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Microbial strategies for BPA removal from recycling and wastewater streams

BPA Bioremediation for Circular Economy Strategies

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ABSTRACT Micropollutants such as BPA hinder plastic recycling and pose serious risks to ecosystems and human health. In the context of a circular economy, bioremediation was investigated as a recycling/upcycling approach, leveraging microbial BPA removal by diverse bacteria and the fungus *Pleurotus ostreatus*. Microbiological and chromatographic methods were used to assess BPA tolerance and quantify BPA removal, highlighting the potential of these processes for future applications.

STICHWÖRTER/KEYWORDS

Bioremediation, Re/Upcycling, Bisphenol A

BPA Bioremediation für die zirkuläre Bioökonomie - Mikrobielle Strategien zur Entfernung von BPA aus Recycling- und Abwasserströmen

ZUSAMMENFASSUNG Mikroverunreinigungen wie BPA behindern das Kunststoffrecycling und gefährden Ökosysteme und Gesundheit. Im Kontext der Kreislaufwirtschaft wird die Bioremediation als Recycling-/Upcycling-Ansatz untersucht, der den mikrobiellen BPA-Abbau nutzt. Mittels mikrobiologischer und chromatografischer Methoden wird die BPA-Toleranz ausgewählter Mikroorganismen bewertet und die BPA-Entfernung quantifizieren (speziell für den Pilz *Pleurotus ostreatus*), um das Potenzial dieser Prozesse für künftige Anwendungen aufzuzeigen.

1 Introduction

Circular economy is understood as the central paradigm for sustainable production, aiming to decouple growth from the consumption of finite natural resources while maintaining material value within closed-loop systems. By recycling materials, minimizing waste generation, and reducing environmental pressures through strategies such as product life extension and reduced reliance on primary raw materials, it provides a foundational framework for environmentally compatible production and consumption systems. In this context, the concept of a circular bioeconomy integrates biological resources and processes to further enhance resource efficiency by valorizing biomass and biological waste streams within systemic value chains [1]. Biotechnologies constitute major enablers of circularity, as they facilitate the conversion of biological feedstocks and wastes into valuable products, energy, and materials, while supporting recycling, reuse and regeneration at multiple scales [2].

However, recycling efficiency is increasingly compromised by micropollutants. These are trace contaminants at nanogram to microgram per liter concentrations, including pharmaceuticals, pesticides, heavy metals, microplastics, and endocrine-active substances. Due to their chemical stability and inadequate removal in conventional treatment systems, micropollutants are a concern not only in mixed waste destined for recycling facilities but also in wastewater, industrial effluents, and agricultural runoff [3, 4]. They accumulate in ecosystems and contribute to endocrine disruption, microbial imbalance and the development of antimicrobial resistance, posing significant risks to both environmental and human health [3, 5, 6]. In the context of wastewater recycling, micropollutants represent a critical barrier to safe water reuse. Conventional wastewater treatment processes are primarily designed for organic matter and nutrient removal and therefore show limited effectiveness against many micropollutants. As a result, micropollutants pass through wastewater treatment plants and are detected in reclaimed water, sludge, and receiving waters [6]. Their presence compromises the quality of recycled wastewater intended for agricultural irrigation, industrial reuse, or indirect potable applications, raising concerns regarding long-term exposure, bioaccumulation, and regulatory compliance.

Bisphenol A (BPA) is one such micropollutant and a key monomer for polycarbonate and epoxy resins, which yield materials that are mechanically robust, heat-resistant, chemically stable, and transparent. Its versatility explains widespread use across packaging, electronics, coatings and construction, contributing to a multimillion-ton global market. However, the same properties that make BPA useful also pose enduring challenges. Residual monomers and additive fragments can leach under thermal, mechanical or pH stress, leading to pervasive environmental contamination. Because BPA exhibits estrogenic- and endocrine-disrupting activity with links to reproductive, metabolic and immune disorders, it is designated in the European Union as a Substance of Very High Concern (SVHC) and is restricted in food contact contexts [4, 7].

BPA therefore poses a dual challenge for a circular economy. First, it creates environmental and health risks as an endocrine-disrupting substance, raising concerns about the safety of water reuse due to incomplete removal in treated effluents. Conventional wastewater treatment plants often fail to fully remove BPA, leaving residues that contaminate the environment and recycled

water streams [8]. The persistence of BPA and other organic compounds in treated effluents and sewage sludge can result in environmental accumulation, depending on the disposal pathway. Chronic exposure has been linked to disruptions in reproductive hormones and lower total sperm count in men, highlighting potential implications for male fertility [9]. Second, BPA complicates plastic recycling. Post-consumer streams containing BPA are unsuitable for most food-contact applications, blocking high-value reuse, forcing downcycling, and undermining the economic viability of recycled plastics. In effect, BPA acts as a value-limiting impurity that reduces the range of applications for recycled products [10].

As plastics are among the most widely used materials in modern economies, removing BPA from these streams is essential to enable high-quality recycling that conserves resources and reduces environmental impacts. Plastics are synthetic polymers derived largely from fossil hydrocarbon feedstocks, and global production now exceeds 400 million tons per year, reflecting continuous growth since the mid twentieth century [11]. In Germany, plastics are extensively used in packaging, construction, the automotive sector, and electronics, generating several million tons of waste annually. However, the recycling rate for plastic packaging was only about 50 % in 2022 [12].

