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To cite this article: Lindomar Alberto Lerin , Domenico Meola , Chaimae Chiabi , Simona Aprile , Francesco Presini , Federico Zappaterra & Pier Paolo Giovannini (2026) Microwave-assisted biocatalysis: A pathway to efficient and sustainable chemical synthesis, *Biocatalysis and Biotransformation*, 44:4, 376-406, DOI: [10.1080/10242422.2026.2695083](https://doi.org/10.1080/10242422.2026.2695083)

To link to this article: <https://doi.org/10.1080/10242422.2026.2695083>



Published online: 05 Jul 2026.



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REVIEW ARTICLE

Microwave-assisted biocatalysis: A pathway to efficient and sustainable chemical synthesis

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ABSTRACT

Microwave (MW)-assisted biocatalysis emerged as a highly attractive route for green chemical synthesis. This review discusses how MW can combine its rapid and selective dielectric heating with specificity, efficiency, and mild conditions of enzymatic catalysis to accelerate enzyme kinetics, reduce energy consumption, and enable process intensification in MW-assisted enzymatic processes. Emphasis is placed on the fundamental mechanisms of MW irradiation interactions with enzymatic and chemical systems, including dipolar rotation and ionic conduction, dielectric parameters, and penetration depth, which govern temperature distribution and reaction performance. Strength and weakness-consideration brings about issues such as hotspots, absorption depending on the solvent used, enzyme stability, and finally scale-up challenges. MW hybrid heating (MHH), which combines MW with other heating sources, such as conventional conduction or ultrasound, is proposed to address these limitations and improve thermal uniformity and selectivity. Examines the key process variables influencing MW-assisted biocatalysis, including solvent polarity, hydration state, molar ratio, catalyst loading, immobilization strategies, and reactor configuration, in both batch and continuous modes. Various enzymatic reactions demonstrate the advantages of MW-assisted biocatalysis, including shorter reaction times, higher yields, and greater operational stability. Furthermore, the use of immobilized enzymes under MW or MHH conditions is crucial for enhancing process robustness, reusability, and control. Finally, the limitations of scaling up processes using MW and MHH for industrial applications are discussed, and future research directions are outlined, focusing on expanding the enzymatic repertoire, improving kinetic and thermodynamic models, optimizing and developing processes and reactors, and further integrating green solvents to promote sustainable biocatalysis.

ARTICLE HISTORY

Received 18 February 2026
Revised 16 May 2026
Accepted 22 June 2026

KEYWORDS

Microwave-assisted; microwave hybrid heating; enzyme catalysis; reaction efficiency; biocatalysis; reaction intensification

1. Introduction

During World War II, British scientists developed the magnetron, a key component of RADAR (Radio Detection and Ranging) systems. This technology gave Allied forces a strategic edge by detecting enemy aircraft. Radar works by emitting microwaves (MW) that reflect off objects, like planes, and return as echoes. By analysing these signals, operators could determine an aircraft's position, speed, and direction. This advancement revolutionized warfare and became the foundation for modern radar and communication systems (Sanseverino 2002).

However, the history of this device took a decisive turn in 1945, when engineer Percy L. Spencer

(1894–1970) discovered a way to adapt MW technology for domestic use. While working with a magnetron tube, he noticed that a candy bar in his pocket had melted. Intrigued, he conducted a series of simple experiments, such as popping popcorn by placing kernels near the tube and observing an egg explode from rapid internal heating. Recognizing the potential of cooking, Spencer refined the technology, paving the way for the MW oven. In 1947, Raytheon, a small North American company, launched the first commercial MW oven, the "Radarange". While early models were large and expensive, advancements in the late 1960s made compact, affordable versions viable for home use. By the 1970s and 1980s, the domestic MW

oven had become a ubiquitous kitchen appliance worldwide (Sanseverino 2002). The use of domestic MW ovens for heating or preparing food is now commonplace. Beyond household applications, MW heating has been widely adopted in industrial settings since the 1990s. It plays a key role in diverse fields, including food processing, rubber vulcanization, environmental remediation, medicine, printing, paints, thin-film production, agriculture, wood drying, chemical synthesis, materials processing, and nanomaterial fabrication, among others (Dagani 1997; Horikoshi and Serpone 2019).

In analytical chemistry, MWs have been utilized since the 1970s. Some of their most important applications include sample digestion for elemental analysis, the extraction of various organic and inorganic compounds, and the thermal desorption of volatile substances. These techniques have significantly improved efficiency and precision in chemical analysis (Zlotorzynski 1995; Sanseverino 2002).

The application of MW irradiation for the synthesis of organic products was soon introduced in the 1980s. The first reports of organic reactions performed in domestic MW ovens appeared in 1986 in two independent studies (Gedye et al. 1986; Giguere et al. 1986). However, these early experiments also highlighted safety concerns, as some reaction vials deformed or even exploded under irradiation. Despite these risks, subsequent research has demonstrated that many organic reactions can be conducted safely in unmodified domestic MWs. In parallel, researchers also explored the use of modified reactors, digesters, and MW ovens to improve control and safety (Sanseverino 2002; Loupy et al. 1998; Varma 1999).

MW technology has been established as a highly efficient heating method, significantly accelerating synthesis rates, improving yields, and reducing reaction times. Compared to conventional heating techniques, it also offers notable energy savings. As a result, MWs have found widespread application in diverse synthetic reactions, including enzyme-catalysed processes (Shinde and Yadav 2015; Khan and Rathod 2018; Venturi et al. 2024). However, MW-assisted processes face several challenges, including uneven heating (due to low MW absorption by certain substances), limited penetration depth, and potential MW leakage. These limitations can be addressed by combining MW irradiation with other reaction intensification techniques, such as photocatalysis, ultrasound (US), conventional heating, plasma, or mechanochemical activation. Such hybrid approaches, known as MW hybrid heating (MHH), enhance reaction efficiency, selectivity, and scalability (Horikoshi and Serpone 2019).

The study of MW effects on biological systems has attracted significant interest since its initial application in food heating. Early research in this field primarily focused on safety aspects related to MW heating, where changes in enzymatic activity played a key role in determining optimal food processing methods (Parker et al. 1996).

By the early 1990s, researchers began exploring the combined effects of MWs and enzymatic catalysis after observing that many enzymes exhibited enhanced activity upon exposure to MWs. They found that MW irradiation of enzymes, both in aqueous and non-aqueous media, was a simple yet effective way to accelerate reaction rates and improve stereospecificity. Intriguingly, these effects were attributed to non-thermal mechanisms, suggesting a direct influence of MWs on enzymatic behaviour (Parker et al. 1996; Lin and Lin 1998).

MWs interact strongly with polar molecules, especially those with high dipole moments, such as enzymes and substrates involved in enzymatic synthesis. This increases molecular motion and collision frequency, enhancing energy transfer. Additionally, MWs can enhance enzyme stability by delaying denaturation compared to conventional heating methods, as demonstrated with *Candida antarctica* lipase B (CalB). Due to these characteristics, MW irradiation is considered a promising technology for intensifying enzymatic processes. Literature extensively documents MW-assisted enzymatic synthesis, highlighting its advantages (Khan and Rathod 2015; Khan and Rathod 2018; Zare et al. 2020; Zarei et al. 2021; Aprile et al. 2023; Venturi et al. 2024). MW-assisted synthesis is also in line with green chemistry principles, as it helps increase selectivity and reduce the energy demand in many organic reactions. However, the MW hybrid heating (MHH) approach remains underutilized in enzymatic catalysis processes, despite promising results from studies integrating MWs with other heating methods (Horikoshi et al. 2016; Zieliński and Dębowski 2018; Xie et al. 2020). MHH can also be a key strategy for overcoming the limitations of MW-based industrial applications.

Given the above, this review examines the key aspects of MW and MHH effects on such reactions. First, the principles of MW and MHH are introduced, followed by their effects on enzyme activity, selectivity, and stability. Key process parameters, including reaction conditions and enzyme immobilization, are then discussed. The role of MHH in improving enzymatic performance is also addressed. Finally, reactor design, scale-up considerations, and the main challenges and future perspectives are presented.

2. Effects of microwaves on substances

MW irradiation has revolutionized synthetic chemistry, ensuring rapid, selective, and energy-efficient heating compared to conventional thermal methods. Traditional heating techniques, such as oil baths or heating mantles, rely on thermal conduction, where heat transfers from an external source to the vessel and then gradually to the reaction mixture. This often creates temperature gradients, with the outer layers significantly hotter than the core, which is most evident in batch-process scale-up, even with efficient agitation systems.

MW heating overcomes these limitations by using a completely different mechanism: energy is transferred from within the material through interaction with MW-electromagnetic waves with wavelengths typically ranging from 1 mm to 1 m, consisting of synchronized oscillating electric and magnetic fields (Figure 1). This bypasses the limitations of heat transfer by conduction, enabling immediate, localized heating at the molecular level (Hayes 2002).

The magnetic field component of MW radiation does not interact significantly with most chemical systems and thus plays a negligible role in heating. In chemical reactions, the electric-field component dominates the heating interaction without directly affecting molecular structures because the photons' energy is insufficient (typical bond energies range from 1 to 10 eV, whereas MW photon energies are around 10^{-5} eV).

The two mechanisms responsible for MW-induced heating are dipolar rotation and ionic conduction, which depend on the nature of the medium and its ability to couple with the applied oscillating electric field, typically at 2.45 GHz. Dipolar rotation occurs in

polar molecules with a dipole moment, such as water, ethanol, and ethyl acetate. When subjected to the rapidly oscillating electric field of MW radiation, these molecules continuously reorient in response to the changing field. However, due to the high frequency, they cannot fully realign with each cycle. This mismatch causes a phase *Lag* and internal molecular friction, which is dissipated as heat (Mingos and Baghurst 1991). Water's high dielectric loss makes it exceptionally efficient at absorbing MW energy and converting it to heat quickly.

The efficiency of these mechanisms depends on the dielectric properties of the medium, including the dielectric constant (ϵ') which measures how well a material stores electrical energy, the dielectric loss (ϵ'') which indicates how much energy is converted to heat, the loss tangent ($\tan \delta$) that expresses the efficiency of MW absorption, along with the penetration depth (δ_p) which defines how deeply MWs penetrate before power drops to $\sim 37\%$ (Figure 1).

Materials with high $\tan \delta$ and shallow δ_p absorb energy quickly at the surface, while those with low $\tan \delta$ and deep δ_p allow for uniform heating throughout, but may absorb insufficient energy for effective heating. Balancing these dielectric parameters is crucial for optimizing MW-assisted processes, especially in solvent-free or viscous media, where uniform heating is most important.

On the other hand, ionic conduction plays a significant role in systems containing dissolved ions, such as salt solutions, acidic or basic media, or ionic liquids. In this case, the oscillating electric field causes charged particles (cations and anions) to move back and forth. This oscillatory motion results in collisions with surrounding molecules, generating friction and, consequently, heat (Figure 2). For instance, aqueous NaCl

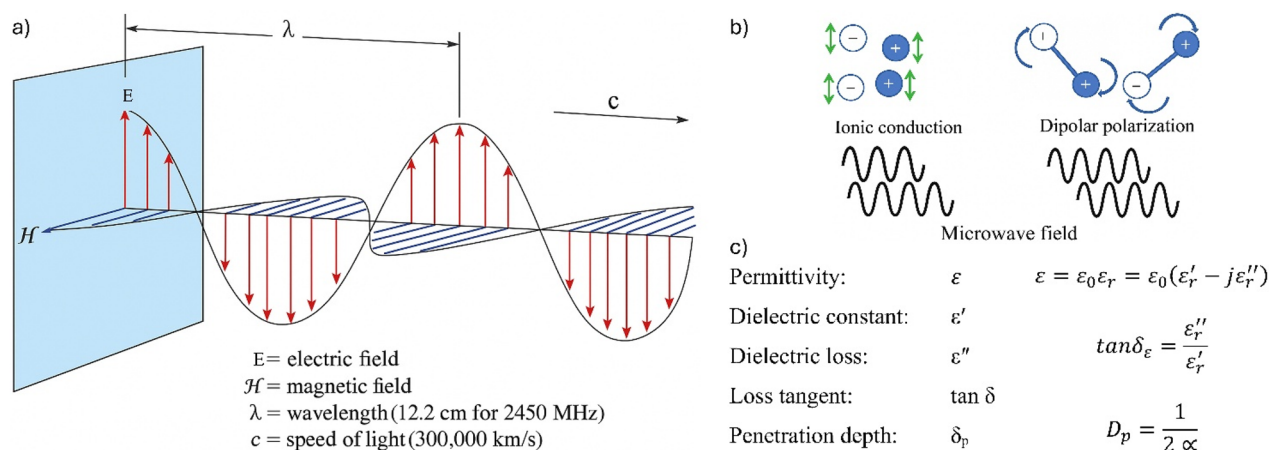


Figure 1. Schematic representation of MW propagation (a), polarization mechanisms (b), and dielectric parameters (c) based on Falciglia et al. (2018) and Jaiswal and Rathod (2022).

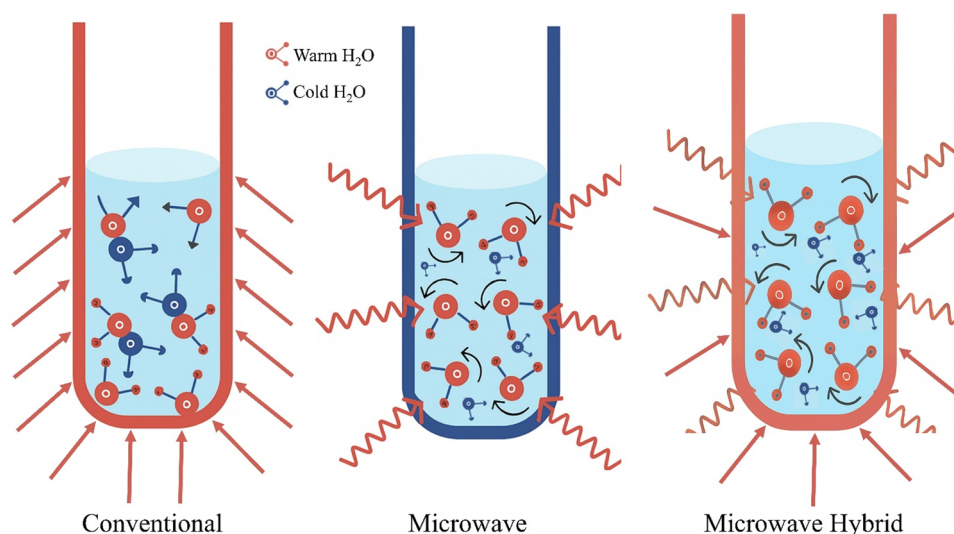


Figure 2. Schematic representation of conventional heating, MW, and MHH.

solutions heat more rapidly under MW irradiation than pure water, not only due to dipolar effects but also enhanced ionic conduction (Mingos and Baghurst 1991).

