

Harnessing Microbe-Mediated Nanoparticles To Overcome Lignocellulosic Recalcitrance In Bioenergy Systems

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Abstract

The most abundant renewable feedstock for producing sustainable biofuels is lignocellulosic biomass. Nonetheless, structural resistance, enzyme inhibition, and expensive downstream processing continue to limit its industrial conversion. Utilising biogenic microbe-mediated nanoparticles (BMNPs), this study suggests a novel, microbe-centric approach to (i) improve lignocellulose accessibility, (ii) stabilise and recycle hydrolytic enzymes, and (iii) detoxify fermentation inhibitors as part of an integrated bioprocess. Three novel ideas are presented by employing filamentous fungi and genetically tractable bacteria to generate metal-oxide and functionalized magnetic BMNPs in situ at biomass–microbe interfaces, generating self-assembling nano-hotspots for catalysis. Secondly, developing BMNPs capped with native microbial EPS to improve enzyme orientation and retention while decreasing ineffective binding to lignin.

Also, utilising BMNP-mediated redox regulation to neutralise inhibitory phenolics and break down certain lignin linkages during saccharification. In addition to BMNP recyclability and ecotoxicity profiling, bench-scale validation is proposed.

This approach combines microbe-derived Fe₃O₄ and TiO₂ BMNPs with immobilised cellulase–ligninase cocktails. The goal is to quantify improvements in saccharification rate, fermentable sugar yield, and ethanol/butanol productivity. According to preliminary research, BMNPs improve enzyme stability and enable magnetic recovery. In contrast to chemically manufactured NPs, microbial manufacturing pathways significantly reduce environmental costs and improve biocompatibility. By lowering enzyme loading and the intensity of chemical pretreatment, this integrated microbial-nanotechnology paradigm may enhance the techno-economic and ecological characteristics of lignocellulosic biofuels. Recent research on biogenic NP production, magnetic-NP enzyme immobilisation, and NP-assisted catalytic hydrolysis provides the foundation for important load-bearing claims and design decisions.

Keywords: Biogenic microbe-mediated nanoparticles (BMNPs), microbial nanotechnology, nano-hotspots, cellulase-ligninase cocktails and extracellular polymeric substances (EPS)

INTRODUCTION

The transition to renewable bioenergy has made LCB an enticing alternative due to its high carbon content, non-food status, and abundance. However, recalcitrance that prevents enzymatic hydrolysis and fermentation is produced by the complex, hydrophobic matrix of lignin, crystalline cellulose structure, and hemicellulose heterogeneity. Pre-treatments, whether chemical, thermal, or enzymatic, help to reduce this, but they are expensive, produce inhibitors, and jeopardise the sustainability of the environment. Therefore, in order to increase saccharification efficiency while decreasing pretreatment intensity, innovative technologies are required (Dutta et al. 2023; Nair and Verma 2025).

At low concentrations, NPs provide structural, catalytic, and adsorptive benefits because of their high surface-to-volume ratio. For example, lignin–carbohydrate complexes are disrupted, magnetic recovery is made possible, and enzyme stability is enhanced by FeO₄ and TiO₂ NPs. The incorporation of nanocatalysts into hydrolysis has been demonstrated to improve the release of fermentable sugars and boost ethanol yields by 30–40%. Concerns about sustainability and toxicity are raised by the fact that the majority of NPs used now rely on chemically produced NP (Dutta et al. 2023; Ingle et al. 2025).

Microbes are biological nanofactories that may naturally produce NPs with proteins or polysaccharides as a cap in ambient circumstances. Metal ions are reduced into NPs with regulated size and surface functionalization by actinomycetes, fungi (*Aspergillus*, *Fusarium*), and bacteria (*Pseudomonas*, *Shewanella*). This method avoids using dangerous reagents and is scalable, adjustable, and environmentally friendly. In addition to their catalytic qualities, BMNPs can be designed to localise at biomass surfaces and create "nano-hotspots" where hydrolysis is most required (Carmona et al. 2023; Kapoor et al. 2021).

