284 mV	H_2 yield and productivity sharply increased at –284 Mv	Optimal ORP boosted H_2 and *Clostridium* dominance
Dark fermentation [18]	Synthetic food waste; *C. butyricum*	–322 ±8 mV	H_2 yield increased by 57%, productivity by 30%	Lower ORP suppressed unwanted microbes and enhanced H_2 pathways
Butanol fermentation [19]	Glucose; *C. acetobutylicum SE25*	–400 mV (using DTT & ferricyanide)	Butanol increased by 10.8%; carbon flux directed toward butanol	ORP regulated $NADH/NAD^+$ and redistributed metabolic flux

The third study [17] examined batch dark fermentation using glucose under different initial ORP conditions. The results showed that an ORP near −284 mV produced the highest hydrogen yield and favored the dominance of Clostridium species. Similarly, the fourth study [18] reported that maintaining ORP at approximately −322 ± 8 mV during food waste fermentation significantly improved hydrogen productivity and promoted the growth of Clostridium butyricum.

The fifth study [19] investigated ORP regulation during butanol fermentation by Clostridium acetobutylicum. The researchers maintained the fermentation broth at approximately −400 mV using redox-modifying agents and achieved higher butanol production. The study also demonstrated that ORP influenced intracellular redox balance and carbon flux distribution toward solvent production.

Overall, the selected studies indicate that ORP functions not only as a monitoring parameter but also as an active regulator capable of influencing microbial ecology, metabolic direction, and fermentation efficiency. These findings highlight the importance of ORP optimization in both bioenergy production and industrial fermentation applications.

3.2 Redox Potential Control Agents in Fermentation Systems

Redox-controlling agents play an important role in regulating Oxidation–Reduction Potential (ORP) during microbial fermentation processes. Since ORP reflects the electron balance within the fermentation system, the addition of oxidizing or reducing compounds can directly influence microbial metabolism, intracellular electron transfer, and metabolic pathway selection [20, 21]. In anaerobic fermentation systems, maintaining an appropriate redox balance is essential for sustaining the regeneration of electron carriers such as ferredoxin and NADH, which are strongly associated with hydrogen and solvent production pathways [22].

Highly reducing ORP conditions, generally around −300 mV or lower, promote hydrogenase enzyme activity and favor hydrogen-producing metabolic pathways [23]. In contrast, less reductive or oxidative conditions may redirect metabolism toward alternative products such as lactate, ethanol, acetate, or methane. Therefore, ORP manipulation provides a practical strategy for directing microbial metabolism toward desired fermentation products.

Several studies have explored the application of redox-controlling agents such as potassium ferricyanide, hydrogen peroxide (H_2O_2), dithiothreitol (DTT), and cysteine to regulate ORP during fermentation [24]. These compounds function either as oxidizing or reducing agents depending on the targeted ORP range and fermentation objective. The use of external redox agents has been shown to improve microbial stability, regulate electron flow, and enhance fermentation efficiency by maintaining more favorable metabolic conditions [25].

The selected studies demonstrated several different strategies for ORP regulation. In Study [15], hydrogen peroxide and potassium ferricyanide were used to maintain ORP at approximately −540 mV during glycerol fermentation. This strategy significantly improved hydrogen production and shifted microbial populations toward hydrogen-producing microorganisms. Similarly, Study [19] used DTT and potassium ferricyanide to maintain ORP near −400 mV during butanol fermentation, resulting in increased butanol production and improved intracellular redox balance.

In contrast, Studies [17] and [18] investigated fermentation systems without the addition of explicit redox-modifying agents. Instead, the researchers evaluated naturally occurring ORP dynamics during fermentation and identified optimal ORP windows for hydrogen production. These findings suggest that microbial metabolism remains highly sensitive to ORP even without direct chemical regulation.

Meanwhile, Study [16] demonstrated that controlled oxygen supplementation indirectly altered ORP conditions during wine fermentation. Although this system differed from dark fermentation, the study still confirmed that ORP modification could significantly influence fermentation kinetics, oxidative stability, and biochemical product profiles.