3. Advantages and disadvantages of microwaves

MW heating represents a significant departure from conventional thermal techniques, offering notable benefits such as rapid, uniform internal heating, shorter reaction times, improved energy efficiency, and the potential to selectively target specific components within a mixture (Dhakite and Kadam 2014). The efficiency of this process depends on the dielectric properties of the materials involved. Some materials are effectively transparent to MWs, while others act as strong absorbers (Mingos and Baghurst 1991). Polar solvents and certain catalysts absorb MW energy rapidly and convert it into heat, whereas other substances may remain largely unaffected. This selective absorption enables fine-tuned control of reaction environments, particularly in complex systems such as heterogeneous reactions or when working with low-solubility compounds (Tiwari and Talreja 2022).

However, in polar solvent systems, the boiling point is reached quickly, leading to a loss of temperature control and masking the MW effect on substrates (Loupy 2004). To overcome this limitation, solvent-free systems are often preferred, as they minimize solvent MW absorption and allow stronger interactions between MWs and the reactant materials (Taylor et al. 2005).

In addition to greater control and efficiency, MW heating offers several other important benefits:

1. **Faster reaction rates** – MW-induced reactions allow reaching high temperatures rapidly and uniformly, reducing reaction durations from several hours (or days) to just minutes. For example, fluorescein alkylation can be completed in just 35 minutes using MW heating, compared with almost 10 hours under conventional conditions (Zdończyk et al. 2021).
2. **Higher yields and improved purity** – MW conditions often reduce the formation of side- or co-products, leading to higher yields and cleaner reactions. Moreover, in enzymatic catalysis, MW heating facilitates the evaporation of water, ethanol, methanol, and other solvents, thereby shifting the equilibrium towards products while avoiding hydrolysis/alcoholysis reactions (Gelo-Pujic et al. 1997b).
3. **Energy efficiency** – Since only the sample is heated, and not the surrounding vessel or air, MW techniques consume less energy compared to conventional methods. This targeted heating approach contributes to more sustainable and cost-effective chemistry (De La Hoz et al. 2016).
4. **Eco-friendly chemistry** – As discussed previously, the direct interaction between MW irradiation and the reaction system eliminates the need for conventional external heating, along with the possibility of performing reactions under solvent-free conditions (Dhakite and Kadam 2014).

Due to their advantages, MWs have become a popular technique in chemical laboratories and are used in numerous areas of chemical research. In organic

synthesis, MWs accelerate various reactions, such as amid bond formation, protection/deprotection reactions, and cycloadditions, thereby improving protocols that are more aligned with green chemistry principles (Datta and Rakesh 2013). In materials science, MWs are used to synthesize complex metal nanoparticles and hybrid materials with high control of crystalline structure (Kustov and Vikanova 2023). Polymer synthesis, biochemical processes, and analytical chemistry also greatly benefit from the MW technique (Khan and Rathod 2018; Kabir 2023).

Although the MW technique offers several advantages, it also presents critical limitations that must be considered. One of the main challenges lies in the non-uniform temperature distribution in particular systems, especially in multi-mode cavities, where standing-wave patterns create hot and cold spots within the reaction medium. This heterogeneity may result in uncontrolled reaction pathways, formation of by-products, or incomplete conversions. This is not in contrast with what was stated above regarding the efficiency of MW heating, since it depends heavily on the dielectric properties of the materials involved. In systems containing low-loss or non-polar solvents, MWs are poorly absorbed, leading to inefficient heating. On the other hand, in high-loss media, δ_p is shallow, leading to superficial overheating and thermal gradients that prevent uniform bulk heating.

Additionally, materials that strongly absorb MW energy, such as certain polar solvents, catalysts, or solid supports, can experience localized overheating or hotspots. These phenomena have been observed in heterogeneous systems, such as those with γ -alumina or MoS_2 , where structural or morphological changes occur under MW irradiation. Such effects indicate intense local heating that conventional external sensors cannot reliably track (Zhang et al. 2003). These hotspots may significantly alter the system's reactivity but are challenging to detect and control, making it difficult to distinguish between purely thermal effects and possible non-thermal MW effects. While thermal effects, such as the direct heating of the catalyst or solvent, are widely accepted, non-thermal effects remain controversial and are often attributed to measurement artefacts or misinterpretation of temperature gradients.

Moreover, when solid catalysts are used or biomass-derived materials are treated, selective heating can occur at the particle level, further complicating heat management. In gas-phase reactions, MW interaction with solid catalysts may lead to intense localized heating, while the surrounding gas remains underheated due to its low MW susceptibility. This imbalance in

thermal distribution raises concerns regarding reproducibility, scaling-up, and the safe operation of MW-assisted processes. Accurate, spatially resolved temperature measurements are essential yet challenging in these heterogeneous systems.

4. Microwave hybrid heating: Overcoming the limitations of conventional microwave processing

In the past few years, the coupling of conventional MW irradiation with other energy sources useful to promote reaction rates, commonly referred to as MHH, has emerged as a promising approach to overcome some of the inherent limitations of conventional MW-assisted processes. Hybrid techniques attempt to surmount these challenges by combining MW energy with photocatalysis, US, conventional heat, plasma, or mechanochemical activation to enhance reaction efficiency, selectivity, and scalability (Trost 1995). A comparative summary of the main differences between direct MW heating and MHH is reported in Table 1.

From a broader perspective, this hybrid configuration, illustrated in Figures 2 and 3, aligns with the principles discussed by Datta and Rakesh (2013), who explored MHH as a strategy to improve heating uniformity and energy efficiency across several domains. In

Table 1. Comparison between direct MW heating and MHH.

Parameter	MW	MHH
Heating mechanism	Dielectric heating via dipolar rotation and ionic conduction	Combined mechanism: MW plus additional energy sources (e.g. conduction, induction, ultrasound, UV, susceptors)
Target materials	Only MW-absorbing	Can also heat MW-transparent or low-absorbing materials through indirect or synergistic means
Temperature uniformity	Hot spots and uneven heating, especially in multi-mode systems	Improved thermal uniformity via combined energy inputs
Selectivity of heating	Heating is selective only to polar or ionic components	Broader selectivity through external triggers (e.g. photocatalysis, ultrasound-induced cavitation, surface conduction)
Energy efficiency	High for compatible materials; low for non-polar systems	Enhanced by combining efficient energy coupling routes, minimizing energy losses, and improving reaction control
System complexity	Relatively simple setup (magnetron, cavity, vessel)	More complex setups with combined technologies and often tailored reactor geometries
Scalability	Limited by δ_p and field distribution	Better scalability via flow setups or hybrid modes

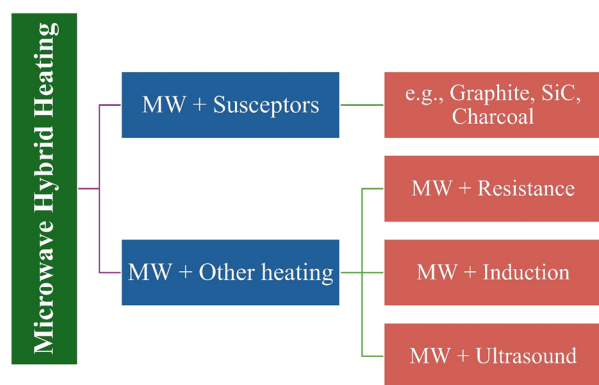


Figure 3. Representation of the primary combinations for MHH.

analogy to their findings in food science for MHH methods, where MW heating provides volumetric, rapid energy input while conventional methods such as hot air or infra-red heating contribute to controlled surface effects, the combined use of MW and conductive heat in laboratory chemistry enables finer thermal management. This synergy leverages the rapid internal heating characteristics of MW with the stabilizing influence of conductive methods, thereby reducing issues such as local overheating or poor temperature penetration often encountered in single-mode MW processing (Figures 2 and 3). Furthermore, this setup facilitates modulated heating profiles that better emulate classical thermal reactions while retaining the kinetic advantages of MW activation (Datta and Rakesh 2013).

Another promising, yet relatively unexplored, hybrid strategy involves combining MW and induction heating. While MWs are highly effective in rapidly heating dielectric materials, they struggle with conductive substrates such as metals, which tend to reflect MW energy. Induction heating, on the other hand, excels at selectively heating conductive materials through the generation of eddy currents. By coupling MW with induction heating, it becomes possible to achieve simultaneous, efficient heating of mixed systems containing both dielectric and conductive phases. This dual-mode approach has demonstrated potential in metallurgical processes, such as the rapid reduction of iron oxides, providing more uniform temperature profiles and enhanced energy efficiency. As such, MW induction heating systems represent a valuable frontier for further investigation, especially in heterogeneous catalysis and materials processing (Zhang et al. 2024).

Another hybrid strategy that has received more attention is combining MW irradiation with ultraviolet (UV) light to create a MW-photocatalytic system. In such configurations, MW plays a complementary role to UV light by enhancing the photocatalyst's

crystallinity, dispersion, or activation, while UV radiation provides the energy needed to excite electron-hole pairs and drive photochemical reactions. The poor penetration of UV light in turbid media can be mitigated by the volumetric heating and agitation induced by MWs. Moreover, MWs can accelerate charge-carrier mobility and reduce recombination rates, improving overall photocatalytic efficiency. Combining MWs with UV light in photocatalytic membrane reactors has shown promise for the conversion of CO, the synthesis of KA oil (cyclohexanol and cyclohexanone) by photocatalytic oxidation, the conversion of acetophenone to phenylethanol, the synthesis of vanillin and phenol, as well as hydrogen production, thanks to the synergistic effects of rapid MW heating and selective photochemical activation (Maliessa and Idris 2023; Li et al. 2025).

One additional variant of MHH is US-assisted MW irradiation, which offers several distinct benefits, including rapid synthesis, operational simplicity, and low cost, with the added benefits of intense mixing and cavitation effects provided by US. This combination has been demonstrated to be successful in different areas: for organic synthesis, it could accelerate reactions like Suzuki and Heck couplings with reduced catalyst loading (Cravotto et al. 2008); for nanomaterials, it enabled the preparation of low-agglomeration $\text{Cu}_2\text{FeSnS}_4$ nanocrystals under simultaneous irradiation (800W MW and 400W US) (Wang et al. 2014). By improving heat and mass transfer, MW/US coupling offers a versatile approach to greener, more efficient chemical processes.

Another distinct form of MHH uses susceptor materials to overcome the limitations of direct MW absorption. In MHH systems, susceptors such as graphite, silicon carbide, charcoal, or MoSi_2 serve as localized heat sources. They have high $\tan \delta$ and therefore rapidly absorb MW energy, converting it into thermal energy. This heat is then transferred by conduction and radiation to surrounding materials, including those that are MW-transparent or weakly absorbing. Such indirect heating enables a more homogeneous temperature distribution, minimizing thermal gradients and reducing the risk of material degradation or interfacial defects (Zhang et al. 2024).

A key advantage of MHH is its ability to modulate δ_p as a function of temperature. Metallic materials, such as Inconel-625, which are reflective at room temperature, become MW-absorbing at critical temperatures ($\sim 900^\circ\text{C}$), enabling a transition from surface to volumetric heating. This phenomenon leads to bidirectional heating, in which MWs volumetrically heat the joint, while the susceptor provides external thermal input, shortening processing times by more than 75% compared to conventional methods (Mohanty et al. 2024).

Finally, the selection of susceptor materials is critical in processing control. Susceptors must preferably be thermally stable, oxidation-resistant, low in impurities, and have the capability to heat rapidly. Their strategic placement enables targeted, localized heating, which is particularly convenient for systems that require uniformity and precision. As a result, MHH is emerging as an enabling tool for processing advanced materials, with recent research focusing on high-entropy alloy interfaces and on predictive computational modelling to further improve its efficiency and reproducibility (Zhang et al. 2024).

5. Microwaves and biocatalysis as green tools

Green chemistry, also known as sustainable chemistry, is an efficient approach designed to minimize environmental pollution. It involves the invention, design, and application of chemical products and processes that reduce or eliminate the use and generation of hazardous substances. Guided by 12 core principles, green chemistry provides a framework to develop sustainable chemical processes. A key advantage of green chemistry technologies is their ability to facilitate environmentally friendly reactions by minimizing hazardous chemical synthesis and reducing by-product formation. MW-assisted synthesis aligns with these goals by employing non-toxic solvents and efficient catalysts/biocatalysts, enabling the sustainable production of diverse molecules (Bassyouni et al. 2012; Banik and Bandyopadhyay 2018; Sahoo et al. 2018; Sheldon and Woodley 2018).

In this context, organic synthesis is considered atom-economical or perfectly efficient if it generates negligible waste. Two critical metrics for evaluating reaction efficiency, in addition to the classical expression $\% \text{ Yield} = (\text{Practical Yield} / \text{Theoretical Yield}) \times 100$, are the Atom Economy = (mass of desired products / stoichiometric mass of reactants) and the Environmental Factor = (mass of waste / mass of products). These metrics help assess the sustainability and efficiency of synthetic processes, ensuring alignment with the principles of green chemistry (Trost 1995; Sheldon 2007; Sahoo et al. 2018).