Following collection, waste plastics are sorted into fractions such as polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and residual mixed streams using near infrared optical sorting, air jet separation, and density-based classification. Mechanical recycling converts these fractions into secondary raw materials via shredding, washing, melt filtration, and pelletizing [13]. However, contamination (e.g. BPA), mixed-plastics, and thermomechanical degradation reduce material quality and limit high-value applications [13]. Plastics that are difficult to process are increasingly directed to chemical recycling pathways (e.g. pyrolysis), although these processes remain limited by scalability, energy demand and economic feasibility [14]. In this context, the development of innovative next-generation recycling as well as upcycling methods and approaches is essential to build a sustainable circular economy.

Biological technologies offer a promising pathway towards a circular economy by addressing the constraints outlined above. To strengthen circular material flows, microbes can be integrated into recycling processes via two principal approaches. First, they can break down contaminants that compromise recycling streams. Second, they can generate valuable compounds using waste or side streams (e.g. agricultural biomass) as substrates, creating added value within circular systems [15]. For example, enzymatic PET depolymerization to terephthalic acid enables engineered *Escherichia coli* to produce vanillin, and mixed microbial consortia convert PET and polyurethane monomers into polyhydroxyalkanoates and biosurfactants [16]. A prominent industrial example is Carbios, whose patented enzymatic PET depolymerization technology can efficiently break down complex, colored and soiled PET plastics into monomers suitable for repolymerization into new high-grade PET [17].

These approaches illustrate pollutant-to-product biosynthesis that couples environmental cleanup with economic incentives. This concept, known as bioremediation, leverages biological systems to degrade, adsorb, or accumulate pollutants and transform contaminants under ambient conditions, thereby coupling pollutant removal with opportunities for resource recovery. Microbial bioremediation employs bacteria, fungi, and algae to degrade persistent pollutants through enzymatic cascades, providing a cost effective and environmentally friendly contrast to physicochemical methods. It reduces dependence on fossil raw materials and promotes ecological and economic sustainability through low-energy and low-emission processes [18, 19].

In this study, BPA bioremediation mediated by various bacteria as well as a fungal strain is investigated as a strategy contributing to circular economy concepts. Literature-derived Gram-negative (*Escherichia coli, Pseudomonas monteilii, Ochrobactrum* sp.) and Gram-positive (*Lactococcus lactis, Bacillus subtilis, Priestia flexa, Rhodococcus rhodochrous*) bacteria, which differ in cell-envelope architecture, were evaluated alongside the white-rot fungi *Pleurotus ostreatus* for BPA tolerance and degradation efficiency using chromatographic analyses.

This study demonstrates the ability of various microorganisms to degrade BPA. It provides a basis for further research and for developing scalable processes that address BPA contamination across industries, enable food-grade recycling and support regenerative material cycles within a circular economy.

2. Material and methods

2.1 Bacterial and fungal strains

Chemicals and media components used in this study, including their purity and supplier, are summarized in **table 1**. Bacterial and fungal strains used in this study are listed in **table 2** together with their respective growth media and incubation temperature. All strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). Media were prepared according to the specifications provided by the DSMZ [20].

For agar plates, 1.5 % (w/v) agar was added to the corresponding medium. Cultivation in a minimal Medium (MM) was performed with the following composition: 1 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4$, 0.05 g/L NaCl, 0.05 g/L $CaCl_2$, and 0.01 g/L $FeSO_4$. For the preparation of MM agar plates, a higher agar concentration of 3 % (w/v) was used.

2.2 BPA tolerance test on agar plates

To assess the effect of BPA on colony-forming unit (CFU) counts of selected bacteria, agar plates with different concentrations of BPA were prepared. BPA (2,2Bis(4hydroxyphenyl)propane) was dissolved in ddH_2O to prepare an aqueous stock solution with a concentration of 0.2989 g/L. The microorganism test panel comprised Gram-negative *E. coli, O.* sp., and *P. monteilii*, as well as Gram-positive *L. lactis, P. flexa, B. subtilis*, and *R. rhodochrous*. Based on preliminary assays (conducted within this study), it was observed that Gram-negative bacteria were more sensitive to BPA. For this purpose, a BPA concentration of 0.15 g/L was used for the Gram-negative strains, while a concentration of 0.26 g/L was applied for the Gram-positive strains. Overnight cultures were adjusted to an OD_{600} of 0.1, serially diluted, and spreadplated (100 µL per plate for each dilution). For each dilution and strain, BPA-free controls and plates containing the respective BPA concentration were prepared and analyzed in duplicate (n = 2). Incubation parameters for each organism are listed in table 1. Colonies on plates were counted after 24 hours of incubation. For the slower-growing or-

Table 1 Chemicals and media components used in this study, including purity, specification, and supplier.

Chemical	Purity and Specification	Supplier
2,2 Bis(4 hydroxyphenyl)propane (BPA)	≥99%	Sigma-Aldrich
Agar-Agar	Kobe I, powdered, for microbiology	Carl Roth
Ammonium sulfate ($(NH_4)_2SO_4$)	≥99 %, cryst.	Carl Roth
Calcium chloride ($CaCl_2$)	93 %, granular, anhydrous	Merck KGaA
Ethylacetat	≥99.5%, HPLC grade	Fisher Scientific
Iron(II) sulfate Heptahydrat ($FeSO_4$)	≥ 99 %, pPh.Eur., USP	Carl Roth
Magnesium sulphate ($MgSO_4$)	≥99 %, p.a., anhydrous	Carl Roth
Malt extract	powdered, for culture media	Carl Roth
Meat extract	powdered, for culture media	Carl Roth
Monopotassium phosphate (KH_2PO_4)	≥ 99 %, p.a., ACS	Carl Roth
Peptone ex casein	tryptically digested, for microbiology	Carl Roth
Peptone ex soya	papainic digested, animal-free, GVO-free, for microbiology	Carl Roth
Sodium chloride (NaCl)	≥ 99 %	Carl Roth
Yeast extract	micro-granulated, for bacteriology	Carl Roth

Table 2 Bacterial strains used in this study, including the corresponding cultivation media.