The importance of redox-controlling agents becomes even more relevant in fermentation systems involving complex substrates such as food waste, lignocellulosic biomass, or mixed microbial cultures [26, 27]. These systems often experience unstable electron balance and fluctuating microbial activity, which may reduce fermentation efficiency [28]. Through ORP regulation, researchers can suppress undesirable microbial pathways, stabilize microbial communities, and improve process selectivity toward target products.

Overall, studies involving direct ORP regulation demonstrated stronger control over microbial metabolism and product formation compared with systems relying solely on natural ORP fluctuations. These findings support the growing potential of ORP engineering as a practical strategy for improving fermentation efficiency in both bioenergy and industrial biotechnology applications.

3.3 The Effect of Redox Potential on Microbial Dominance

Oxidation–Reduction Potential (ORP) strongly influences microbial community structure and microbial dominance during anaerobic fermentation processes. Changes in ORP conditions directly affect the growth of specific microbial groups because different microorganisms possess distinct metabolic preferences and redox tolerances [3]. In dark fermentation systems, highly reducing conditions generally favor hydrogen-producing obligate anaerobes, particularly Clostridium species, whereas less reductive environments may support the growth of competing microorganisms associated with lower hydrogen productivity.

Several studies consistently demonstrated that low ORP conditions promote the dominance of Clostridium spp. and improve hydrogen production performance. Study [15] showed that maintaining ORP near −540 mV significantly increased the abundance of Clostridium species while reducing the population of Veillonellaceae, a microbial group associated with lower hydrogen yields. This finding indicates that strongly reducing conditions selectively enrich microorganisms possessing active hydrogenase enzymes and efficient hydrogen-producing pathways.

Similarly, Study [17] reported that an ORP of approximately −284 mV enhanced hydrogen production during glucose fermentation and promoted the dominance of Clostridium populations. Under these conditions, acetate and butyrate fermentation pathways became more favorable, both of which are strongly associated with hydrogen generation through [FeFe]-hydrogenase activity. When ORP conditions become less reductive, the activity of these enzymes decreases, allowing non-hydrogen-producing microorganisms such as Lactobacillus or Enterobacteriaceae to compete more effectively.

Study [18] further confirmed the importance of ORP in microbial selection during food waste fermentation. Maintaining ORP at approximately −322 ± 8 mV promoted the growth of Clostridium butyricum while suppressing microorganisms associated with ethanol, lactate, and methane production. This result suggests that ORP functions as both an environmental selection factor and a metabolic regulator capable of directing substrate conversion toward hydrogenogenic pathways.

At the cellular level, ORP affects the expression and activity of several redox-sensitive enzymes, including hydrogenases, dehydrogenases, and proton-translocating proteins [29]. Under highly reducing conditions, Clostridium species can efficiently remove excess electrons through hydrogen evolution, thereby maintaining intracellular redox balance. In contrast, higher ORP conditions may generate oxidative stress, which suppresses obligate anaerobes and favors facultative microorganisms such as Pseudomonas that possess greater metabolic flexibility [30].

The reviewed studies collectively indicate that ORP is one of the most important ecological parameters controlling microbial community composition during fermentation. By regulating ORP within an appropriate reductive range, microbial competition can be directed toward hydrogen-producing microorganisms while suppressing competing pathways associated with non-target metabolites. Therefore, ORP regulation not only improves fermentation efficiency but also serves as an effective strategy for microbial selection and metabolic pathway control in biohydrogen production systems.

3.4 Research Gaps and Future Directions

Although the reviewed studies have significantly improved the understanding of Oxidation–Reduction Potential (ORP) regulation in microbial fermentation systems, several important scientific and technological limitations remain unresolved. These limitations restrict the broader implementation of ORP-controlled fermentation technologies, particularly for industrial-scale bioenergy and waste-to-energy applications. Future research is therefore required to address microbiological, biochemical, engineering, and operational challenges associated with ORP regulation.