Furthermore, the drive towards a circular economy uses chemistry to provide the basis for innovative products and new, greener pathways and techniques. In this regard, biocatalysis has experienced significant growth, emerging as a strategic, clean, and eco-friendly technology that offers sustainable, highly regio- and stereo-selective routes for chemical synthesis and has been utilized in the production of a wide range of chemicals (Khan and Rathod 2018). The advantages

include easy product purification, bulk production, mild reaction conditions, negligible side reactions, and low environmental waste generation, all of which are well documented in the literature (Bhavsar and Yadav 2018).

Enhancing enzymatic efficiency has therefore garnered considerable interest, given the widespread use of enzymes across various sectors (Ahmed et al. 2024). Similar to biocatalysis, MW irradiation has emerged as a simple, fast, energy-efficient, and environmentally friendly alternative heating source for the process (Yadav and Devendran 2015), due to its high potential to intensify processes by improving yields and reaction selectivity in faster reaction times by orders of magnitude (Rejasse et al. 2007). This led to investigating, besides the US, which has also shown beneficial effects during enzymatic processes, MW irradiation as well, not just as a more sustainable option, but also as a promising tool to be used in combination with biocatalysis, delivering better outcomes than those achievable by each approach alone (Khan and Rathod 2018). Hence, this methodology represents a forward-looking, innovative approach for modern industrial processes. Indeed, MW energy can regulate enzymatic activity, directly addressing the drawbacks of conventional biocatalysis in industrial processes, including low yields and lengthy processing times (Bhavsar and Yadav 2018), making biocatalysis reactions more attractive to synthetic organic chemistry.

A more detailed understanding of how MW irradiation interacts with enzymatic systems has emerged over the years. Specifically, this interaction, and consequently the MW irradiation effects on enzymatic catalysis, are determined from the direct coupling of molecules with the MW field. Generally, MW irradiation induces molecular rotation arising from dipole alignment with the oscillating electric field. As a result, MW significantly impacts molecules with high dipole moments. Proteins and peptides have significant dipole moments; hence, they may be highly susceptible to MW irradiation (Young et al. 2008). However, historically, the use of MW in enzyme chemistry has been limited due to reaction conditions, such as the thermal sensitivity of biocatalysts in MW systems. These limitations have been addressed and overcome through advances in MW/enzymatic system technology, particularly by enabling precise temperature regulation *via* controlled power input and by immobilizing enzymes on solid supports, which enhances their thermal stability (Rejasse et al. 2007).

It is also crucial to investigate the various operational conditions for MW-assisted biocatalysis to better

understand how irradiation affects enzymatic catalysis. In particular, three different environments have been investigated, each presenting a distinct advantage:

1. **Solvent-free media** – MW-assisted biocatalysis offers faster reaction rates, minimal co-products, simple product isolation, and scalability, while also reducing environmental impact. Specifically, it has been demonstrated that using immobilized enzymes instead of their free forms under controlled MW irradiation increases conversion, improves selectivity, and reduces synthesis time (Bhavsar and Yadav 2018).
2. **Aqueous media** – Offer the advantages of reduced process times, higher final product quality, and undesirable reactions and co-products are minimized. However, enzymatic inactivation appears faster under MW irradiation than under traditional heating methods (Rejasse et al. 2007). However, enzyme deactivation has been observed, particularly in water-dependent systems such as galactosidases, likely due to localized overheating or water loss (Carrillo-Munoz et al. 1996; Gelo-Pujic et al. 1996; Gelo-Pujic et al. 1997b).
3. **Non-aqueous media** – In non-aqueous media, such as organic solvents like acetonitrile, toluene, butanol, etc., improvements in enzyme-catalysed reactions have been observed. As reported above for both solvent-free and aqueous media, this environment also leads to enhanced enzymatic activity, shorter reaction times, and higher conversion rates. This opens the possibility of exploiting alternative pathways in organic synthesis, thereby diversifying the range of potential applications (Yadav and Lathi 2004; Lin et al. 2005; Da Wan et al. 2012).

6. Effects of microwaves on proteins/enzymes

In enzymatic catalysis, dielectric heating primarily affects the enzyme through its microenvironment rather than *via* direct interaction with the protein backbone. The intense coupling of MWs with polar solvents and bound water can locally modulate temperature, viscosity, and polarity around the biocatalyst. These changes alter enzyme conformational dynamics and hydration, which, in turn, affect catalytic turnover, substrate binding, and product release (Cao et al. 2023). For immobilized systems, selective heating of the support and surrounding medium relative to the protein can generate microthermal and polarity

gradients that enhance activity and sometimes selectivity, while overly strong or prolonged exposure may destabilize the native fold and accelerate deactivation (Shang et al. 2015). Thus, the dielectric properties of the reaction medium not only control bulk heating efficiency but also govern the delicate balance between enzyme activity and stability under MW irradiation.

In this context, enzyme deactivation remains a critical factor, as high temperatures can induce protein unfolding and inactivation of the active site. The microenvironment surrounding the enzyme plays a critical role in its thermal stability. Under MW irradiation, using non-polar solvents can increase enzyme rigidity and enhance catalytic stability (Khan and Rathod 2018), but in some cases, even polar solvents can improve enzyme activity. This is probably due to their strong interaction with radiation, which allows for lower radiation energy. For example, butanol requires only 24W of MW power to maintain 100°C, while ethyl butyrate requires 210W, illustrating the importance of solvent-MW interactions (Rejasse et al. 2004). In this view, the parameters that appear to influence enzymatic activity under MW-assisted catalysis are the following:

1. **Hydration state** – The presence or absence of water in the reaction medium, commonly referred to as the enzyme's hydration state, can significantly influence the behaviour of enzyme-catalysed reactions under MW irradiation. In aqueous systems, water, as a strong MW absorber, captures most of the MW energy, resulting in the solvent's heating rather than direct energy transfer to the enzyme or substrates. In contrast, in low-hydration systems, MW energy couples directly to the enzyme (or enzyme-support) rather than the solvent, leading to improved catalytic outcomes (Figure 4) (Rejasse et al. 2007).
2. **Influence of polarity** – The solvent polarity, much like the hydration state, plays a critical role in modulating enzymatic performance under MW irradiation. Polar solvents absorb MW energy efficiently, reducing direct interaction of MW energy with the enzyme. In contrast, less polar or hydrophobic solvents absorb MW energy less effectively, allowing MW irradiation to interact directly with the enzyme-substrate complex. As a result, increased solvent hydrophobicity has been correlated with enhanced reaction rates in MW-assisted enzymatic catalysis (Figure 4) (Rejasse et al. 2007).

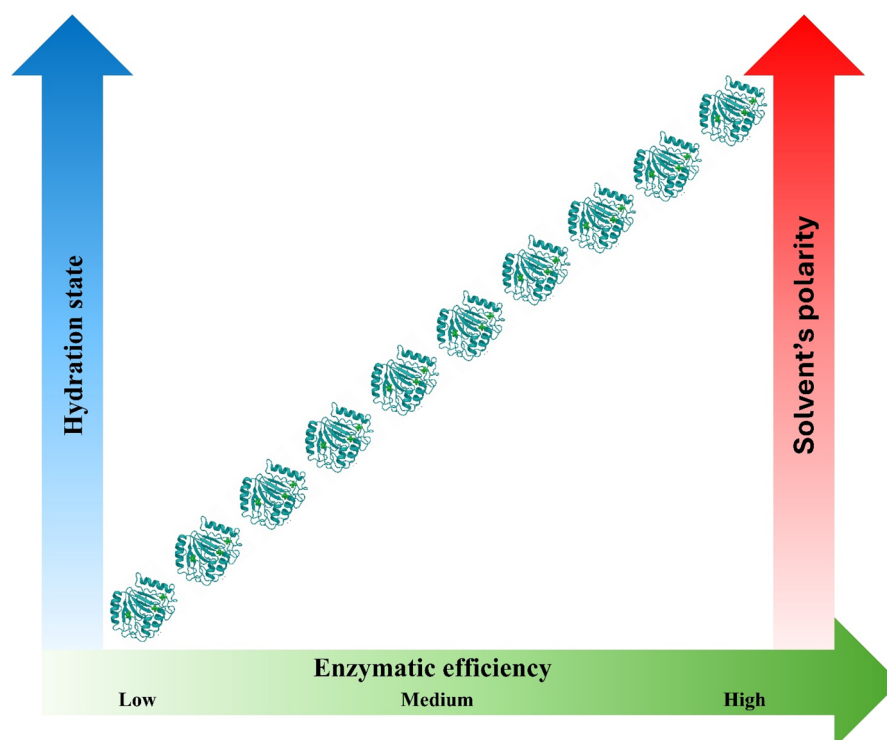


Figure 4. Representation of enzyme efficiency concerning the hydration state and the solvent polarity.

6.1. Effects of microwave irradiation on enzyme activity, selectivity, and stability

As discussed previously, MW irradiation heats the reaction media *via* dielectric mechanisms that depend strongly on solvent polarity, ionic strength, and δ_p . These parameters define the local thermal and dielectric environment surrounding the enzyme and its support in the case of immobilized enzymes, thereby directly influencing conformational flexibility, active-site integrity, and the balance between catalytic activity and long-term stability. The following examples illustrate how careful tuning of the MW-absorbing medium and immobilization strategy can translate these fundamental dielectric effects into enhanced activity, improved selectivity, and, in many cases, increased operational stability. In this context, numerous studies in dry media using both commercial and non-commercial immobilized enzymes have demonstrated that supports with weak MW absorbance can promote optimal enzymatic activity, selectivity, and stability (Carrillo-Munoz et al. 1996; Gelo-Pujic et al. 1996; Gelo-Pujic et al. 1997b). For instance, one of the first published works on MW-assisted biocatalytic processes reported enhanced enantioselectivity using two lipases (from *Pseudomonas cepacia* and CalB) immobilized on Celite 545, Hyflo Super Cel, and polypropylene resin. Esterification reactions involving isopropenyl acetate and octanoic acid showed markedly improved

selectivity compared to those using ethyl esters, highlighting the influence of substrate polarity on selectivity (Carrillo-Munoz et al. 1996).

It was also demonstrated that a 95% esterification yield was achieved within 3 h using a commercial immobilized lipase, CalB, under MW irradiation, whereas only 55% conversion was observed after 5 h under conventional heating, indicating a synergistic effect of MW irradiation, enzyme immobilization, and solvent polarity (Gelo-Pujic et al. 1996). In subsequent work, the same group found that MW-assisted starch hydrolysis using immobilized almond β -glucosidase achieved a 98% conversion in 15 min, compared to 55% with conventional heating. They also observed a significant reduction in product hydrolysis during glycosylation, achieving nearly full conversion in transglycosylation and less than 10% undesired hydrolysis (Gelo-Pujic et al. 1997b).

Years later, thermal and non-thermal MW effects were further explored by comparing esterification reactions catalysed by commercial immobilized lipase from *Rhizomucor miehei*. Reactions under MW irradiation consistently proceeded at twice the rate of conventional heating. Optimal performance occurred at 55°C, whereas higher temperatures led to enzyme deactivation, likely due to polarity-related destabilization caused by activated short-chain substrates (Huang et al. 2005).

Similar enhancements were reported in both esterification and transesterification reactions catalysed by subtilisin and α -chymotrypsin under MW. Reaction rates increased 2- to 5-fold, depending on the solvent and temperature, with *t*-amyl alcohol at 60°C yielding the greatest improvement (Roy and Gupta 2003). It was also noted that MW irradiation improved conversion in the transesterification of methyl acetoacetate with alcohols, particularly with long-chain alcohols (e.g. 1-butanol, 1-hexanol, 2-pentanol), resulting in a 3-fold increase in conversion under MW irradiation compared to conventional heating (Yadav and Lathi 2004).

Further evidence was obtained by measuring the deactivation constants for enzymes in different solvents. It was found that MW irradiation improved enzyme stability, with deactivation 1.7-fold higher in ethyl butyrate and 6-fold lower in butanol than under conventional heating (Rejasse et al. 2004). Subsequently, Table 2 summarizes the effects of MW on enzymatic biocatalysis.

6.1.1. Enzyme activity under microwave vs conventional heating

MW can dramatically affect the apparent activity of enzymes. In some cases, MW irradiation increases the initial reaction rate of enzymatic transformations beyond what is observed under conventional heating at the same bulk temperature. For example, in the late 1990s, it was found that irradiating a hydrated cutinase (lipase) from *Fusarium solani*, suspended in an organic substrate medium (butanol and ethyl butyrate) at 50°C with MWs, enhanced the esterification rate by 2- to 3-fold compared to an oil bath at 50°C (Parker et al. 1996). This early study suggested non-thermal MW-specific activation of the enzyme, showing that the degree of enzyme hydration is crucial: under low water activity ($a_w \sim 0.6$), MWs increased the initial rate by 2- to 3-fold, whereas at high hydration ($a_w \sim 0.9$) the MW- and conventional-heated rates were identical (Rejasse et al. 2007). This implies that MWs may exert special effects (e.g. rapid localized heating or field interactions) when the enzyme is in a mostly organic environment with limited water, a scenario common in lipase-catalysed esterifications and transesterifications. In fully aqueous systems, by contrast, enzyme rates under MW vs conventional heating are usually the same when the bulk temperature is equal (Rejasse et al. 2006).