Bacterial Strains					
	Abbreviation	**Gramstaining**	**DSM-Number**	**Medium**	**Incubation Temp. [°C]**
Escherichia coli	*E. coli*	Gram-negative	1116	M1	37°C
Pseudomonas monteilii	*P. monteilii*	Gram-negative	11388	M1	28°C
Ochrobactrum sp.	*O.* sp.	Gram-negative	717	M1	30°C
Lactococcus lactis	*L. lactis*	Gram-positive	20175	M92	30°C
Bacillus subtilis	*B. subtilis*	Gram-positive	10	M1	30°C
Priestia flexa	*P. flexa*	Gram-positive	1316	M1	30°C
Rhodococcus rhodochrous	*R. rhodochrous*	Gram-positive	363	M535	28°C
Fungal Strain					
Pleurotus ostreatus	*P. ostreatus*	n/a	1020	M90	25°C

ganisms *L. lactis* and *Ochrobactrum* sp., counts were performed after 48 hours.

Growth of *P. ostreatus* on M90 agar supplemented with BPA (0, 0.1, and 0.2 g/L) was monitored for seven days. An agar plug with a diameter of 5 mm from an actively growing, BPA-free culture on agar was placed centrally on each plate to allow radial expansion. Mycelial growth was recorded at defined intervals by measuring colony diameter.

2.3 Gas chromatography – flame ionization detector (GC-FID)

To evaluate the potential for BPA-degradation of the selected microorganisms in liquid, cultures were incubated with BPA and culture supernatants were sampled at distinct time points and analyzed via a gas chromatography – flame ionization detector (GC-FID) to monitor decreases in detectable BPA. Fresh medium containing the respective BPA concentration was inoculated with overnight cultures to a starting OD_{600} of 0.25 and incubated with shaking at 180 rpm at organism-specific temperatures. For preparation of the overnight co-culture of *R. rhodochrous* and *Ochrobactrum* sp., single colonies of each strain were picked from agar plates and inoculated together into liquid medium. This mixed overnight culture was then used to inoculate the main experimental cultures in M1 for subsequent GC-FID analyses. Initial experiments in section 3.3 were performed using the respective culture media (indicated in table 1) alongside with MM at 144 h, 50 mL samples were collected, centrifuged (3,000×g, 10 min, 4 °C), and

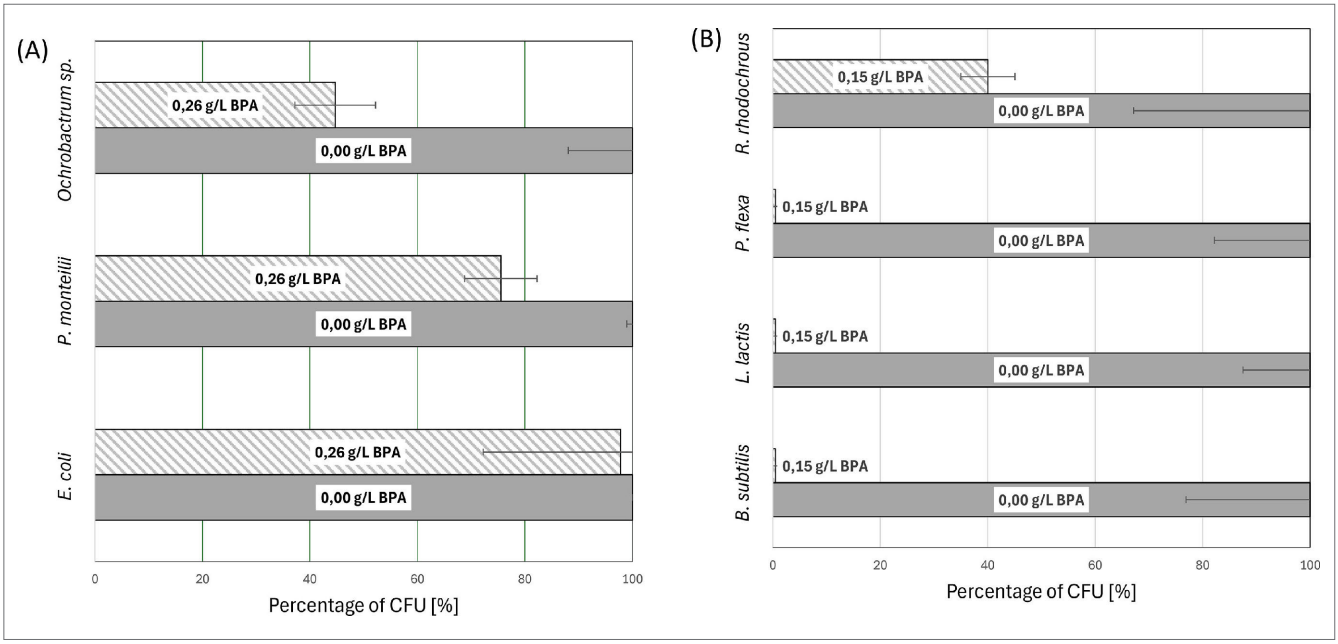

Fig. 1 Effects of BPA (Bisphenol A) on bacterial growth, quantified as CFU (colony-forming unit) relative to BPA-free controls. Gram-negative (A) and Gram-positive (B) strains were tested at indicated BPA concentrations. *Source: University of Stuttgart*

the supernatants were sterile-filtered through 0.22 µm filters prior to GC-FID analysis.