One of the major research gaps involves the limited understanding of microbial community dynamics under different ORP conditions. Most existing studies focus primarily on identifying optimal ORP ranges for hydrogen or solvent production while providing only limited information regarding temporal microbial succession during fermentation. In practice, microbial populations continuously change throughout fermentation due to substrate depletion, metabolite accumulation, pH variation, and environmental stress. These microbial shifts may strongly affect fermentation stability, hydrogen productivity, and by-product formation. However, many studies only analyze microbial communities at the end of fermentation, making it difficult to understand how ORP influences microbial interactions throughout the entire process. Future studies should therefore incorporate real-time microbial monitoring approaches such as metagenomics, metatranscriptomics, and high-throughput sequencing to better evaluate the dynamic relationship between ORP and microbial ecology.

Another important limitation concerns the incomplete understanding of intracellular biochemical mechanisms associated with ORP regulation. Although several studies demonstrated that ORP affects hydrogenase activity, electron transfer, NADH/NAD^+ balance, ATP generation, and carbon flux distribution, the underlying molecular pathways remain insufficiently explored. Redox-sensitive enzymes and intracellular electron carriers play central roles in microbial metabolism, yet their responses to ORP fluctuations are still poorly characterized. More detailed biochemical investigations involving metabolomics, proteomics, transcriptomics, and metabolic flux analysis are needed to clarify how microorganisms regulate their metabolic pathways under different redox environments. Understanding these intracellular mechanisms is essential for improving process optimization and developing more efficient fermentation systems.

The majority of current ORP studies are also limited to laboratory-scale fermentation systems using relatively simple substrates such as glucose, glycerol, or synthetic food waste. However, industrial fermentation systems generally utilize heterogeneous and compositionally complex feedstocks including agricultural residues, lignocellulosic biomass, industrial wastewater, municipal food waste, and agro-industrial by-products. These substrates contain varying concentrations of carbohydrates, proteins, lipids, inhibitory compounds, and mineral components that may significantly affect redox balance and microbial behavior. Consequently, fermentation performance under real industrial conditions may differ substantially from laboratory observations. Future research should therefore evaluate ORP regulation using real waste streams and complex substrates under more realistic operational conditions.

Another critical issue is the limited number of studies investigating ORP regulation in continuous and pilot-scale fermentation systems. Most currently available studies rely on short-term batch fermentation experiments that do not accurately represent long-term industrial operations. Continuous fermentation systems experience dynamic changes in substrate loading, microbial populations, metabolite accumulation, and ORP fluctuations over extended operational periods. Therefore, maintaining stable ORP conditions becomes considerably more challenging at larger scales. Future research should focus on pilot-scale bioreactors equipped with automated ORP monitoring and feedback control systems to evaluate long-term operational stability and industrial feasibility.

Engineering challenges associated with ORP monitoring systems also require further attention. ORP sensors used in fermentation systems are often susceptible to fouling, calibration drift, signal instability, and electrode degradation, especially under highly anaerobic and particle-rich conditions. These technical limitations may reduce measurement accuracy and affect process control efficiency. Furthermore, large-scale bioreactors may exhibit uneven ORP distribution due to mixing limitations and reactor hydrodynamics, creating heterogeneous microbial environments within the reactor. Future studies should therefore investigate sensor optimization, reactor design, mixing strategies, and automated process control systems to improve the reliability of ORP-based fermentation operations.

Economic and environmental aspects of ORP regulation also remain insufficiently explored. Although many studies report increased hydrogen or butanol production under controlled ORP conditions, relatively few studies evaluate the economic feasibility of applying ORP control at industrial scale. The use of redox-controlling agents such as potassium ferricyanide, hydrogen peroxide, dithiothreitol, and cysteine may increase operational costs and introduce additional downstream treatment requirements. Some of these chemicals may also pose environmental concerns if used in large quantities. Future research should therefore include techno-economic analysis and life cycle assessment to determine the sustainability and practical applicability of ORP-controlled fermentation technologies.