6.1.2. Regio- and enantio-selectivity

Enzyme-catalysed reactions are often highly selective (e.g. lipases that distinguish between fatty acid

positions or resolve enantiomers). Generally, MWs do not fundamentally alter enzyme selectivity if temperature is controlled correctly; the same products and enantiomeric excess are obtained as with conventional heating. For instance, studies on lipase-mediated kinetic resolutions under MWs achieved the same enantiomeric purities as thermal reactions while enhancing reaction rates, resulting in a 12-fold reduction in time and a nearly 2-fold increase in yield for both mono- and di-acetate products (Rouillard et al. 2014). However, evidence suggests that the pattern or intensity of MW irradiation can influence selectivity in some cases. It is noteworthy that in only one study, a weakened 1,3-regioselectivity of a lipase in triglyceride transesterification under consecutive MW pulses resulted in a different product distribution than typical 1,3-specific enzymatic esterification (Fang et al. 2008b). This loss of positional specificity was attributed to the intense, non-uniform heating of the enzyme or substrate. Such cases are the exception; most experiments show that, when the bulk temperature is the same, MW-heated enzymes exhibit the same selectivity as conventionally heated enzymes (Rejasse et al. 2006). Indeed, one advantage of MW heating is the avoidance of thermal gradients, which can prevent side reactions and maintain selectivity. For instance, MW-irradiation effect on enzymatic hydrolysis of *RS*-($\pm$)-methyl mandelate employing the commercial immobilized CalB showed an augmented activity (with a 6-fold reaction time reduction) and a retained high selectivity, enabling the sole formation of *R*-($-$)-enantiomer of mandelic acid, leaving the *S*-($+$)-enantiomer unhydrolyzed (Yadav et al. 2008).

6.1.3. Enzyme stability

Enzyme stability is a critical concern because proteins can denature when overheated. Pure MW heating can lead to rapid enzyme deactivation if not carefully managed. Many studies report that prolonged MW exposure reduces enzyme stability compared with conventional heating. For example, in a biodiesel synthesis using immobilized *Burkholderia* sp. lipase, the enzyme's half-life dropped from 430h under conventional heating to only 23.5h under MW heating (Souza et al. 2016). The MW-irradiated enzyme achieved faster conversion but lost activity ~ 18 -fold faster. Interestingly, there are also cases where MWs improve enzyme stability. It was reported that commercial immobilized CalB showed higher stability at 100°C under MW heating than under conventional heating, in identical conditions. The stability gain was more pronounced in polar organic solvents (which couple strongly with MWs). In the study, MWs applied either during

Table 2. Reported effects of MW irradiation on enzymatic biocatalysis compared to conventional heating.

Enzyme	Support	Reaction	System polarity	MW effect			Notes	Ref.
				Activity	Selectivity	Stability		
Lipase	Acrylic resin	Hydrolysis	Solvent-free (medium-high)	Enhanced	–	Enhanced	Stability increases with increasing polarity	Rejasse et al. (2004)
Lipase	Acrylic resin Titanium silicate Silica gel	Esterification	Solvent-free (medium)	Enhanced	–	Enhanced	Enzyme support matters	Bhavsar and Yadav (2018)
Lipase	SiO ₂ No support	Esterification	Solvent-free (medium-high)	Enhanced	Enhanced	Decreased	Immobilization method matters	Cebrián-García et al. (2018)
Lipase	Biosilicified enzyme Polystyrene nanoparticles Acrylic resin	Esterification	Solvent-free (medium-high)	Enhanced	–	Enhanced	Support influences exposure	Bukhari et al. (2017)
Lipase	Acrylic resin	Esterification	Solvent-free (medium-high)	Enhanced	–	Decreases increasing polarity	Temperature/MR/polarity interplay	Da Rós et al. (2013)
Lipase	Ion exchange resin	Esterification	Different solvents (medium-low)	Enhanced	–	Unchanged	Reaction mixture polarity modulates non-thermal effects	Da Wan et al. (2012)
Lipase	Acrylic resin Ion exchange resin	Esterification	Solvent-free (low)	Enhanced	–	Enhanced	MW non-thermal effects related to enzyme hydration state	Yadav and Thorat (2012)
Lipase	Acrylic resin	Esterification	t-butanol (medium-high)	Enhanced	–	Enhanced	Hotspots aid equilibrium shift	Rufino et al. (2010)
Lipase	Anionic resin	Esterification	Solvent-free (medium-high) n-octane (low-medium)	Enhanced	–	Deactivation > 55 °C	Short-chain substrate instability	Huang et al. (2005)
Lipase	Acrylic resin	Esterification	Water (High)	Enhanced	–	Enhanced	Synergy MW/solvent/support	Gelo-Pujic et al. (1996)
Lipase	Acrylic resin	Transesterification	Solvent-free (medium-low)	Enhanced	–	Enhanced	Sustainability enhanced	Venturi et al. (2024)
Lipase	Acrylic resin	Transesterification	Solvent-free (medium-low)	Enhanced	–	Unchanged	Techno-economic benefits	Aprile et al. (2023)
Lipase	Acrylic resin Anionic exchange resin	Transesterification	Solvent-free (low)	Enhanced	Decreased	Denaturation over 95 °C	Temperatures have fine balance	Gupta et al. (2017)
Lipase	SiO ₂ Acrylic resin	Transesterification	n-heptane and Solvent-free (low)	Enhanced	–	Deactivation over 60 °C	Substrate polarity effects	Shinde and Yadav (2014, 2015)
Lipase	Acrylic resin	Transesterification	Solvent-free (medium-low)	Enhanced	Enhanced	Enhanced	Mild MW conditions	Risso et al. (2012)
Lipase	Acrylic resin	Transesterification	Toluene (low)	Enhanced	–	–	Non-thermal activation	Yadav and Lathi (2004)
Lipase	No support	Transesterification	Solvent-free (low)	Enhanced	–	–	Non-thermal MW effect	Parker et al. (1996)
Lipase	Silica-PVA	Transesterification	Solvent-free (medium)	Enhanced	Enhanced	–	Complete conversion in 8h, while conventional needed 48h	Da Rós et al. (2012)
Lipase	Acrylic resin	Transesterification	Solvent-free (medium)	Enhanced	Enhanced	–	Complete conversion in 12h	Yu et al. (2010)

(Continued)

Table 2. Continued.

Enzyme	Support	Reaction	System polarity	MW effect			Notes	Ref.
				Activity	Selectivity	Stability		
Lipase	Silica gel carrier	Interesterification	Solvent-free (low)	Enhanced	–	–	Viable alternative to traditional	Ramírez et al. (2025)
Lipase	Acrylic resin Ceramic support	Kinetic resolution	Cyclohexane (low)	Enhanced	Enhanced	–	Non-thermal MW effect	De Souza et al. (2009)
Lipases	No support Florisil Celite 545	Kinetic resolution	Solvent-free (medium-high)	Enhanced	Enhanced	–	Support and polarity effects	Carrillo-Munoz et al. (1996)
β -glucosidase	Hyflo Super Cel Different Celites	Glycosidation	Aqueous (high)	Enhanced	Enhanced	–	Favoured transglycosylation	Gelo-Pujic et al. (1997b)
β -glucosidase	Neutral Al_2O_3 No support Type C Al_2O_3 Neutral Al_2O_3 Eupergit	Glycosidation	Solvent-free (medium)	Enhanced	Enhanced	–	Reduced unwanted hydrolysis (best results in apolar solvent)	Gelo-Pujic et al. (1997a)
β -glucosidase	No support	Saccharification	Choline chloride: Lactic acid (high)	Enhanced	Enhanced	–	Deep eutectic solvents application	Martins et al. (2025)
Peroxidase	No support	Schiff base conjugation	Aqueous (high)	Enhanced	–	Enhanced	Bioconjugation benefits	Çelebi et al. (2022)
Peroxidase	No support	Oxidation	Acetone (medium-high)	Enhanced	–	Enhanced	Advanced Oxidation Process for dye removal	Klanovicz et al. (2020)
Subtilisin/ α -chymotrypsin	No support	Esterification Transesterification	Solvents (low to high)	Enhanced	–	Unchanged	Solvent and temperature dependent	Roy and Gupta (2003)
β -fructosidase	No support	Hydrolysis	Aqueous (high)	Enhanced	–	–	Food chemistry MW use	Ying et al. (2025)
Alcalase	No support	Hydrolysis	Aqueous (high)	Enhanced	Enhanced	–	Sustainable process from industrial waste	Torices-Hernández et al. (2025)
Trypsin	No support	Digestion	Aqueous (high)	Unchanged	Unchanged	Deactivation over 60 °C	Proteomics	Damm et al. (2012)

catalysis or as a pre-treatment appeared to slow the enzyme's thermal inactivation compared with an oil bath. The authors suggested a MW-specific effect on the enzyme or its microenvironment that reduces deactivation (Rejasse et al. 2004). This intriguing result contrasts with many reports of faster deactivation and highlights that the effect of MWs on enzyme stability is context-dependent; factors such as enzyme formulation (free vs. immobilized), solvent polarity, and MW power density are significant.

6.1.4. Comparative kinetic data: Conventional vs. microwave

A significant motivator for MW use is the kinetic acceleration it provides. Many enzymatic reactions exhibit higher rates under MW heating than under conventional heating. The magnitude of acceleration varies depending on the conditions. In favourable cases listed in Table 2, rate enhancements of a few-fold have been reported under MW at the same bulk temperature.

In contrast, other studies found there was no significant difference in reaction rate or yield between MW heating and conventional heating when temperature profiles were matched. During the investigation of several lipase-catalysed polycondensations, it was observed that most systems yielded similar polymer yields using MWs as an oil bath. Only in cases of very intense MW in bulk media was a faster enzyme deactivation and a drop in final conversion observed (Pellis et al. 2016). Thus, the kinetic advantage of MWs can range from negligible (purely thermal effects) to several-fold increases, depending on whether conditions allow non-thermal effects or simply faster temperature attainment.

One clear advantage of MW heating is the reduction in activation time and possible lowering of the apparent activation energy (E_a). By some accounts, reactions under MWs exhibit a lower apparent E_a than under conventional heating (Fang et al. 2008a; Saifuddin et al. 2011). Another report on MW-driven enzymatic catalysis noted that the Arrhenius plot was biphasic (indicating a change in mechanism or a change in E_a at certain temperatures), whereas it was linear under conventional heating (Rejasse et al. 2006). These observations have led to the proposal of genuine "specific MW effects" beyond simple thermal acceleration (Dudley et al. 2015).

However, the consensus is not universal; many researchers, including those performing controlled hybrid experiments, conclude that when temperature is uniform, MWs do not speed up enzymes beyond thermal expectations, and any enhancement is due to rapid heating, avoidance of heat-limiting steps (like

slow substrate diffusion), or improved mass transfer (Pellis et al. 2016). For instance, a comparative study of lipase-catalysed transesterification showed that MW-irradiated and conventionally heated reactions proceeded with similar results when conducted at the same temperature, except that the MW reached that temperature faster (Chen et al. 2016).

MWs (and hybrid heating) undoubtedly excel in space-time yield and productivity. By dramatically shortening reaction times, they increase throughput. Several examples illustrate this: the enzymatic synthesis of isopropyl palmitate, a reaction that traditionally might take hours, was completed in <10 min under MHH (Sitepu et al. 2023). In enzymatic biodiesel production from jatropha oil, heating the reaction with MWs led to a higher yield and an overall productivity boost compared to conventional runs. The MW-heated transesterification reached ~80–90% conversion in 24 h, whereas conventional heating required 72 h to achieve similar yields. The MW system achieved these yields with slightly less enzyme deactivation (within the short timeframe) than expected (Souza et al. 2016).

Another metric is conversion yield and selectivity over time. MW methods often achieve the same final yields as conventional methods, albeit in a shorter timeframe. In a lipase-catalysed kinetic resolution of a diol, MW assistance had no negative impact on enantioselectivity but allowed completion of the reaction in a fraction of the time, with a 3-fold increase of enzyme activity under MW irradiation (Yu et al. 2007). Similarly, in MW-assisted polymerizations (e.g. ϵ -caprolactone ring-opening by lipase), the molecular weights and yields of the polymer were comparable to those obtained by traditional heating, but reaction times were shorter with MW irradiation when using a solvent such as diethyl ether. These indicate that kinetic parameters (e.g. turnover frequency) are improved, while equilibrium yields remain governed by thermodynamics (Kerep and Ritter 2006).

7. Effects of microwaves on enzymatic reactions

MW irradiation has proven efficient at promoting a wide range of biotransformations, especially in the synthesis of biologically active compounds, including those with pharmaceutically relevant activity (Das and Banik 2024). This confirmed the broad applicability of MW-assisted enzymatic catalysis, specifically in key reaction types (Table 2), such as esterification, glycosylation, hydrolysis, and polymerization reactions, as discussed below:

1. Esterification/transesterification reactions – One of the most relevant applications in the chemical industry is the production of specialty esters. Based on their application, esters are used in a wide range of products, including agrochemicals, fragrances and flavours, personal care products, food, nutraceuticals, textiles, polymers, dyes, lubricants, and more. Notably, the value of MW-assisted enzymatic synthesis of esters has been demonstrated in the production of high-value compounds such as benzyl benzoate, an important anti-scabies agent (Aprile et al. 2023), and geraniol esters, which act as insect pheromones and exhibit pharmacological activities, particularly as neuroprotective agents (Venturi et al. 2024; Lerin et al. 2024). This highlights the industrial potential of this combined approach as an excellent, sustainable catalytic method for producing bioactive molecules.
2. Glycosylation and hydrolysis of glycosidic bonds – These reactions play a fundamental role in the synthesis and modification of biologically relevant molecules, which are critical in pharmaceutical, food, and biotechnological applications. In this context, MW-assisted enzyme catalysis has shown promising results when applied in aqueous media, particularly by reducing undesired co-products that can lower the reaction's yield (Braun et al. 2010). For instance, MW-assisted transglycosylation of lactose using immobilized galactosidase, performed in a biphasic system (70:30 organic solvent/water, v/v), initially yielded results like those obtained with conventional heating. However, once lactose was fully converted, the galacto-oligosaccharides produced remained stable under MW irradiation, whereas they were hydrolysed under conventional heating conditions (Maugard et al. 2003).
3. Polymerization reactions – Enzymatic polymerization has emerged as a potential alternative to conventional chemo-catalytic methods, offering milder reaction conditions, selectivity, and reduced environmental impact. MW-assisted biocatalysis has proven its value in polyester synthesis, either through ring-opening polymerization and polycondensation. In both cases, the combined approach demonstrated advantages in reaction acceleration, energy efficiency, and maintenance of polymer properties, positioning as a green alternative to conventional heating for producing biodegradable polymers without compromising yield or polymer integrity (Kerep and Ritter 2006; Pellis et al. 2016).