For *P. ostreatus*, mycelial plugs were pre-cultured in M90 for five days until spherical mycelial aggregates formed. Individual aggregates were then inoculated into fresh medium for incubation with and without BPA in MM and M90 under the same conditions. For experiments with *P. ostreatus* (see results in section 3.4), multiple aggregates (40 mL of culture containing numerous (at least 50) small aggregates, not individually counted) were used to inoculate the test culture to increase the degradation rate. On days 8, 16, and 34, 50 mL samples were collected. Mycelium was removed by filtration through Whatman filter paper, followed by centrifugation of the filtrates (3,000×g, 10 min, 4 °C) and sterile filtration (0.22 µm) prior to GC-FID analysis.

To prepare collected samples for GC-FID analysis, cell-free culture supernatants were extracted by mixing equal volumes of sample and ethyl acetate, vortexing for 15 s, and centrifuging at 4000×g for 2 min. BPA partitioned into the upper organic phase, which was transferred into GC vials with 250 µL glass inserts. GC-FID analyses were performed using a Shimadzu "GC-2010" equipped with an FID detector and a ZB-1 (Phenomenex, 30 m × 250 µm × 0.25 µm) column. Hydrogen was used as the carrier gas at a constant linear velocity of 30 cm/s. Injections of 1 µL were analyzed in split mode (5:1). For each biological sample, three technical replicates were measured by GC-FID (three independent extractions). The GC oven was programmed to hold at 150 °C for 1 min, then ramp to 300 °C at a rate of 20 °C/min, and finally hold at 300 °C for 3 min.

Prior to analysis, a standard curve was generated. For this purpose, medium supplemented with 0.05 g/L BPA was used as the stock solution. Serial dilutions were performed using BPA-free medium as the diluent to generate a concentration gradient. The diluted samples were extracted and analyzed using GC-FID for quantification. Each concentration was prepared in triplicate, and the areas of the peaks were used to construct the calibration curve.

3 Results

To mitigate BPA, a value-limiting impurity in waste streams, microbial bioremediation was evaluated as an enabling step for its removal from recycling-relevant streams. BPA tolerance and degradation capacity of selected, promising microorganisms, including Gram-negative and Gram-positive bacteria as well as the fungus *P. ostreatus*, were examined. The combination of agar-based tolerance tests with GC-FID measurements of liquid cultures over time enabled the identification of strain-specific responses to BPA, providing an initial basis for prospective use in bioremediation and innovative future technologies.

3.1 Tolerance of microbial strains to BPA

Initially, toxicity assays on agar plates were conducted to determine the tolerance of selected organisms to BPA. As described in section 2.2, strains were plated on BPA-containing and BPA-free agar plates. After incubation, colony-forming units (CFU) were quantified to evaluate growth inhibition relative to BPA-free controls. The results are depicted in **figure 1**.

Gram-negative strains (figure 1 (A)) maintained substantial growth at 0.26 g/L BPA. This was the highest concentration that could be applied in agar plates, as it is limited by the maximal solubility of BPA in water. In contrast, Gram-positive bacteria (figure 1 (B)) exhibited growth inhibition already at 0.15 g/L (for Gram-positive strains, only the concentration of 0.15 g/L is shown, as strains displayed little to no growth already at this lower concentration). For Gram-negative strains (*O.* sp., *P. monteilii*, *E. coli*), 100 µL of a 10^{-5} dilution of an $OD_{600} = 0.1$ culture was plated on agar containing 0.26 g/L BPA and on BPA-free control agar. For Gram-positive strains, 100 µL of a 10^{-5} dilution (*B. subtilis*, *L. lactis*) or 10^{-4} dilution (*P. flexa*, *R. rhodochrous*) of an $OD_{600} = 0.1$ culture was plated on agar with/without 0.15 g/L BPA. All conditions were analyzed in duplicate. CFU on BPA-free controls were set to 100 %, and CFU on BPA plates are expressed

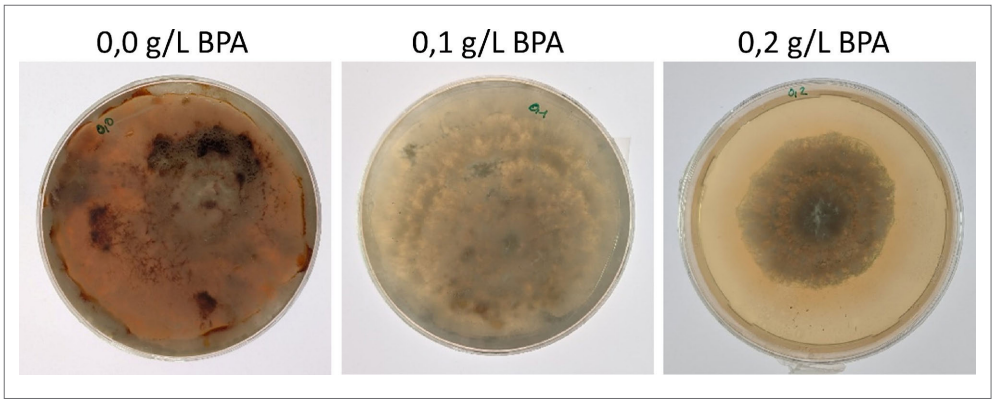

Fig. 2 Growth of *Pleurotus ostreatus* on agar plates containing increasing BPA concentrations. *Source: University of Stuttgart*

as a percentage of this value. Bars show the mean percentage of CFU relative to BPA-free controls; error bars indicate the range across replicates.