The sustainability of chemical redox agents represents another important research concern. Continuous addition of external oxidizing or reducing compounds may not always be environmentally or economically sustainable for industrial applications. As a result, alternative ORP regulation strategies should be explored. Emerging technologies such as electro-fermentation, bioelectrochemical systems, microbial electrolysis cells, and renewable-energy-assisted ORP control may offer more sustainable and energy-efficient approaches for regulating microbial redox conditions without excessive chemical consumption.

Another promising future direction involves integrating ORP control with advanced microbial engineering approaches. Most reviewed studies relied on naturally occurring microbial consortia or conventional inoculum pretreatment techniques. However, advances in synthetic biology and metabolic engineering may enable the development of microbial strains with enhanced tolerance to redox fluctuations, improved hydrogenase activity, and greater product selectivity. Combining ORP optimization with engineered microbial systems could substantially improve fermentation efficiency and process stability.

The integration of artificial intelligence (AI), machine learning, and smart bioreactor technologies also offers significant future potential. Real-time monitoring and predictive modeling based on ORP data may allow automated optimization of fermentation conditions and early detection of process instability. Integrating ORP monitoring with other operational parameters such as pH, dissolved gas concentration, conductivity, temperature, and metabolite accumulation could contribute to the development of intelligent fermentation systems with improved control accuracy and operational efficiency.

Research on ORP regulation in food and beverage fermentation systems remains relatively limited compared with biohydrogen production studies. Existing studies mainly focus on wine fermentation, where ORP influences oxidative stability, fermentation kinetics, and flavor formation. However, the potential application of ORP control in other food fermentation systems such as dairy fermentation, cocoa fermentation, soybean fermentation, probiotic production, and fermented beverages remains underexplored. Future interdisciplinary studies involving microbiology, food chemistry, and biochemical engineering may reveal broader industrial applications of ORP optimization.

Finally, despite growing evidence regarding the importance of ORP in microbial fermentation, there is still no universally accepted ORP range applicable across different fermentation systems. The optimal ORP condition appears to depend strongly on substrate characteristics, microbial composition, reactor configuration, fermentation objectives, and operational conditions. Therefore, future research should focus on developing more standardized ORP control frameworks while still considering the unique characteristics of individual fermentation systems.

Overall, ORP regulation represents a highly promising strategy for improving microbial fermentation performance and directing metabolic pathways toward valuable bio-based products such as hydrogen, butanol, and organic acids. However, further integration of microbiology, biochemical engineering, process automation, sustainability assessment, and industrial process design is necessary to fully realize the industrial potential of ORP-controlled fermentation systems. Continued research in this area may contribute significantly to the development of more efficient, stable, and sustainable bioenergy and waste-to-energy technologies in the future.

4. Conclusion.

Oxidation–Reduction Potential (ORP) is a critical yet often overlooked parameter in regulating microbial activity and metabolic pathways in fermentation systems, particularly for biohydrogen production. Evidence indicates that maintaining ORP within optimal reductive conditions (approximately −280 to −400 mV) significantly enhances hydrogen productivity by promoting hydrogen-producing bacteria such as *Clostridium* spp. while simultaneously suppressing competing pathways, including methanogenesis. These findings demonstrate that ORP is not merely a monitoring parameter but a strategic operational variable capable of steering microbial consortia and metabolic flux toward more efficient biohydrogen generation. Consequently, proper ORP management presents strong potential for improving fermentation performance and advancing sustainable bioenergy production.

References

[1] A. Copeland and D. Lytle, "Measuring the oxidation–reduction potential of important oxidants in drinking water," *Journal - American Water Works Association*, vol. 106, no. 1, pp. E1–E10, 2014.

[2] C. G. Liu, C. Xue, Y.H. Lin, and F. Bai, "Redox potential control and applications in microaerobic and anaerobic fermentations," *Biotechnology Advances*, vol. 31, no. 2, pp. 318–334, 2013.