BMNP capping mediated by microbial EPS: When mounted on BMNPs, EPS can naturally orient cellulases and ligninases, minimising the binding of ineffective enzymes to lignin. BMNP-mediated detoxification: Iron oxide and TiO₂ BMNPs can perform modest Fenton-like or photocatalytic reactions, degrading phenolic inhibitors during saccharification. *In situ* microbial nano-hotspots: Without the need for external NP addition, engineered microorganisms may produce BMNPs at lignocellulose interfaces to create catalytic microenvironments. Incorporating BMNPs into LCB can be accomplished by Pretreatment reduction, using microbial nano-catalysis to decrease lignin barriers. Enzyme reuse: cellulase–ligninase immobilisation and recovery are made possible by magnetic BMNPs, which reduces the expense of enzyme loading (Ingle et al. 2025; Sulis et al. 2025).

According to recent assessments, the use of NT in biofuel production enhances yield, enzyme stability, and process efficiency (Dutta et al. 2023; Ingle et al. 2025). Simultaneously, microbial NP production is becoming more popular as an inexpensive, environmentally friendly substitute (Mariño, Fulaz, and Tasic 2021). New approaches like incorporating machine learning into biorefineries point to the possibility of building and optimising BMNPs *in silico* (Nair and Verma 2025). However, there is a dearth of thorough research on microbe-derived NPs specifically for overcoming lignocellulosic recalcitrance in the literature, which makes this a novel and highly significant area of study.

Biosafety assessment is still crucial even if BMNPs are more biocompatible than chemical NPs. Designing degradable nanostructures, magnetically retrieving BMNPs to reduce release, and choosing non-pathogenic chassis are other strategies. To show actual sustainability, lifecycle assessments must strike a balance between lower chemical pretreatment and BMNP production costs. Few studies have found a connection between improved enzyme-substrate interactions in biomass hydrolysis and the functionalization of BMNPs. There is no experimental evidence to support the idea of *in situ* microbial nano-hotspots. The incorporation of BMNP systems into actual biorefineries is still at the conceptual stage (Kapoor et al. 2021; Singhvi and Kim 2020).

BMNPs can be used to: (1) Reduce the amount of enzymes by 30 to 50%. (2) Reduce the use of pretreatment chemicals considerably. (3) Increase the output of biofuel and sugar release by 25–40%. (4) Combine NT and microbial biotechnology to promote the circular bioeconomy. All things considered, microbe-mediated NPs provide a revolutionary, environmentally friendly technique to break down lignocellulosic resistance, connecting NT and microbial bioprocessing to the next generation of sustainable energy.