The results (see figure 1) show that Gram-positive bacteria are more sensitive to BPA, with growth inhibition at concentrations of 0.15 g/L BPA, whereas the tested Gram-negative strains maintained substantial growth at 0.26 g/L BPA. Results for Gram-negative and -positive strains are shown in figure 1 (A) and (B), respectively.

Following the bacterial tolerance assays, the tolerance of the fungus *P. ostreatus* on BPA supplemented M90 agar plates was evaluated. Since CFU enumeration is not applicable to this organism, mycelial growth was monitored over seven days on plates containing 0.0, 0.1, or 0.2 g/L BPA (**figure 2**), each tested in duplicate (n = 2). For this purpose, a 5 mm agar plug from an actively growing culture was placed centrally on each plate, and radial expansion was recorded as colony diameter. As shown in figure 2, cultures on 0.0 and 0.1 g/L BPA reached full plate coverage by day 7, whereas growth at 0.2 g/L BPA covered only about half of the plate, indicating BPA-mediated growth inhibition.

3.2 Selection of organisms

Based on agar plate tolerance tests (shown in the previous section), a subset of microorganisms was selected for further investigation. Assuming that growth tolerance in the presence of BPA correlates with degradative potential, more tolerant strains were prioritized for further analysis. Specifically, the Gram-negative bacteria *P. monteilii* and *E. coli*, as well as the white-rot fungus *P. ostreatus* were selected. The latter showed robust growth at 0.1 g/L BPA and diminished but sustained growth at 0.2 g/L (see figure 2). For downstream assays, Gram-positive strains were excluded due to poor or no growth at low BPA concentrations (see figure 1 (B)). The Gram-negative organism *Ochrobactrum* sp. was not investigated further in monoculture, as its BPA tolerance was markedly lower compared to *P. monteilii* and *E. coli*. However, it was retained in co-culture with the Gram-positive *R. rhodochrous* for further experiments, as previous studies have reported promising synergistic effects [21].

This strain selection was further examined in liquid culture assays monitoring BPA depletion in culture medium versus MM over time using gas chromatography.

3.3 BPA degradation using GC-FID analyses

The potential of bacterial and fungal strains for degrading BPA was investigated by GC-FID, enabling time-resolved monitoring of BPA in cell-free supernatants. The bars illustrated in **figure 3** provide insight into the signal intensity associated with BPA in the supernatant, which was evaluated by integrating the corresponding peaks.

BPA was added at 0.05 g/L and decreases in BPA concentration in the supernatants were quantified relative to the initial level. For each organism, the BPA signal at t = 0 h was set to 100 %, so subsequent measurements represent the percentage of BPA remaining relative to this baseline. Samples from bacterial cultures (*E. coli*, *P. monteilii*, and the co-culture of *O.* sp. and *R. rhodochrous*) were analyzed after 144 h, while samples from the fungal culture (*P. ostreatus*) were taken after 192 h and 480 h. Cultures were grown in both the respective culture medium (shaded bars) and MM (solid bars) to evaluate potential nutrient-dependent effects. Error bars indicate the standard deviation of three technical replicates (n = 3; independent extractions). The initial BPA concentrations (at t = 0) did not differ significantly from those in the corresponding controls without microorganisms after the incubation time (medium + BPA; identical incubation and shaking) and remained stable throughout the incubation period.

Since BPA concentrations remained unchanged over time in control samples (data not shown in figure 3), it was concluded that BPA was stable under the tested conditions. The GC-FID limit of detection for BPA was determined to be approximately 4.9×10^{-5} g/L. Therefore, if BPA was not detectable in culture supernatants with the method used here, its concentration was below this limit of detection. As shown in figure 3, BPA reduction in supernatants was consistently poorer in MM (solid bars) than in the respective culture medium (shaded bars) and no clear decrease of BPA was detectable in MM for all strains. This was likely due to significantly slower and poorer growth of the organisms in MM, which lacks an additional carbon source. The initial hypothesis that BPA degradation might be enhanced in MM, where BPA serves as the sole carbon source, could not be confirmed. Over a period of 144 h, *E. coli* showed no notable decrease in BPA concentration in either the culture medium or MM. In contrast, *P. monteilii* exhibited approximately 30 % reduction at 144 h and the co-culture of *Ochrobactrum.* sp. and *R. rhodochrous* showed a reduction of approximately 40 % at the same time point. *P. ostreatus* showed approximately 50 % reduction after 192 h. By 480 h, BPA in the supernatant of the *P. ostreatus* culture had further fallen below the GC–FID limit of detection.