[3] A. Vesga-Baron, C. Etchebehere, M. C. Schiappacasse, R. Chamy, and E. Tapia-Venegas, "Controlled oxidation-reduction potential on dark fermentative hydrogen production from glycerol: Impacts on metabolic pathways and microbial diversity of an acidogenic sludge," *International Journal of Hydrogen Energy*, vol. 46, no. 16, pp. 10665–10674, 2021.

[4] C. G. Liu, J. C. Qin, and Y. H. Lin, "Fermentation and Redox Potential," in *Redox - Principles and Advanced Applications*, InTechOpen, 2017.

[5] D. Lipson, T. Raab, M. Parker, S. Kelley, C. J. Brislawn, and J. Jansson, "Changes in microbial communities along redox gradients in polygonized Arctic wet tundra soils," *Environmental Microbiology Reports*, vol. 7, no. 4, pp. 601–609, 2015.

[6] X. Y. Sim, J. Tan, N. He, S. Yeap, Y. W. Hui, A. A. Luthfi, S. Manaf, N. A. Bukhari, and N. S. Jamali, "Unraveling the effect of redox potential on dark fermentative hydrogen production," *Renewable and Sustainable Energy Reviews*, vol. 182, p. 113755, 2023.

[7] A. J. M. Stams, C. M. Plugge, F. A. M. de Bok, B. H. G. W. van Houten, P. N. L. Lens, H. Dijkman, and J. Weijma, "Metabolic interactions in methanogenic and sulfate-reducing bioreactors," *Water Science and Technology*, vol. 52, no. 1–2, pp. 13–20, 2005.

[8] A. Spagni, J. Buday, P. Ratini, and G. Bortone, "Experimental considerations on monitoring ORP, pH, conductivity and dissolved oxygen in nitrogen and phosphorus biological removal processes," *Water Science and Technology*, vol. 44, no. 2–3, pp. 187–194, 2001.