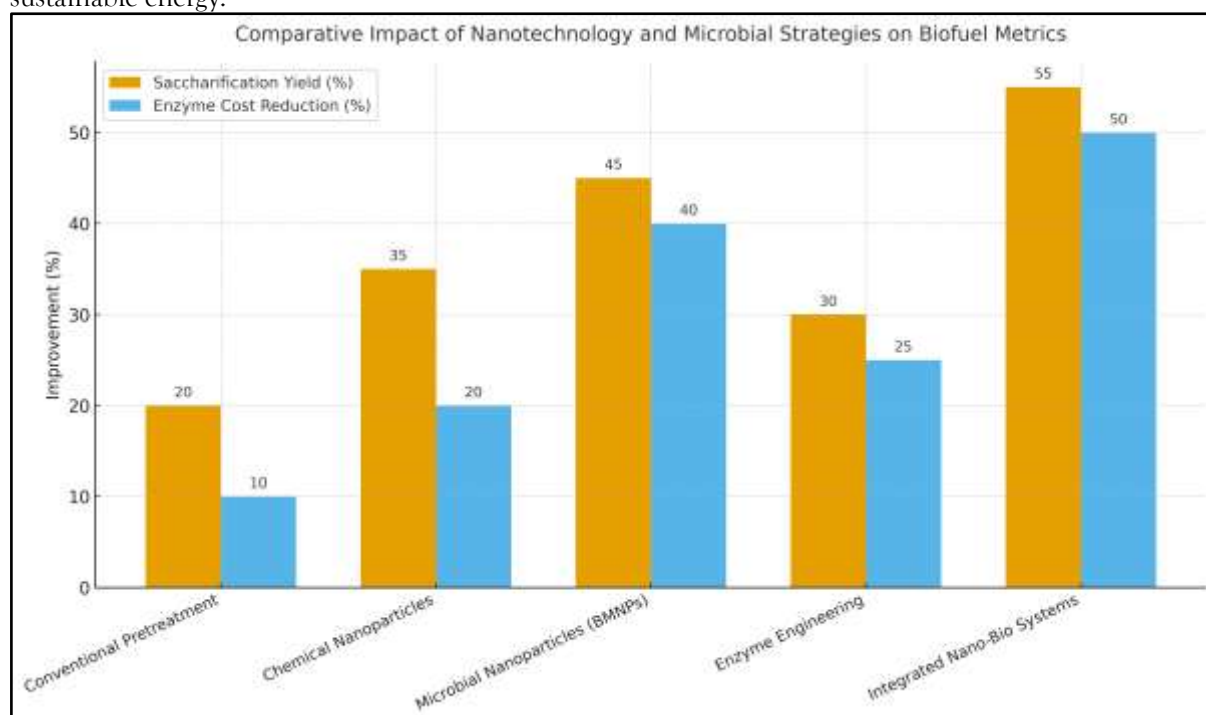


Fig. 1 : BMNPs function better than chemically generated NPs because they provide a higher saccharification yield and more efficiently lower enzyme costs. Microbial NT and biocatalysis work in concert to produce the greatest results in integrated nano-bio systems (BMNPs + enzyme engineering).

METHODS

Enzyme immobilisation, biomass hydrolysis, and microbial nanoparticle manufacturing will all be integrated into the three-phase technique of the proposed study.

1. Synthesis of microbial NPs

To biosynthesise FeO₄ and TiO₂ nanoparticles, specific strains of bacteria and fungi (*Shewanella oneidensis*, *Aspergillus niger*) will be cultivated under ideal circumstances. To guarantee low-cost scalability, media based on agro-waste will be used. TEM, XRD, and FTIR will be used to characterise the NPs to verify their shape, crystallinity, and EPS capping (Carmona et al. 2023; Kapoor et al. 2021).

2. Enzyme immobilisation on BMNPs

EPS-mediated binding or moderate crosslinking will be used to immobilise commercial cellulases and ligninases onto BMNPs. Measurements will be made on activity retention, stability during temperature and pH changes, and reusability over several cycles. Free enzymes and artificially produced NPs will be used as controls (Dutta et al. 2023).

3. Lignocellulosic hydrolysis and fermentation

BMNP–enzyme complexes will be used to enzymatically hydrolyze sugarcane bagasse and pretreated rice straw. Saccharification rate, glucose yield, and inhibitor degradation are important measures. *S. cerevisiae* (ethanol) and *C. acetobutylicum* (butanol) will ferment the hydrolyzed materials. GC and HPLC will be used to quantify product titers (Ingle et al. 2025).

4. BMNP ecotoxicity and recyclability

FeO₄ BMNPs' magnetic recovery will be examined over a minimum of five cycles. To evaluate environmental safety, ecotoxicity tests (algal growth inhibition, microbiological diversity shifts) will be carried out (Ingle et al. 2025).

This integrated pipeline connects environmentally friendly synthesis, catalytic enhancement, and process circularity, providing a revolutionary proof-of-concept for sustainable, microbe-enabled nanotechnology in biofuel systems (Mariño, Fulaz, and Tasic 2021).

Abbreviation

BMNPs	Biogenic microbe-mediated nanoparticles
NP	Nanoparticles
EPS	Extracellular polymeric substances
LCB	lignocellulosic biomass
NT	Nanotechnology
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
<i>C. acetobutylicum</i>	<i>Clostridium acetobutylicum</i>

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