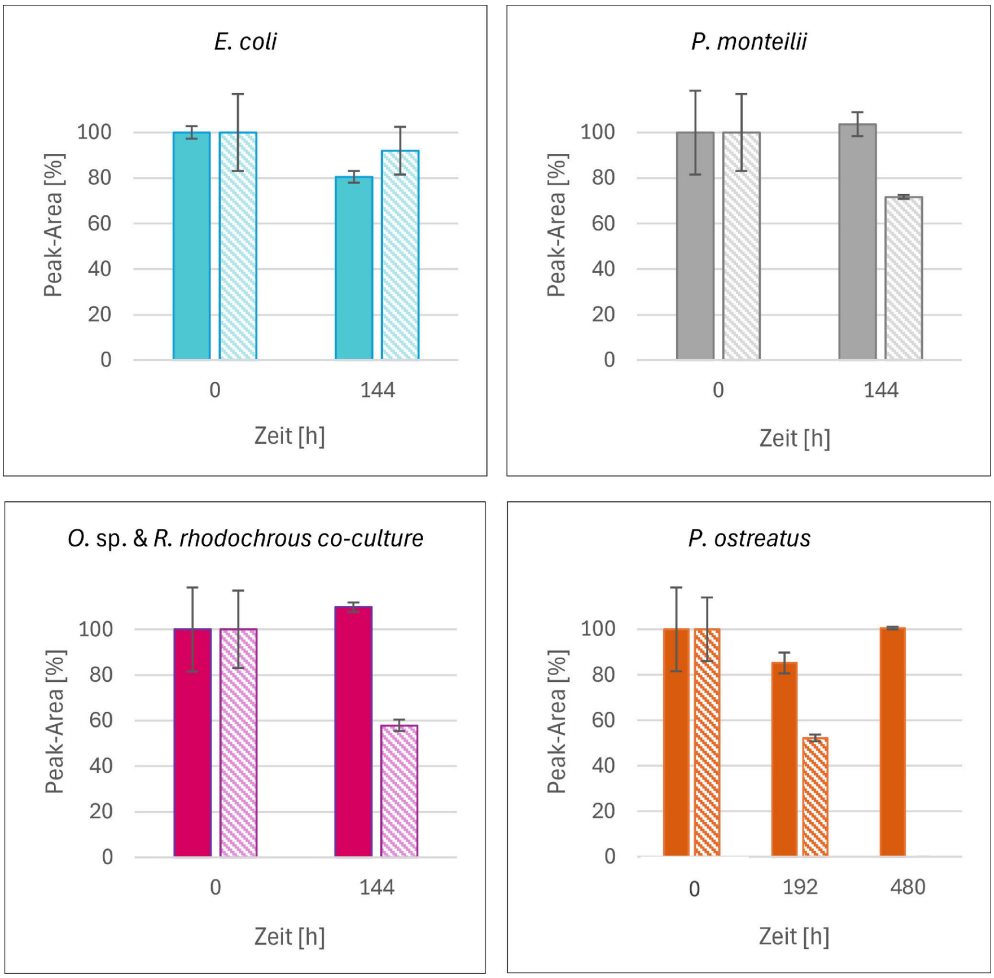

Fig. 3 Evaluation of BPA reduction in liquid media mediated by microorganisms. *Source: University of Stuttgart*

3.4 Pleurotus ostreatus-mediated BPA degradation

Based on the preceding results, subsequent analyses focused on *P. ostreatus*. This choice was motivated by (i) complete BPA removal in M90 after prolonged incubation (below the GC-FID limit of detection), (ii) robust growth at a moderate 25 °C, both demonstrated in this study. Additional considerations included (iii) the ability to grow on diverse lignocellulosic side streams (e.g. agricultural residues) that align with circular-economy integration, and (iv) the safety and practicality of an edible, non-pathogenic fungus for industrial bioremediation [22, 23].

By contrast, *E. coli* as well as *P. monteilii* did not exhibit promising BPA degradation, and the planned co-culture of *O.* sp. with *R. rhodochrous* is operationally challenging since co-cultures readily destabilize. In addition, *Pleurotus* species have a broad temperature optimum for growth, combining strong growth at around 20–25 °C with a strain-dependent tolerance to higher temperatures, which improves their applicability in different environments and makes the fungus particularly promising for industrial applications [24].

To further investigate and optimize BPA removal by *P. ostreatus* in liquid, BPA load, inoculum density, and medium composition were varied. Compared to the preliminary experiments described above, in which only one spherical mycelium aggregate was used for inoculation, the experiments described here were conducted at two BPA concentrations (0.05 and 0.1 g/L) and with a substantially increased inoculum density of *P. ostreatus* to enhance BPA removal (details see section 2.3). Furthermore, in order to resolve BPA degradation kinetics and assess medium effects, the time course was expanded with shorter sampling intervals and medium composition was systematically varied. In this context, the M90 medium was prepared according to DSMZ specifications (30 g/L malt extract, 3 g/L soy peptone). For experiments conducted in this section, M90 was additionally modified by replacing half of the malt extract (15 g/L) with glucose (15 g/L), hereafter referred to as M90-Glc.

Figure 4 summarizes BPA removal by *P. ostreatus* at two initial concentrations (0.05 and 0.1 g/L) in culture medium M90 (grey bars) and M90Glc (blue bars).

The BPA level at day 0 was set to 100 % and subsequent values represent the percentage of BPA remaining in the culture supernatant. Across all tested conditions, BPA was not detectable (n.d.) in supernatants by day 7. A notable difference was only observed at the higher BPA concentration of 0.1 g/L in M90 after four days. Under these conditions, the BPA level was not reduced, indicating slower degradation capability, compared to the other tested conditions. Of note, these data were obtained from a single experiment (with three technical replicates) and should be repeated. However, these initial experiments suggest that a higher inoculum density of *P. ostreatus* accelerates BPA depletion and indicate that, at elevated BPA concentrations, glucose supplementation of the medium may further support BPA removal.