7.1. Effect of microwave process conditions on enzymatic catalysis

As observed, the behaviour of enzymes in MW-assisted reactions depends on several factors. Thus, it becomes crucial to evaluate the effects of process parameters, including MW power, temperature, molar ratio (MR), catalyst amount, solvent type (solvent or solvent-free), immobilization strategies, mechanical stability, and biocatalyst reuse.

7.1.1. Effect of microwave power

In terms of setups, MW power is usually set to a low or moderate level to avoid continuous overheating. Studies often report using MW power levels of 100–300W, which are then stabilized to 30–50W, for enzymatic reactions (Yadav and Thorat 2012; Shinde and Yadav 2014; Shinde and Yadav 2015; Gupta et al. 2017; Aprile et al. 2023; Venturi et al. 2024). For example, in the synthesis of isopropyl myristate and palmitate, power levels ranging from 10% to 50% of a 900W output were used for the enzymatic esterification. They found an optimal power of around 20% (≈ 180 W), which yielded >94% conversion in less than an hour; higher power (>30%) led to lower conversion due to enzyme denaturation from overheating (Sitepu et al. 2023). Notably, at 20% power, they achieved a $\sim 95\%$ yield in 5 min of reaction, whereas at 50% power, the yield dropped despite faster initial heating. An interesting report concerns the enzymatic Biginelli reaction mediated by α -chymotrypsin under MW assistance. The MW power effect on enzymatic activity was monitored from 0 to 300W; the highest enzymatic activity was observed at 110W, with an 82-fold increase in reaction rate compared to conventional heating. Enzyme activity was drastically reduced at power levels below 90W, and only traces of the desired product were detected. For MW power above 110W, the reaction did not proceed faster; rapid protein denaturation occurred at 250W. The same behaviour was observed, underscoring the importance of selecting the appropriate power value for enzymatic MW-assisted syntheses (Xie et al. 2020; Sitepu et al. 2025).

7.1.2. Effect of temperature

Another key variable in biocatalysis is temperature, particularly for immobilized enzymes. In MW systems, both thermal and non-thermal effects are operative. In 2009, the synthesis of citronellyl acetate using the commercial immobilized lipase CalB was investigated over the 30–60°C temperature range. Reactions without an enzyme showed no conversion, excluding purely thermal MW effects. While conversion improved

with temperature, a decline in activity at 60°C suggested enzyme deactivation due to denaturation, so 50°C was selected as the more efficient temperature. Nevertheless, MWs' assistance permitted a 190-fold increase in the transesterification's frequency factor compared to the conventionally heated system (Yadav and Borkar 2009).

Enzymatic catalysis under MW conditions benefits from milder optimal temperatures than those of traditional chemical catalysts. An enantiomeric excess >97% was observed in the kinetic resolution of *rac*-1-phenylethanol using commercial immobilized lipase CalB, and similar conversions (51–54%) were achieved at 25 and 40°C (De Souza et al. 2009; Sheldon and Woodley 2018).

Interesting outcomes were obtained comparing MW and US-assisted chemoenzymatic esterification of xylytol. Both methods yielded 5-fold increases in productivity compared to conventional heating, suggesting a thermodynamic advantage and reaction equilibrium shift due to hotspot formation in both alternately heated systems (Rufino et al. 2010). Additional evidence came from 50°C solvent-free esterification reactions catalysed by lipase, achieving higher yields than at 70°C, particularly for less branched substrates, demonstrating that MW systems can reduce thermal input while improving efficiency (Risso et al. 2012).

The Design of Experiments (DoE) approach was employed to study the effects of temperature and the alcohol-to-oil MR on biodiesel production. At 1:8 MR, the optimal yield (86%) was observed at 43°C; at a higher MR (1:16), the best yield (67%) shifted to 57°C. This was attributed to the increased polarity of ethanol-rich systems, which require more energy and may destabilize the enzyme at lower temperatures. Moreover, a 6-fold increase in productivity was observed when comparing MW-assisted and conventional heating systems (Da Rós et al. 2013).

Subsequent studies on more complex substrates confirmed 60°C as the optimal MW-assisted reaction temperature for alkyl benzoate and geranyl cinnamate esters, with lower performance at 70°C due to enzyme degradation (Shinde and Yadav 2014; Shinde and Yadav 2015). A similar trend was observed in the enantioselective esterification of *R*-flurbiprofen with various linear alcohols, where activity increased up to 75°C, but enantioselectivity decreased beyond that point due to possible conformational changes in the enzyme (Gupta et al. 2017).

7.1.3. Effect of molar ratio and catalyst loading

It is now well established that reaction parameters such as MR and catalyst loading significantly influence reaction rates. In conventionally heated systems,

increased catalyst loading consistently enhances reaction performance, mainly due to the higher protein concentration in the reaction mixture (Rodrigues De Sousa et al. 2021). This increase statistically raises the frequency of enzyme-substrate interactions, thereby accelerating the reaction rate (Tacias-Pascacio et al. 2017; Khan and Rathod 2020b; Sousa et al. 2021). Given that approximately 90% of biocatalysis literature focuses on lipases, esterification and transesterification reactions have been extensively investigated. In these systems, a high excess of alcohol generally favours product formation (Khan and Rathod 2015; Parikh et al. 2019; Rodrigues de Sousa et al. 2020; Rodrigues De Sousa et al. 2021).

However, although these observations are well documented for conventional systems, the effects of MR and catalyst loading, both individually and synergistically, require specific evaluation under MW-assisted conditions.

An illustrative example is the MW-assisted (50W) synthesis of citronellyl acetate catalysed by a commercial immobilized lipase, CalB (Yadav and Borkar 2009). When evaluating the MR between vinyl acetate and citronellol, MRs above 1:2 (vinyl: citronellol) resulted in decreases in both conversion and reaction rate. This decline was attributed to citronellol's inhibitory effect on CalB, possibly *via* hydrophobic interactions. As the enzyme is hydrophobic, it may interact non-specifically with citronellol, thereby increasing the substrate's residence time. Furthermore, close contact with neighbouring hydrophobic residues may require partial dehydration of the enzyme-citronellol complex, potentially destabilizing the enzyme's native conformation and interfering with "molecular lubrication" (Yadav and Borkar 2009; Yadav and Thorat 2012; Shinde and Yadav 2014; Tiwari and Yadav 2025).

In a related study, the same research group reported similar results in the MW-assisted (50W) esterification of isoamyl alcohol with myristic acid, also catalysed by the commercial immobilized lipase CalB. In this case, hydrophobic interactions between the enzyme and the hydrocarbon chain of isoamyl alcohol were hypothesized to form inhibitory dead-end complexes, again reducing conversion and reaction rates. As MW power was kept constant across these studies, variations in performance cannot be attributed to differences in applied MW energy. Regarding catalyst loading, the commercial immobilized lipase CalB was tested at 0.19–0.58% (w/v), and reaction rates increased with enzyme concentration. However, increasing the load from 0.38% to 0.58% produced only marginal improvements in conversion, leading to the selection of 0.38% as the optimal concentration based on economic and

efficiency considerations (Yadav and Thorat 2012). A comparable trend was similarly observed during the MW-assisted transesterification of methyl benzoate with *n*-hexanol, catalysed by commercial immobilized lipase CalB (Shinde and Yadav 2014; Shinde and Yadav 2015).

These findings suggest that MW-assisted and conventionally heated systems exhibit similar MR and catalyst-loading behaviour. However, to move beyond the analysis of isolated parameters, a synergistic approach that accounts for variable interactions is necessary for a more accurate understanding of reaction optimization under MW irradiation.

Recently, the transesterification of methyl benzoate with benzyl alcohol to produce benzyl benzoate was studied using a DoE approach under MW conditions. MW power was held constant, while MR, temperature, and enzyme loading were varied. The resulting response surface indicated that both MR and enzyme loading had a significant positive effect on benzyl benzoate yield, as evidenced by the linear terms. However, their quadratic terms negatively affected conversion, suggesting diminishing returns at higher MR and enzyme concentrations. A kinetic study based on optimal DoE-derived conditions confirmed that further increases in MR or enzyme loading did not significantly enhance yield. This phenomenon may be linked to the interaction between commercial immobilized lipase CalB and MW energy in a solvent-free environment with excess alcohol, possibly resulting in localized overheating (hotspots) near the enzyme's catalytic site, thereby reducing enzyme activity (Aprile et al. 2023).

Further investigation into the MW-assisted transesterification of geraniol with various methyl and ethyl esters, using a DoE approach while maintaining constant MW power, was performed. Reactions were carried out both with and without 5 Å molecular sieves to mitigate the potential inhibitory effects of methanol and ethanol co-products. In the sieve-free system, temperature and enzyme loading were the most influential parameters, whereas in the sieve-containing system, MR and enzyme loading were the dominant factors. In both cases, the linear term of enzyme loading had the most significant positive effect. However, the corresponding quadratic term had a negative impact: for example, increasing enzyme concentration from 7% to 8.4% resulted in a slight decrease in conversion. This behaviour, already reported in the literature, may be due to diffusion limitations arising from excessive active site density (Shinde and Yadav 2015; Venturi et al. 2024). From the analysis of the collected data, the relative importance of the studied variables

for geranyl ester production under MW irradiation can be ranked as follows:

- Without molecular sieves: enzyme loading > temperature > MR;
- With molecular sieves: enzyme loading > MR > temperature (Venturi et al. 2024).

In both systems, catalyst loading played a critical role. Under solvent-free conditions, high substrate concentrations can enhance substrate-related inhibition, especially when lower enzyme amounts are used (Sousa et al. 2021; Venturi et al. 2024).

7.1.4. Solvent vs solvent-free systems

The absorbance capacity of organic solvents used in MW chemistry can be classified into three categories based on the $\tan \delta$ as high- ($\tan \delta > 0.5$), medium- ($\tan \delta = 0.1\text{--}0.5$), and low-absorbing ($\tan \delta < 0.1$). Highly polar solvents such as water, methanol, dichloromethane, and acetonitrile easily absorb MW irradiation due to their high dielectric constants. In contrast, non-polar solvents such as hexane, heptane, and iso-octane have no dielectric properties or are transparent to MWs and cannot be employed as solvents in MW-assisted reactions unless the reaction mixture contains other dielectric components, which are strongly MW-absorbing molecules/materials. The direct correlation between the solvents' absorbance and $\log P$ is briefly summarized in Figure 5.

MW-assisted enzymatic reactions have straightforward workups and reduced times, resulting in energy savings, improved enzyme stability, and reduced solvent consumption. Furthermore, in combination with solvent-free conditions, or employing "green solvents" (ionic liquids, eutectic mixtures, etc.) MW methods can offer an efficient, safer alternative to traditional synthetic chemistry, thereby confirming their environmental friendliness (Yadav and Devendran 2015; Sheldon and Woodley 2018; Liow et al. 2022).

It is well known that enzyme activity is higher in a non-polar environment ($\log p > 4$) and mid-polar solvents ($2 > \log p < 4$), whereas the lowest activity is expressed in polar solvents ($\log p < 2$). Non-polar solvents do not distort the essential water coat around the protein, thereby leaving the biocatalyst in an active state. The highest MW effect is expressed in polar solvents instead of non-polar ones, although polar solvents are anticipated as more "unfavorable" to enzymatic reactions under conventional heating, as already described in Figure 5.

To gain a better understanding of this effect, the isoamyl alcohol and myristic acid MW-assisted esterification under lipase catalysis provides an optimal

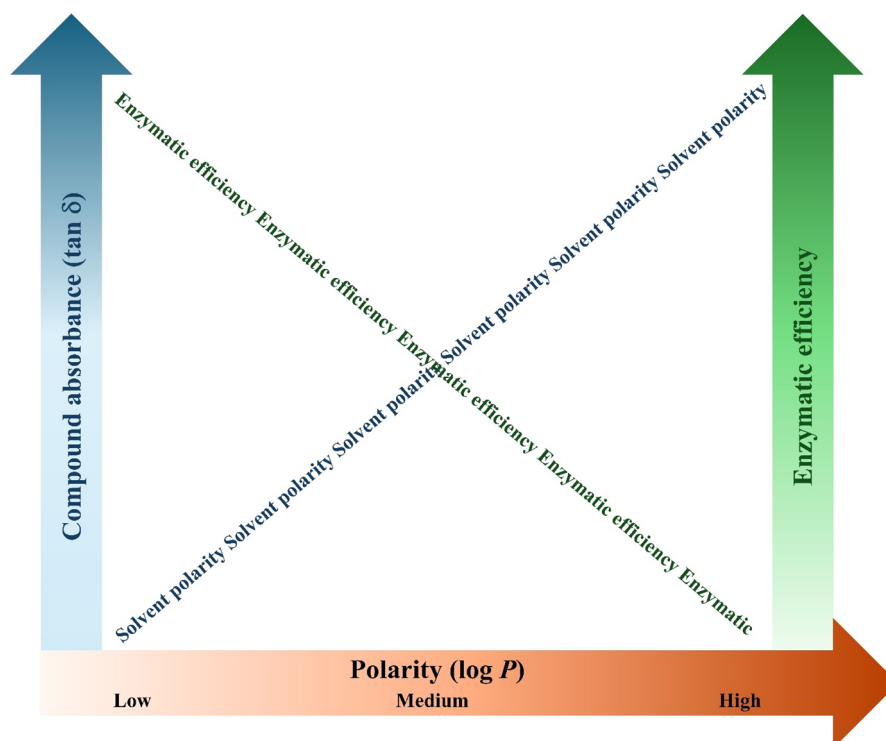


Figure 5. Representation of $\log P$ and MW absorbance correlation in a biocatalytic system.

example. Under solvent-free conditions, the enzyme microenvironment is surrounded by myristic acid, which has a high $\log P$ value of 5.8 and thus preserves the essential water coat, keeping the biocatalyst active. In particular, non-polar solvents, such as toluene, hexane, heptane, and 1,4-dioxane, possess very low dielectric constants, $\tan \delta$, and dielectric values, resulting in transparency to MW radiation. Solvent-free reactions demonstrated higher activities compared to reactions performed in non-polar solvents. MW irradiation exerts stronger selective heating of isoamyl alcohol due to its $\log P$ value of 1.03, which affects dipole molecular rotation and ion-induced polarization of this polar substrate; thus, energy transfer is direct and fast to the reactant in solvent-free conditions (Huang et al. 2005; Yadav and Thorat 2012).