[9] D. Alwazeer, "Importance of consideration of oxidoreduction potential as a critical quality parameter in food industries," *Food Research International*, vol. 137, p. 109108, 2020.
[10] X. Zhuge, J. Li, H.-D. Shin, L. Liu, G. Du, and J. Chen, "Improved propionic acid production with metabolically engineered *Propionibacterium jensenii* by an oxidoreduction potential-shift control strategy," *Bioresource Technology*, vol. 175, pp. 251–257, 2015.
[11] S. Hirano, N. Matsumoto, M. Morita, K. Sasaki, K. Sasaki, and N. Ohmura, "Electrochemical control of redox potential affects methanogenesis of the hydrogenotrophic methanogen *Methanothermobacter thermautotrophicus*," *Letters in Applied Microbiology*, vol. 56, no. 1, pp. 53–59, 2013.
[12] J.-x. Song, D. An, N. Ren, Y. Zhang, and Y. Chen, "Effects of pH and ORP on microbial ecology and kinetics for hydrogen production in continuously dark fermentation," *Bioresource Technology*, vol. 102, no. 22, pp. 10852–10858, 2011.
[13] S. Bhatia *et al*., "Renewable biohydrogen production from lignocellulosic biomass using fermentation and integration of systems with other energy generation technologies," *Science of the Total Environment*, vol. 765, p. 144429, 2021.
[14] S. Mohan and A. Pandey, "Sustainable Hydrogen Production," in *Biohydrogen*, 1st ed., Elsevier, pp. 1–20, 2019.
[15] A. Vesga-Baron, C. Etchebehere, M. C. Schiappacasse, R. Chamy, and E. Tapia-Venegas, "Controlled oxidation-reduction potential on dark fermentative hydrogen production from glycerol: Impacts on metabolic pathways and microbial diversity of an acidogenic sludge," *Int. J. Hydrogen Energy*, vol. 46, no. 10, pp. 7499–7508, 2020.
[16] I. Benucci, M. Cerreti, and M. Esti, "Dosing oxygen from the early stages of white winemaking: Effect on oxidation–reduction potential, browning stability, volatile composition, and sensory properties," *Food Chem.*, vol. 432, p. 137243, 2024.
[17] A. R. Fleitas García, J.-S. Guez, P. Fontanille, and G. Christophe, "Enhanced dark fermentative hydrogen production by initial reduction of oxidation-reduction potential," *Int. J. Hydrogen Energy*, vol. 87, pp. 1338–1346, 2024.
[18] A. R. Fleitas García, J.-S. Guez, C. G. Dussap, P. Fontanille, and G. Christophe, "Effect of redox potential on biohydrogen production during dark fermentation of food wastes in bioreactor," *Int. J. Hydrogen Energy*, vol. 141, pp. 1199–1210, 2025.
[19] Z. Y. Zhou, Y. Jing, S. Wei, Q. Zhang, S.-y. Peng, X. An, and H. Li, "Enhancement of butanol production in *Clostridium acetobutylicum* SE25 through oxidation-reduction potential regulation and analysis of its metabolic mechanisms," *Fuel*, vol. 341, p. 121345, 2023.
[20] P. Arbter, N. Widderich, T. Utesch, Y. Hong, and A. Zeng, "Control of redox potential in a novel continuous bioelectrochemical system led to remarkable metabolic and energetic responses of *Clostridium pasteurianum* grown on glycerol," *Microb. Cell Fact.*, vol. 21, no. 1, p. 178, 2022.
[21] M. Saito and Y. Magara, "Removal of organic pollutants and metabolic adaptation of microorganisms by micro-aeration," *J. Environ. Sci. Health A*, vol. 38, no. 7, pp. 1331–1341, 2003.
[22] G. Schut and M. Adams, "The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production," *J. Bacteriol.*, vol. 191, no. 15, pp. 4451–4457, 2009.
[23] M. Akiyama and T. Osanai, "Regulation of organic acid and hydrogen production by NADH/NAD^+ ratio in *Synechocystis* sp. PCC 6803," *Front. Microbiol.*, vol. 14, p. 1332449, 2024.
[24] W. Ping, "Effect of redox potential regulation on the succinic acid production by anaerobic fermentation of *Actinobacillus succinogenes*," *Chem. Ind. Eng. Prog.*, vol. 27, no. 8, 2008.
[25] C. Zerfaß, J. Chen, and O. S. Soyer, "Engineering microbial communities using thermodynamic principles and electrical interfaces," *Curr. Opin. Biotechnol.*, vol. 50, pp. 121–127, 2018.
[26] J. Yin, X. Yu, Y. Zhang, D. Shen, M. Wang, Y. Long, and T. Chen, "Enhancement of acidogenic fermentation for volatile fatty acid production from food waste: Effect of redox potential and inoculum," *Bioresour. Technol.*, vol. 216, pp. 996–1003, 2016.
[27] V. P. Soudham, B. Alriksson, and L. Jönsson, "Pretreatment liquid," *J. Biotechnol.*, vol. 153, no. 1–2, pp. 132–137, 2011.
[28] J. Nelson, R. Coleman, L. Chacón-Rodríguez, R. C. Runnebaum, R. Boulton, and A. Knoesen, "Advanced monitoring and control of redox potential in wine fermentation across scales," *Fermentation*, vol. 9, no. 1, p. 7, 2022.
[29] K. Arunasri, D. K. Yeruva, K. V. Krishna, and S. V. Mohan, "Monitoring metabolic pathway alterations in *Escherichia coli* due to applied potentials in microbial electrochemical system," *Bioelectrochemistry*, vol. 134, p. 107530, 2020.

[30] J. A. Ciemniecki and D. Newman, “The potential for redox-active metabolites to enhance or unlock anaerobic survival metabolisms in aerobes,” *Journal of Bacteriology*, vol. 202, no. 4, e00797-19, 2020.