These findings provide a first optimization step for process conditions. Further work should systematically probe carbon-

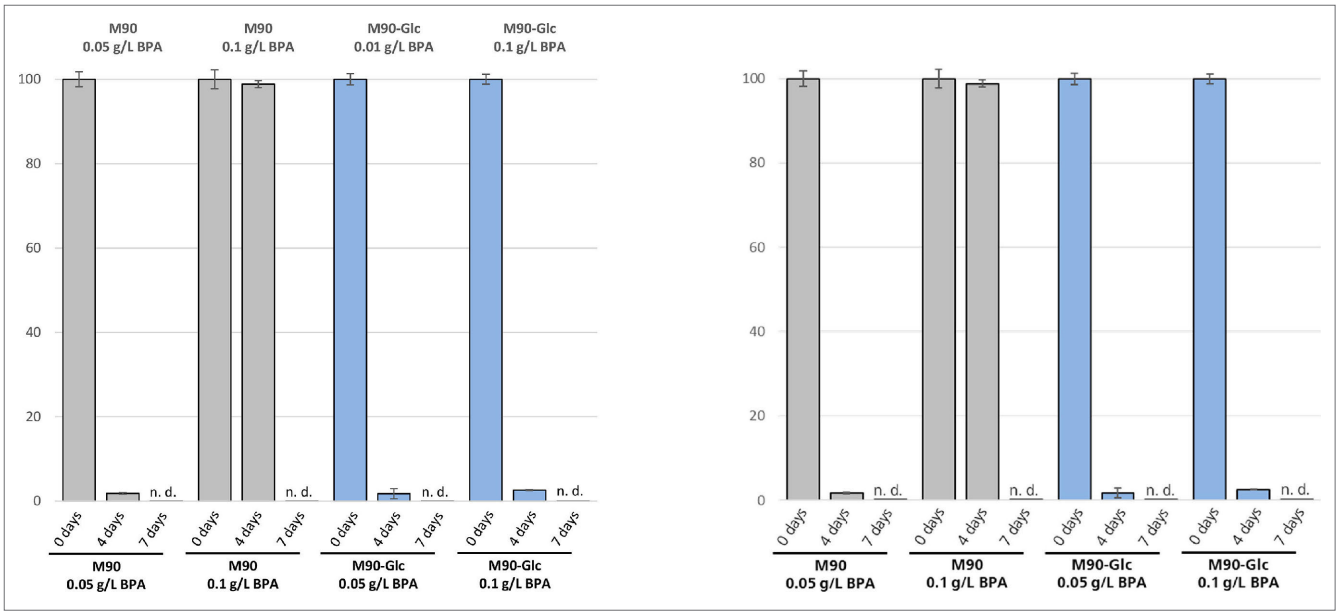

Fig. 4 Effect of medium composition and initial BPA load on degradation capacity by *Pleurotus ostreatus*. *Source: University of Stuttgart*

source effects (e.g. alternative sugar sources) and evaluate lignocellulosic side streams as substrates to align bioremediation with circulareconomy integration.

4 Discussion

In the context of a biotechnology-driven circular economy, these results address a central bottleneck: the persistent contamination of recycling streams and the environment by endocrine-active compounds such as BPA, which hinder recycling or have long-term ecological impacts. By integrating tolerance profiles with time-resolved analyses of BPA degradation in bacterial and fungal systems, initial mechanistic and performance-oriented insights into microbially mediated BPA degradation are provided.

The observed results provide the basis for the development of biotechnological strategies, from tailored biological remediation approaches to bio-catalytic recovery processes, that could be integrated into recycling plants, wastewater treatment, and downstream material recovery. The following discussion places these findings in the context of taxon- and group-specific tolerance differences, medium effects, and the path towards scalable, sustainable solutions for BPA removal within circular recycling technologies.

4.1 Microbial BPA tolerance and degradation

The diversity in cell envelope architecture and physiology of different bacterial groups suggests significant differences in their resistance and tolerance to pollutants. The presented results indicate that BPA tolerance varies among different microorganisms, thereby probably affecting their potential degradation capacities. Our plate assays (section 3.1) reveal a difference in BPA tolerance across the here tested bacteria: Gram-positive strains showed reduced growth or no growth at the applied BPA concentrations (i.e. they are more sensitive), whereas tested Gram-negative strains continued to grow under the same conditions, albeit with reduced growth in the presence of BPA, indicating that BPA also causes damage in these bacteria. This pattern indicates that, for the strains and experimental conditions used here, Gram-negatives are comparatively more tolerant to BPA exposure than the Gram-positives.

A number of structural and physiological features plausibly underlie this divergence. Gram-negative bacteria have an outer membrane that contains lipopolysaccharides and selective porins. This outer layer can act as diffusion and chemical barriers, limiting the passive uptake of toxins or antimicrobial compounds, and it encloses a periplasmic space where detoxification enzymes and efflux systems can act before damage to the cell occurs [25]. These same features also contribute to antibiotic resistance of Gram-negative bacteria and could similarly reduce the penetration of BPA and its toxicity.

In contrast, Gram-positive bacteria lack an outer membrane and instead have a thick peptidoglycan cell wall with teichoic acids. In Gram-positives, BPA has been shown to disturb cell wall biosynthesis and thereby cell growth [26], likely contributing to their higher sensitivity to BPA exposure. Consequently, BPA (a moderately hydrophobic phenol) may reach the cytoplasmic membrane of Gram-positives more readily or interact differently with essential envelope components, increasing cell disruption, metabolic stress or intracellular toxicity and causing earlier growth arrest or death.

The structure of the fungal cell wall is different: it is multilayered and composed mainly of chitin, β-glucans, and mannoproteins, which confer mechanical robustness and create a large, reactive surface that can sorb hydrophobic organics, thereby lowering the freely bioavailable fraction of contaminants such as BPA. The filamentous mycelium formed by fungi provides a very large surface area, further enhancing such sorption. Prior studies have demonstrated both BPA sorption and biodegradation across multiple fungal species [27, 28]. In line with these reports, a decrease in BPA in the culture supernatant was observed for the *Pleurotus* species examined here. However, further experiments are required to explicitly distinguish whether this decrease reflects biodegradation, sorption, or a combination of both.