Solvent influence (hexane; $\log p=3.76$, toluene; $\log p=2.68$) in a MW-assisted enzymatic system was also examined in the esterification reaction of oleic acid ($\log p=6.78$) with cetyl alcohol ($\log p=7.25$) catalysed by lipase. As described previously, the solvent-free condition provided an energetic boost to substrates, enabling higher reaction rates without compromising catalyst stability. An interesting outcome with the same substrates was obtained when cetyl alcohol was replaced with decyl alcohol ($\log p=4.57$). In particular, the initial reaction rate with decyl alcohol was slightly higher than with cetyl alcohol; however, the final

conversions in both cases were the same. The difference in reaction rates occurs because energy activation is directly proportional to molecular weight. Thus, decyl alcohol esterification had a higher initial rate due to its lower activation energy barrier compared to cetyl alcohol (Khan and Rathod 2020a).

Generally, enzymatic reactions are conducted in apolar media, and MW-assisted enzymatic reactions exhibit higher reaction rates in solvent-free systems where MW energy is directly coupled to the reactants. On the other hand, reactions employing polar substrates may require a non-polar solvent to mediate the strong MW-substrates interaction, which can destabilize the biocatalyst, leading to loss of activity.

7.2. Enzyme immobilization strategies for microwave systems

Using immobilized enzymes is a popular strategy in MW-assisted catalysis, as it provides greater enzyme stability and reuse potential under harsh conditions. Various supports and methods have been tested for MW synthesis. The widely used immobilized enzyme in biocatalysis, commercial immobilized CalB (immobilized on a macroporous acrylic resin), has been employed in numerous MW studies. Notably, commercial immobilized CalB has shown resilience under MWs. In MW-assisted biodiesel production from waste oil, a

commercial immobilized CalB retained ~88% of its initial activity after catalysing 6 successive batches, resulting in a 6-fold increase in conversion compared to conventional heating. This indicates minimal deactivation across multiple cycles, demonstrating that a robust immobilization can withstand MW exposure. The porous acrylic resin (a moderate thermal insulator) likely shielded the enzyme from rapid temperature spikes, and its hydrophobic surface can prevent the accumulation of water that might boil under MW (Panadare and Rathod 2016). Other studies, however, have found some loss of activity; for example, immobilized lipase PS from *Burkholderia cepacia* on epoxy-silica-PVA support lost ~50% of its activity after ~24 h of MW operation in biodiesel production (Souza et al. 2016). This disparity suggests that support material and MW coupling are important factors in determining the outcome. Resins supports that heat gradually or have good thermal conductivity (to spread heat) tend to protect enzymes better. In contrast, supports that localize MW energy (e.g. certain high dielectric materials) could create hotspots at the enzyme site. Empirically, enzyme immobilization can improve thermal stability under MWs because the enzyme is more rigidly held and often in a microenvironment that resists unfolding. For example, it was found that free CalB in solution exhibited biphasic inactivation under MWs (indicating some destabilization at temperatures above 60 °C) (Rejasse et al. 2006), whereas immobilized CalB showed improved stability under MWs compared to conventional heating (Rejasse et al. 2004). Immobilization likely prevents the enzyme from aggregating or unfolding as readily when “shock-heated” by MWs.

An interesting approach is to immobilize enzymes on supports that are susceptible to magnetic fields, such as magnetic nanoparticles, carbon materials, or metal-organic frameworks, thereby inducing localized heating. For instance, enzymes have been attached to magnetic iron oxide nanoparticles, which heat up under a MW field, effectively converting MW energy to heat directly at the catalyst (Kamel Ariffin and Idris 2022). This can improve heating efficiency in low-loss (non-polar) reaction mixtures. However, care must be taken to ensure that the support does not overheat and denature the enzyme. To date, most reported MW-enzymatic studies have used traditional supports (resins, silica, zeolites) rather than highly MW-active supports, likely to avoid overheating.

Enzyme activity and stability in low-water media depend on a small amount of bound water. Under MWs, this water can superheat. Some hybrid approaches include controlling the enzyme’s hydration state *via* support. For instance, a patent for enzymatic

interesterification under MWs suggests placing molecular sieves or an anhydrous salt in the reactor to scavenge excess water and maintain low a_w . In one implementation, enzymes on a support were mixed with 3 Å molecular sieves and a saturated salt solution in a closed MW reactor to buffer the water activity (Weijie et al. 2015). This can both protect the enzyme from MW-induced boiling of water and shift the equilibrium by removing excess water (useful in esterification). Immobilized enzymes on hydrophilic supports (like polysaccharide beads) may retain more water and be more prone to MW heating of that water; hydrophobic resin supports, conversely, keep enzyme hydration low. Thus, supports such as polypropylene or silica with hydrophobic silanization have been favoured in some MW enzymatic studies to minimize dielectric heating of excess water (Carrillo-Munoz et al. 1996).

Interesting information on enzyme-support interactions in a MW-assisted system was highlighted by comparing the commercial and nanopolystyrene immobilized lipase CalB. CalB immobilized on nanopolystyrene, which is more exposed to MWs, achieved yields similar to those of the commercial immobilized enzyme, albeit at lower enzyme loadings. While both systems shared an optimal temperature of 45 °C, CalB showed shorter reaction times due to its greater MW exposure. Using this technique, enzyme rigidity was improved, and heat- and MW-induced conformational changes were minimized (Bukhari et al. 2017).

7.2.1. Mechanical stability and reuse

During repeated MW heating cycles, immobilized enzyme preparations must remain mechanically stable. Most common supports (polymer beads, sol-gel entrapments, etc.) have demonstrated adequate structural integrity under these conditions. For instance, in the 6-cycle MW biodiesel test, the commercial immobilized CalB beads were filtered and reused without disintegration (Panadare and Rathod 2016). In another study on MW-assisted ester synthesis, an immobilized *Rhizopus oryzae* lipase on a macroporous resin was reused 5 times with only a slight loss of activity per cycle (Souza et al. 2016). These outcomes are comparable to conventional processes, indicating that MW heating itself does not necessarily destroy the support or enzyme when conditions are optimized. When enzyme activity does drop, it is often due to chemical inactivation (e.g. thermal denaturation or product inhibition) rather than MWs causing unique damage. Indeed, a review of enzyme inactivation kinetics under MW vs conventional heating noted that the kinetics follow similar Arrhenius behaviour, although rate constants may differ (Xu et al. 2016). Practical guidance is

that immobilized enzymes should be tested for leaching under MW conditions; some supports may soften or swell differently during MW heating. Sol-gel-immobilized and covalently attached enzymes generally perform well.

In continuous-flow MW systems, immobilization is essential to retain the enzymes within the reactor. Enzyme-coated tube reactors (where the inner wall of tubing is enzyme-coated) and packed beds have been tested at small scales, particularly in analytical applications such as protein digestion. These often use microfluidic channels or silica capillaries that can withstand MW fields and allow good contact with a MW source. For example, a continuous MW-assisted trypsin digestion device immobilized trypsin on silica monoliths within a capillary, achieving rapid protein cleavage while retaining enzyme activity across multiple runs. The findings from such systems indicate that immobilization strategies must withstand continuous MW exposure, which can cause gradual heating of the solid support. Ensuring cooling (*via* a jacket or coolant flow) in parallel with the MW input is crucial to prevent the immobilized enzyme from gradually overheating or denaturing (Ray and Rivera-Albarran 2023).

A relevant point concerns why the reuse capacity of immobilized enzymes under MW-assisted conditions is often comparable to, rather than clearly superior to, that observed under conventional heating, despite reports of improved enzyme stability under MW irradiation (Rejasse et al. 2004). This apparent discrepancy arises because enzyme stability and operational reuse capacity are related but non-equivalent descriptors. Enzyme stability primarily refers to the conformational and functional integrity of the protein under thermal or chemical stress, whereas reuse capacity is a system-level parameter that is governed by the entire enzyme-support-medium-conditions-reactor assembly. In immobilized biocatalysts, activity loss during repeated use may originate from enzyme leaching or desorption, support attrition, pore blockage, changes in water activity, substrate or product inhibition, accumulation of hydrophilic co-products, mass-transfer limitations, mechanical stress during catalyst recovery, washing, and drying (Rodrigues et al. 2021; Mohamad et al. 2015). These phenomena are not mitigated by MW irradiation *per se* and frequently become the dominant causes of activity decay after several reaction cycles.

An MW-induced improvement in enzyme stability does not automatically transfer into a higher number of reuse cycles. This expectation would only hold if bulk thermal denaturation were the dominant deactivation pathway, a condition rarely met for properly

immobilized enzymes operating within their typical temperature range (40–70°C), where thermal inactivation rate constants are already low under both heating modes. Indeed, when MW and conventional heating are rigorously compared at controlled temperatures, activity and stability are essentially indistinguishable (De Souza et al. 2009; Damm et al. 2012). The cycle-count metric itself is also partly misleading: under MW irradiation, enzymatic reactions typically proceed several-fold faster than under conventional heating (Yadav et al. 2008; Yu et al. 2010). Thus, the same nominal number of cycles corresponds to a substantially shorter cumulative thermal exposure of the biocatalyst, implying, on an equivalent-time basis, a higher operational stability that is masked when reuse is reported only in cycles. Conversely, when MW power is inadequately controlled, localized hot spots and selective heating of polar components may lead to dehydration of the enzyme microenvironment or support-related degradation, thereby offsetting the stabilizing effect (Da Rós et al. 2012).

This complex scenario is particularly relevant for widely used commercial immobilized lipases such as CalB, whose reuse performance depends not only on enzyme conformational stability but also on the properties of the acrylic support, including enzyme desorption, mechanical fragility under stirring, accumulation of water or co-product or product within the porous matrix, and compatibility with the reaction medium. These considerations are further accentuated within the MHH framework, discussed in Section 8, where the deliberate combination of dielectric and conductive energy sources challenges the binary distinction between MW and conventional protocols, making the number of cycle reuse an inadequate sole evaluation criterion. To capture the full impact of MW or MHH on biocatalyst performance, cumulative productivity, space-time yield, residual activity normalized to total operating time, and product formed per unit mass of enzyme should be considered jointly with the conventional cycle count.

7.2.2. Microwave-assisted immobilization

The use of MW irradiation has been shown not only to enhance reaction rates but also to drive the immobilization process. One study demonstrated that MW irradiation (2.45 GHz, 700 W) could immobilize several enzymes, including horseradish peroxidase, glucose oxidase, and urease, on a pre-activated polystyrene surface through covalent bonding. The study demonstrated that full immobilization was achieved within 60 s, compared with the conventional immobilization process (37°C for 45 min) (Nahar and Bora 2004).

In a different study, a lipase from *Candida rugosa* was effectively immobilized on Eupergit® support *via* MW irradiation. The significant reduction in immobilization time, from 2 days under conventional heating (22 °C) to a mere 2 min under MW irradiation (2.45 GHz, 100 W), resulted in only a minor decrease in lipase loading. Furthermore, the lipase immobilized on Eupergit® under MWs showed approximately 4-fold higher specific hydrolytic activity than the lipase immobilized without MW assistance. Moreover, in the esterification reaction, the MW-immobilized biocatalyst retained over 90% of its initial activity for approximately 100 h (Bezbradica et al. 2009).

Another example of a MW covalently immobilized enzyme is lipase CalB, which was immobilized onto mesocellular siliceous foams. The optimal MW irradiation conditions were determined to be 400 W, 0–4 °C, and 120 s, resulting in a 360-fold reduction in immobilization time. The specific activity of immobilized lipase under optimal MW irradiation conditions was 1.23-fold higher than that with a traditional optimal method without MW irradiation (Du et al. 2009). The same technique was exploited to immobilize different enzymes onto mesocellular siliceous foams. For instance, 2-deoxy-D-ribose-5-phosphate aldolase, thermolysin, papain proteinase, and penicillin acylase were immobilized, with the latter requiring only 140 s for complete immobilization, instead of the 15 h required by the conventional method (Wang et al. 2008; Wang et al. 2011; Chen et al. 2012).

The immobilization of two laccases and a glucose oxidase onto ceramic honeycomb and porous glass samples was achieved using MW irradiation. In the most favourable scenario, this method achieved a 20-fold reduction in immobilization time (Plagemann et al. 2014).

8. Microwave hybrid heating in enzymatic catalysis

The integration of MW heating with supplementary heat sources, known as MHH, represents a significant innovation in biocatalysis. MHH refers to the use of MW irradiation in conjunction with a conventional heating source (e.g. a thermostatic water jacket, oil bath, infra-red heater, or electrical resistance) to heat a reaction system (Figure 2). This technology combines the advantages of MW irradiation with conventional heating methods to enhance the efficiency, selectivity, and sustainability of enzymatic reactions (Table 3).

The literature demonstrates that these hybrid systems provide significant advantages over traditional methods, including shorter reaction times, enhanced

enzymatic activity, and improved thermal control. In particular, the hybrid approach overcomes key limitations of MW used independently, such as potential structural damage to the enzyme during prolonged exposure, thereby creating more favourable reaction conditions for biocatalysts. In enzymatic catalysis, these hybrid setups aim to combine the volumetric, rapid heating capabilities of MW with the uniform, controlled heating of traditional methods, thereby improving thermal homogeneity and enhancing reaction rates and yields while avoiding enzyme overheating or hotspots. This synergistic integration optimizes reaction conditions by balancing rapid energy input with controlled heat distribution.

In biocatalytic processes, MHH has proven especially advantageous because it preserves the structural integrity of enzymes during MW exposure (Horikoshi et al. 2016; Zarei et al. 2021). This approach has been recognized as both environmentally friendly and economically efficient, with the potential to improve the quality of final products compared with conventional methods (Zhang et al. 2024). The combination of MWs with other forms of thermal control enables rapid attainment of optimum enzymatic temperatures while minimizing protein denaturation.

Selecting complementary heat sources and integrating them with MWs is a critical aspect of optimizing MHH systems. The literature suggests that the most effective approach often involves conventional heating

Table 3. Comparison between conventional heating, MW, and MHH in enzyme-catalysed processes.

Feature	Conventional	MW	MHH
Mechanism	Conduction/Convection	Dipolar polarization/ionic conduction	Combination of conventional and MW
Heating characteristics	Slow/Uniform	Rapid/volumetric (potential hotspots)	Rapid/Volumetric with controlled bulk temperature
Reaction rate	Slow	Significantly/accelerated	Significantly accelerated (optimized)
Energy consumption	High	Lower	Optimized lower
Solvent requirement	Often high	Reduced/solvent-free	Reduced/Optimized
Product yield/purity	Variable	Improved	Optimized improved
Enzyme stability	Good (with temperature control)	Potential for denaturation (hotspot)	Improved/adjustable
Control/reproducibility	Established	Difficult (at scale-up)	Improved (with hybrid control)
Cost (equipment)	Moderate	High	High (complex configuration)
Alignment with green chemistry	Moderate	High	Very high

(such as water baths) combined with short MW irradiation (Horikoshi et al. 2016). This configuration leverages the advantages of both methods: conventional heating provides stable, uniform thermal control, while MWs provide additional energy input that can enhance catalytic activity. The timing of heat source application is equally important. In some cases, conventional preheating followed by short MW pulses has proven more effective than simultaneous application. In other scenarios, circulating systems that alternate between MW-heated zones and conventional cooling zones have shown superior performance (Xie et al. 2020). These strategies enable more precise thermal control, which is crucial when working with enzymes that operate within narrow optimal temperature ranges.

A notable example concerns catalase, whose activity under continuous MW exposure proved highly transient, with rapid inactivation occurring in less than 3 min. By contrast, the same enzyme exhibited markedly greater stability when heated conventionally in a thermostatic water bath. However, when MW irradiation was applied in short pulses while maintaining the bulk solution at 25–40°C *via* conventional heating, catalase activity was preserved, and H₂O₂ decomposition proceeded with accelerated kinetics (Horikoshi et al. 2016). This behaviour illustrates how synergistic energy inputs can mitigate the destabilizing effects of MWs while harnessing their capacity to enhance reaction rates.

In fact, such hybrid systems outperformed MW-only conditions: the enzyme benefitted from the MW's initial rate enhancement, while the conventional heating jacket prevented localized overheating (Horikoshi et al. 2016). This observation highlights the importance of controlling the spatial and temporal distribution of energy input in enzymatic systems. Indeed, uncontrolled MW heating can damage enzymes; therefore, hybrid strategies are required to modulate MW intensity and prevent deactivation.

More broadly, successful MW-assisted biocatalytic reactions are often reported at temperatures of 40–60°C, where MWs accelerate thermal equilibration or generate transient, localized hotspots. However, the catalase example reveals an important exception: maximal activity was achieved at a lower bulk temperature (25°C) when MWs were applied as short, pulsed bursts. This suggests that, contrary to conventional heating, optimal conditions in hybrid systems may involve cooler bulk environments combined with temporally modulated MW input. Such strategies are particularly advantageous when the reaction medium exhibits low MW absorptivity (e.g. non-polar systems).

In these cases, conventional heating ensures temperature control and homogeneity, while MWs

contribute to internal heating, enhancing overall heat transfer and potentially inducing additional non-thermal effects (Zieliński and Dębowski 2018). Overall, MHH can enhance enzymatic activity, especially in low-water environments, without compromising stability, provided that MW intensity, exposure time, and bulk temperature are carefully controlled. Short MW bursts or low-power irradiation combined with conventional heating appear to be key parameters for maintaining enzyme structure while maximizing reaction rates (Horikoshi et al. 2016).

8.1. Comparative kinetic data: Microwave hybrid vs. microwave-only

When evaluating MHH vs. MW-only operation, the defining advantage of the hybrid mode lies less in raw kinetic acceleration than in the preservation of enzymatic function through controlled heating. Hybrid systems may sacrifice a fraction of initial speed, yet they prolong catalyst lifetime and thereby enhance overall process efficiency. For instance, in Horikoshi's catalase experiments, the water-bath plus MW configuration achieved the best compromise, combining a rapid initial rate with sustained enzyme activity, clearly outperforming both MW-only operation (rapid onset followed by swift inactivation) and conventional-only heating (slower but stable performance) (Horikoshi et al. 2016).

This trade-off underscores the central feature of MHH. While peak rates may be slightly lower than under unrestricted MW irradiation, the cumulative turnover is substantially higher because enzymatic integrity is maintained. Importantly, the presence of an auxiliary heating element does not markedly slow the reaction, since MWs still provide the dominant energy input. Instead, it prevents the precipitous loss of activity that continuous high-power MWs would otherwise inevitably trigger.

The kinetic advantage becomes evident in repetitive or extended runs. This effect is illustrated by immobilized lipase systems, which retained ~88% of their activity after 6 successive MW-assisted batches when operated under hybrid control (Panadare and Rathod 2016), whereas a comparable enzyme exposed to harsher, MW-only conditions lost nearly 50% of its activity in a single prolonged batch (Souza et al. 2016). These data indicate that in well-engineered hybrid systems, overall productivity, measured as product yield per unit enzyme, can be superior to both conventional heating and MW-only protocols. From a process perspective, reactors that integrate MW and conventional elements deliver the most balanced performance, combining rapid kinetics with stable, uniform, and selective catalysis.

9. Reactor design, scale-up, and process considerations

The key component of MW reactors is the magnetron, a thermionic valve that generates high-power MW, typically at a fixed frequency of 2.45 GHz. Oscillating electromagnetic waves are produced by the interaction between a stream of electrons generated from a high DC voltage and a magnetic field induced by two ring magnets around a magnetron. Both household MW ovens and laboratory-scale MW reactors frequently utilize this component due to its efficiency and compact size.

MW-assisted organic synthesis involves two types of reactors: multi-mode and single-mode reactors (Figure 6). Depending on the application, batch and flow reactors can be used in either mode. Among these, single-mode MW systems consist of a restricted cavity in which a uniform electromagnetic field is generated. In this configuration, MW within the cavity or at a particular spot are well defined, and the reactor is located inside a waveguide with the highest MW concentration. Samples are uniformly irradiated, making this configuration particularly suitable for small-scale or highly controlled reactions. The scalability of this system is limited by the waveguide size and δ_p , resulting in higher system costs. To address this limitation, the use of 75 kW-915 MHz in continuous-flow reactors for extraction allows the use of larger waveguides due to the higher δ_p (Priecel and Lopez-Sanchez 2019).

Multi-mode MW reactors generate a distributed, less homogeneous MW field within a contained space, where MW is reflected in random directions. Multi-mode reactors are ideal for parallel synthesis, scale-up studies,

or bulk processing due to their ability to accommodate multiple or larger vessels (Jiao et al. 2025).

In contrast, laboratory-scale MW mono- or multi-batch reactors were introduced as valuable tools for kinetic studies and small-scale synthesis. The first-generation multi-batch reactors, which featured magnetic stirring and direct monitoring of temperature and pressure inside the reaction vessel, were constrained by operational limits (up to 200 °C and 1 MPa) and lacked functionalities such as sample withdrawal during reaction, inhomogeneity field, or efficient post-reaction cooling. These limitations were later addressed with the advent of more advanced multi-batch reactors, designed to handle higher temperatures and pressures, thereby enabling safer, more versatile, and better-controlled MW-assisted synthesis on a laboratory scale (Raner et al. 1995).

While MW batch reactors are well-suited for discrete reactions and mechanistic studies, they are not ideal for scale-up or continuous production. In this context, continuous-flow MW reactors overcome these limitations by enabling precise control over reaction parameters (e.g. temperature, pressure, and residence time) while maintaining efficient energy transfer. The synthesis of Laurydone, or the condensation of benzoic acid with ethanol, carried out in flow systems, achieves higher yields and faster conversions than batch methods (Pipus et al. 2000). The flow approach allows operation at elevated temperatures (up to 200 °C) and pressures (14 bar) without the use of solvents, thereby minimizing side reactions and simplifying purification. Additionally, flow reactors facilitate catalyst recycling (e.g. Amberlyst-15) and integration with inline monitoring, demonstrating superior scalability and safety for industrial applications (Baxendale et al. 2007).

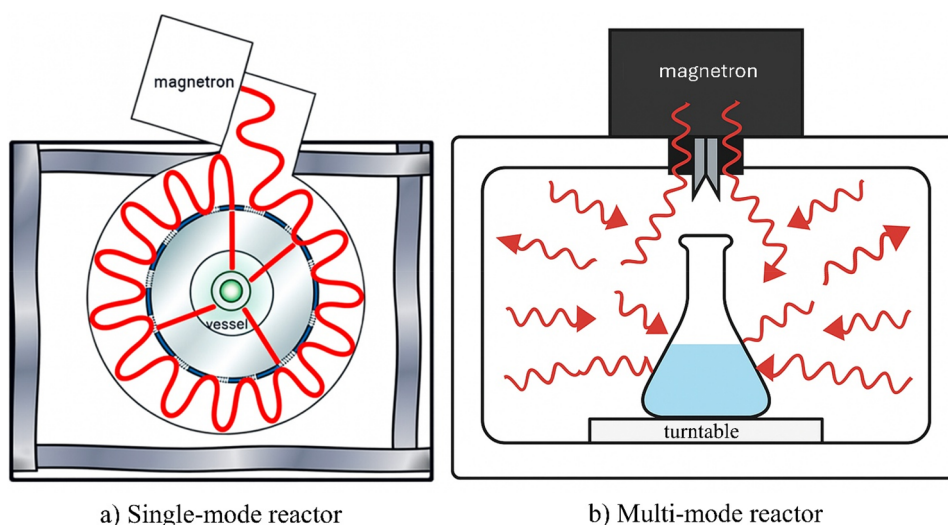


Figure 6. Representation of the two main MW reactors used in organic synthesis.

In parallel, researchers have explored various reactor configurations to implement MHH for biocatalysis. Most laboratory investigations rely on batch configurations, ranging from modified domestic units to dedicated instruments. The vessel is often equipped with an external jacket or insulation for heating and cooling. One reported MHH configuration employs a standard MW cavity combined with a surrounding water-bath jacket, which maintains the bulk solution at a controlled temperature while the MWs irradiate the reaction flask, thus exemplifying a clear hybrid design (Horikoshi et al. 2016).

Many commercial MW synthesis platforms incorporate temperature feedback systems in which an infra-red sensor or fibre-optic probe continuously monitors the bulk temperature and modulates MW power *via* pulsing to maintain the set conditions. Although not always explicitly described as a hybrid system, such an operation effectively alternates between active MW irradiation and passive thermal maintenance, closely resembling conventional resistance heating. Some advanced reactors even integrate additional heating elements, such as convection fans or infra-red sources, to ensure uniform surface heating in the vessel (Rakesh et al. 2009; Gamit and Chudasama 2020). These implicit hybrid designs enhance thermal homogeneity, a crucial factor for enzymatic reactions, as local hotspots could otherwise compromise the biocatalyst's stability.

Building on this concept of thermal control and hybridization, continuous reactor designs for enzymatic catalysis, particularly when enzymes are immobilized, are of considerable industrial interest. The integration of MW irradiation into such systems presents clear challenges; however, some approaches have been explored. One configuration involves a fixed-bed column packed with immobilized enzyme, positioned within a MW waveguide or cavity. Simulations of this setup indicate that it provides more uniform heating compared to single-source MW exposure, particularly when combined with conventional or auxiliary heating to stabilize the temperature (Wongyai et al. 2025).

In line with this finding, experimental demonstrations of hybrid continuous reactors have shown that coupling MWs with conventional heating can markedly improve thermal uniformity in flow. For instance, a bioreactor divided into two functional zones, with an upper section heated by MWs *via* a waveguide and a lower section stabilized by a hot-water jacket, illustrated how such complementary energy inputs mitigate gradients. At mesophilic conditions ($\sim 35^{\circ}\text{C}$), MWs alone maintained adequate uniformity, except for slight overheating below the waveguide, whereas at thermophilic

conditions ($\sim 55^{\circ}\text{C}$), the addition of jacket heating counterbalanced the profile, resulting in consistent bulk conditions (Zieliński and Dębowski 2018). This exemplifies how a hybrid approach, distributing MW and conventional inputs across different reactor sections, can achieve stable operation in continuous systems.

Beyond laboratory-scale demonstrations, a patent describes a flow system for enzymatic processes in which MWs accelerate biomass hydrolysis by directly heating slurries as they pass through the reactor (Cullingford et al. 1991). Such designs highlight both the potential and the engineering difficulties of continuous MW-assisted biocatalysis. Among these, the most important challenge is to avoid arcing or localized hotspots in flowing streams and to ensure that immobilized enzymes remain within a stable, optimal temperature range for activity.

A semi-continuous fed-batch configuration could offer a pragmatic solution when an immobilized lipase is sensitive to both substrate overload and prolonged MW exposure. The practice of dosing small aliquots of methanol or another acyl donor into a packed-bed bioreactor is already well described in the biodiesel literature. Complementing each feed with a brief MW pulse may confer two additional benefits: first, step-wise dosing prevents the sharp alcohol spikes that inactivate commercial immobilized CalB; second, the pulse rapidly restores the slurry to its set-point temperature without subjecting the enzyme to a continuous electromagnetic field.