Fungal bioremediation, also referred to as mycoremediation, has gained increasing attention in recent years, in part because white-rot fungi produce high activities of extracellular oxidative

enzymes, such as laccases and manganese peroxidases, which can oxidize and transform phenolic pollutants including BPA. These enzymes are known to mediate the transformation of phenolic compounds, as demonstrated in various studies using qualitative assays such as the Remazol Brilliant Blue R (RBBR) test, which indicates the presence of extracellular phenol-oxidizing activities [29]. Therefore, we assume that in our experiments, phenol-oxidizing enzymes secreted by the microorganisms are responsible for the degradation of BPA in culture supernatants.

The present findings provide an initial hint toward industry-relevant bioremediation processes. However, further investigations are needed to elucidate the exact BPA degradation pathways and to identify the enzymes involved. In addition to quantifying BPA degradation, future studies should investigate potential degradation intermediates and products. This is essential to determine whether the degradation of BPA leads to less toxic compounds or whether other potentially harmful intermediates are formed. Moreover, degradation efficiency can likely be enhanced through medium engineering and supplementation with enzyme inducers. For example, Cu^{2+} is known to upregulate laccase production in some white-rot fungi [30]. Consistent with this, our preliminary experiments in this study showed that BPA removal varied with medium composition, underscoring medium optimization as a practical lever to improve degradation performance.

In our experiments, we compared degradation in culture medium and MM to assess whether restricting the carbon source would enhance BPA degradation. However, the results showed that BPA removal was generally poorer in MM, most likely due to reduced growth rates and biomass formation in the absence of an additional carbon source. This suggests that sufficient nutrient supply is crucial for effective microbial bioremediation of BPA. In addition to optimizing culture conditions, genetic engineering approaches, such as using stronger promoters for laccase gene overexpression or introducing additional gene copies, can further enhance laccase production.

4.2 Perspectives and challenges for implementation

Within a circular economy framework, the microbial degradation of BPA reported here represents a fundamental step toward integrating biotechnological solutions into recycling and wastewater treatment systems. Coupling microbial BPA or micropollutant degradation with microbially enabled recycling and upcycling processes can support robust, low-energy, bio-based treatment stages that complement or substitute physicochemical and mechanical methods. Establishing such process systems requires detailed characterization of the organisms, enzymatic mechanisms, and matrix- and medium-specific kinetics to enable optimal process design tailored to the matrix, co-substrate, and operating conditions.

Among the organisms examined, *P. ostreatus* emerges as a particularly attractive candidate for further research and scale-up. It is non-pathogenic and Generally Regarded As Safe (GRAS status) [22], which can simplify risk assessment and regulatory review for environmental applications. This edible basidiomycete grows on diverse carbon sources, including lignocellulosic and agricultural side streams, and produces high activities of extracellular oxidative enzymes such as laccases and manganese peroxidases. Its ability to grow and maintain enzyme activity at ambient temperatures and pH values typical of many industrial effluents enhances practicality, while its responsiveness to medium composition enables optimization of degradation kinetics through process adjustments [22, 31].

Translating these capabilities into robust, large-scale processes remains challenging due to several physicochemical and biological constraints that reduce efficacy under real-world conditions. First, the bioavailability of many contaminants is limited, as hydrophobic compounds sorb to soils or organic matter, restricting microbial access [32]. Second, bioremediation systems are sensitive to pH and temperature, as many enzymes operate within narrow ranges and lose activity under fluctuating environmental conditions [6, 33]. Third, incomplete degradation can yield toxic or more persistent intermediates that raise environmental and regulatory concerns. Therefore, comprehensive identification and characterization of all degradation products is essential [6].

Moreover, scale-up constraints including oxygen transfer, reactor design, nutrient delivery, and maintenance of stable microbial growth and activity remain major bottlenecks when moving from laboratory to industrial volumes [33]. Current advances address these limitations through microbial consortia of fungi and bacteria that broaden substrate scope and complete degradation pathways due to synergistic effects [34, 35]. Additionally, cell immobilization techniques such as encapsulation, carrier-based biofilms or support matrices enhance microbial stability, protect cells from environmental fluctuations and increase pollutant contact efficiency [36, 37]. Finally, process optimization, including controlled aeration, nutrient modulation, co-substrate addition, and reactor engineering, is increasingly applied to enable complete and sustained pollutant removal at industrially relevant scales [33].

Taken together, these insights pave the way for bioremediation workflows that couple the removal of BPA and other micropollutants with resource recovery technologies ranging from material recycling to wastewater treatment, enabling safer, higher-quality recycled plastics and cleaner effluent and biosolids. They also support the use of robust, energy-saving bioprocesses that promote a technology-based circular economy.

5 Conclusion

This study demonstrates the potential of microbial bioremediation, particularly using the white-rot fungus *P. ostreatus*, for the efficient removal of BPA from recycling-relevant streams. Among the tested strains, *P. ostreatus* showed the most promising BPA degradation capacity under various conditions, highlighting its suitability for integration into biotechnological processes within a circular economy framework. The results emphasize that both inoculum density and medium composition are critical parameters for optimizing BPA removal, with nutrient-rich media supporting more effective degradation than MM.

Our findings underline the importance of selecting robust, safe, and versatile microorganisms for future large-scale applications. The ability of *P. ostreatus* to grow on diverse, lignocellulosic side streams further supports its practical use in sustainable recycling and wastewater treatment processes. However, challenges remain regarding process scale-up, the influence of environmental conditions, and the need for comprehensive characterization of degradation pathways and products.

Overall, implementing such biotechnological approaches can make a significant contribution to safer, more sustainable recycling and wastewater treatment, supporting the transition to a resource-efficient, circular bioeconomy.

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