This safeguard becomes crucial whenever MW irradiation or jacket heating threatens the stability of either catalyst or substrates. Under these circumstances, pulsed MW assistance acts as an adjunct, combining fast inside-out heating with gentle, temperature-controlled dwell times. Two strands of evidence underpin this hybrid feed-pulse-rest concept. First, repeated methanol-feed regimes preserve higher residual CalB activity and yield greater biodiesel than single-shot additions. Second, power-cycling studies, e.g. 50W bursts followed by forced-air cooling at 60°C , during lipase-mediated transesterification, minimize local overheating and extend catalyst lifetime (Queiroz et al. 2015; Sandoval et al. 2017). Collectively, these findings indicate that a controlled MW pulse can re-establish reaction temperature almost instantaneously, promote consumption of each substrate increment before inhibitory concentrations build up, and then allow the enzyme to function under mild, stable thermal conditions. Although optimization of pulse duration, duty cycle, and feed interval remains required, these observations point to a promising strategy for this type of reaction system.

Although the US is not a heating source in a strict sense, it has been integrated with MWs into so-called US-MW reactors, which have been explored as a strategy for process intensification in enzymatic catalysis. In such systems, the US enhances mixing and localized heating, thereby complementing the bulk and selective energy delivery of MWs. The combined effect is often manifested as faster kinetics and more efficient mass transfer towards the enzyme. From a mechanistic standpoint, this configuration constitutes a multimodal reactor that couples electromagnetic and mechanical energy, rather than a combination of two thermal inputs. Such systems fall within the broader class of hybrid biocatalysis techniques. Notably, the joint application of MW and US has been shown to significantly accelerate lipase-catalysed transesterifications. Reactor designs typically employ a MW-transparent vessel that is directly accessible to a probe sonicator within the MW cavity. However, careful engineering is required to mitigate risks associated with sparking caused by metallic components (Cravotto and Cintas 2007).

Overall, by improving heat and mass transfer, MW/US coupling offers a versatile approach to greener, more efficient chemical processes. These advantages are closely linked to the reactor design employed. In simultaneous MW/US setups, temperature control is crucial for maintaining cavitation performance, typically achieved with a cooling jacket using MW-transparent fluids, such as silicone oil or Galden®. On the other hand, flow reactors with sequential MW and US zones have been developed, allowing the circulation of the reaction mixture through a MW chamber and then an US cell equipped with a conventional metallic horn, which expands the applicability of the hybrid system to thermally sensitive substrates (Wang et al. 2014).

In all these designs, the central idea is to achieve uniform and controllable heating. Conventional heating tends to warm from the outside in, whereas MW heating is volumetric (from the inside out) (Zieliński and Dębowski 2018; Singh and Sehgal 2024). By combining them, hybrid systems mitigate the drawbacks of each, eliminating thermal gradients associated with MW heating (no cold core) while the external heating/circulation prevents cool spots on the walls and helps distribute heat evenly. This is particularly important for immobilized enzyme reactors, where channelling or uneven flow, combined with uneven heating, could deactivate portions of the biocatalyst bed. Many hybrid reactors for enzymes also incorporate stirring, or recirculation pumps, to maintain a homogeneous temperature. Batch MW reactors typically feature magnetic stirrers, while continuous systems often employ static mixers.

It is worth noting that safety and scale-up are key concerns when considering MHH reactors. Industrial MW and MHH reactors for chemical or enzymatic processes are still relatively rare. As MW reactor designs evolve, more examples of integrating external heat exchangers or resistive heaters to help control temperature in large-scale enzymatic and chemical processes will emerge. In summary, temperature-controlled batch MW reactors are more common for enzyme work (explicit or implicit hybrids), while continuous MW reactors are an emerging technology that requires careful design but shows promise, for example, in online digestion and flow biocatalysis.

Another important parameter for batch and flow reactions is enzyme immobilization, which is highly recommended for enzymatic processes in MW and MHH systems. It enhances stability, enables enzyme reuse, and facilitates certain hybrid strategies, such as fixed-bed reactor designs and water activity control. Common immobilized enzymes (lipases on various supports) have shown good performance in MW fields, especially when paired with hybrid temperature control to prevent overheating. Future developments may involve specialized supports optimized for MW catalysis, such as materials that evenly distribute MW energy or possess selective heating properties, to further enhance enzyme performance. Even with current technology, studies have demonstrated that immobilized enzymes can retain high activity over many MW cycles (Khan and Rathod 2018), enabling efficient processes in fine chemicals, biofuel production, cosmetics, and pharmaceutical synthesis under MHH.

Despite these advantages, important challenges remain when scaling up MW-assisted systems. In general, the main limitations for scaling up MW technology are the limited penetration depth of MW radiation into absorbing materials, such as solvents or reaction mixtures, and the non-uniform temperature distribution (Stadler et al. 2003; Horikoshi and Serpone 2019; Liow et al. 2022). These drawbacks also affect the scale-up of biocatalytic systems, especially the inhomogeneous temperature distribution, which is particularly critical given the limited thermal stability of proteins. The combination of MW irradiation with acoustic waves (US) or mechanical stirring (Khan and Rathod 2018) has been adopted to increase δ_p and intensify mass and heat transfer in small-batch reactor vessels, but controlling temperature in MW-irradiated batch reactors larger than 1 liter remains a critical point.

Given these limitations, most large-scale MW-assisted syntheses reported in the literature rely on continuous-flow reactors (Martina et al. 2021), in which the reaction mixture is pumped through a MW-transparent coil

located in the MW cavity. However, to the best of our knowledge, there is no commercial chemical manufacturing process that relies on or includes biocatalytic reactions performed under MW irradiation. MW technology and flow chemistry show some complementary features. Flow processes typically require rapid reactions to ensure high productivity, and MW irradiation is a valuable strategy for accelerating reactions. On the other hand, continuous-flow processes favour better temperature control, a critical aspect in MW-assisted reactions. Furthermore, a process optimized in a single MW-assisted continuous-flow reactor can be readily scaled to large volumes *via* a number-up strategy (Dong et al. 2021) by placing multiple continuous-flow reactors in multi-mode MW ovens.

Despite this, most of the MW-assisted biocatalytic reactions reported in the literature are still performed in small-volume batch reactors (Khan and Rathod 2018; Das and Banik 2024). The only example of a MW-assisted continuous-flow biocatalytic process remains the demonstration of the synthesis of (*R*)-1-phenylethyl acetate, promoted by an immobilized lipase. In this study, the authors carefully optimized the MW-assisted continuous-flow apparatus to maximize the synthesis of the enantiopure ester *via* enzymatic kinetic resolution of the corresponding alcohol, and compared the results with those obtained with a conventionally heated batch (stirred tank reactor). Key parameters, including residence time distribution, flow and mixing (Reynolds number), and heat transfer (Nusselt number), were investigated using reactor tubes of different diameters and shapes (with or without inner blades) and various solvents. After this, the batch-loop setup was envisaged as the best choice because it offers the typical advantages of a flow system in terms of δ_p and allows modulation of residence time without altering the reaction tube dimensions. With this setup, the enzymatic kinetic resolution was successfully performed, yielding about 22 g h^{-1} of (*R*)-1-phenylethylacetate. Even if conducted on a lab scale, this study provides a valid approach to evaluate the feasibility and convenience of implementing a biocatalytic MW-assisted flow process, which can be easily scaled up through parallel circuits (number-up strategy) (Dressen et al. 2010). This example also employs a heterogeneous biocatalyst (a commercial immobilized lipase CalB).

However, beyond technical limitations, economic factors also play a decisive role. The scarcity of commercial MW-assisted synthetic processes stems from their non-economic nature, and the limited adoption of biocatalysis in chemical manufacturing, in turn, explains the absence of studies on the scalability of

MW-assisted enzymatic processes. The switching of a conventionally heated large-scale process to a MW-irradiated version requires a significant investment in the design and implementation of a completely new platform with technical solutions and components not available in the market. This means that a thorough cost-benefit analysis is needed before comparing the optimized conventionally heated process with the MW-assisted one, at least on the pilot scale. The most claimed benefit of MW technology is the reduction in energy demand achieved through shorter reaction times. In this sense, the reaction's acceleration must be significant, since, starting from traditional energy sources such as gas, the energy costs for MW heating are higher than those for conventional industrial heating (condensing steam) due to the lower conversion efficiency (Dressen et al. 2010). Unfortunately, a detailed energy-cost analysis is lacking in most of the literature on this topic.

In this context, hybrid solutions have emerged as promising alternatives. The MHH, which combines conventional heating with MW irradiation to integrate the advantages of the two heating modes while mitigating their drawbacks, is a simple, modern approach that is gaining attention in materials science and lab-scale chemical applications, including biocatalytic processes (Horikoshi et al. 2016; Zhang et al. 2024; Balyan et al. 2024; Leite et al. 2024). The MHH can facilitate the diffusion of MW irradiation in large-scale production processes. Several examples of continuous-flow processes on the lab scale have been proposed, in which the two heating modes are applied simultaneously or sequentially, with a consecutive section of the reactor coil subjected to MW irradiation and conventional heating (Zieliński and Dębowski 2018; Patel et al. 2024). Given the critical thermal stability of various enzymes, MHH is forecast to be a superior technology for future design and implementation of biocatalytic MW-assisted continuous-flow processes.

10. Conclusions and research directions

MW-assisted biocatalysis has emerged as an innovative approach to eco-friendly organic synthesis, combining the efficiency of MW irradiation with the high selectivity and mild conditions of enzymatic processes. This combination has demonstrated important advantages, including reduced reaction times, higher energy efficiency, improved yield and selectivity, and lower by-product formation, making it a promising alternative to conventional synthesis methods.

The literature on MW-assisted biocatalysis has shown remarkable advances, particularly in

esterification and transesterification reactions, which are mainly catalysed by lipases and carried out in batch reactors. These studies reveal improvements in reaction rate and selectivity, as well as in process performance under solvent-free conditions. However, despite this progress, the number of reactions and enzymes explored remains limited. To the best of our knowledge, few, if any, applications have been tested with oxidoreductases, lyases, ligases, transferases, proteases, cellulases, etc., which would considerably expand the diversity of reactions enabled by MW technology.

A critical analysis of the literature indicates that improvements result from thermal intensification and more efficient process control. In contrast, the so-called "non-thermal effects" remain controversial across different systems studied. Furthermore, although MW and MHH have demonstrated the potential to increase the stability, activity, and selectivity of biocatalysts, the underlying molecular mechanisms remain poorly understood.

Despite the progress achieved, the applications of MW technology at pilot and industrial scales are limited, in part due to issues related to electromagnetic field penetration, thermal control, enzymatic sensitivity, and the complexity of reactor engineering. In this context, MHH emerges as a promising approach, combining rapid MW heating with the stability provided by conventional heating. Ultimately, collaboration between academia and industry is crucial to validate and commercialize these processes, thereby ensuring their economic viability.

For the MW-assisted biocatalysis to advance more robustly, several open questions remain and need to be investigated:

1. The non-thermal effects of MW on protein structure and enzymatic activity remain controversial and require further clarification.
2. Expansion of enzyme classes – how do little-studied or unexplored enzymes (oxidoreductases, transferases, isomerases, proteases, cellulases, etc.) behave under MW/MHH? Could they reveal new types of accelerated reactions or unique selectivity?
3. Use of bio-based solvents or natural deep eutectic solvents (NADES) – the compatibility between green solvents and MW irradiation, considering dielectric absorption, stability, and enzyme selectivity, remains lacking systematic studies. MW with green solvents integration can achieve a balance between catalytic efficiency and sustainability.

4. Interaction between immobilization, support materials, and the electromagnetic field – the literature shows that supports differ significantly in MW absorption. However, models that predict hot spots, thermal gradients, and their effects on protein stability remain scarce. Exploring new support materials, such as nano-materials with tuneable dielectric properties, can enhance the enzyme's stability and reusability.
5. Kinetic and thermodynamic modelling under MW – the determination of kinetic parameters remains limited, making it difficult to distinguish between purely thermal effects and specific effects. Such studies are essential for understanding the dynamics of reactions intensified by MW and for designing reactors for large-scale production.
6. Optimization of reaction parameters – the presence or absence of solvents, temperature, agitation, reaction time, molar ratio of substrates, free or immobilized enzyme, residence time in continuous processes, and irradiation power must be optimized to maximize biocatalytic efficiency.
7. The combination of MW with other process intensification techniques, such as ultrasound or photocatalysis, should also be explored to develop more versatile and efficient synthetic routes.
8. Integration with continuous flow processes – how can MW heating be adapted to continuous flow reactors, maintaining thermal uniformity, enzymatic stability, and homogeneous distribution of the electromagnetic field? Industrial viability depends directly on the answer to this question.
9. Scalability and energy efficiency – under what operating conditions can MW overcome the high energy cost compared to steam or resistive heating on an industrial scale?

Thus, the evidence gathered indicates that MW-assisted biocatalysis is a recent technology at the laboratory scale and requires systematic investigation, expansion of the enzymatic repertoire, development of new enzyme-support materials, and integration with continuous processes to enable its complete application in sustainable synthesis. With advances in these areas, the application field has the potential to transform chemical processes on an industrial scale, consolidating itself as a fundamental tool in green chemistry and the circular economy.

Author contributions

CRedit: **Lindomar Alberto Lerin**: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing; **Domenico Meola**: Data curation, Formal analysis, Methodology, Writing – original draft; **Simona Aprile**: Data curation, Writing – original draft; **Francesco Presini**: Data curation, Methodology, Writing – original draft; **Federico Zappaterra**: Data curation, Formal analysis, Writing – original draft; **Pier Paolo Giovannini**: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used DeepSeek, ChatGPT, and Grammarly in order to proofread and improve the text. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

Disclosure statement

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

Funding

The authors acknowledge the University of Ferrara (FAR/FIRD funds) and the National Recovery and Resilience Plan (NRRP), Mission 04 Component 2 Investment 1.5-NextGenerationEU, Call for tender n. 3277 dated 30 December 2021. Award Number: 0001052 dated 23 June 2022, for financial support.

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