PSM
Biological Research

Review Article 2024 | Volume 9 | Issue 1 | 9-29

Open Access

Article Information

Received: October 21, 2023

Accepted: October 28, 2023

Published: November 13, 2023

Keywords
Aspergillus niger;
Biodegradation;
Natural Rubber;
Oxidase enzymes;
Beta oxidation;
PDA media.

Authors' Contribution
OA conceived and designed the study and wrote the paper. SH supervises the paper. SN, AK, MH, MA, AU and SA revised and finalize the manuscript.

How to cite
Alam, O., Khan, S., Nadir, S., Khan, A., Hussain, M., Ahmad, M., Atiq, S., 2024. Unleashing *Aspergillus niger*: Transforming Rubber Waste into Eco-Friendly Solutions. PSM Biol. Res., 9(1): 9-29.

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Unleashing *Aspergillus niger*: Transforming Rubber Waste into Eco-Friendly Solutions

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Abstract:
Natural rubber (NR) is a jack-of-all-trades material found in everything from car tires to rubber ducks. The global NR industry produces a massive 13.6 million metric tons annually. However, the usual methods of disposing of leftover NR i.e., pyrolysis, recycling, and stockpiling have caused serious environmental headaches. Pyrolysis spews nasty pollutants into the air we breathe. Recycling relies on harsh chemicals. And stockpiling leads to soil and water contamination that would make an environmentalist weep. Enter the biodegradation savior: a process using the humble fungus *Aspergillus niger*. This fungus secretes special proteins that break down NR's molecular bonds with impressive results. In just one week at pH 5.6, *A. niger* degraded a whopping 28% of NR with a growth rate to impress even the most ambitious scientists. But how do we turbocharge this process for big industry applications? No problem – a few "mass and stoichiometry" studies will reveal the perfect degradation conditions. Although lab tests show biodegradation's promising potential, the real challenge is scaling up. Thankfully, innovative solutions like "immobilized enzymes" and genetically modified microorganisms could soon make large-scale NR biodegradation a reality. With continued investigation, we'll develop techniques to reduce NR waste's massive environmental footprint. So read on to learn how you can help advance this exciting technology.

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INTRODUCTION

NR stands as a captivating exemplar of polymer chemistry, woven with isoprene units as its monomeric threads. These isoprene units, composed of three carbon atoms, exhibit distinct cis or trans configurations, delineated by the positioning of a solitary double bond. Remarkably, NR adopts its polymeric conformation through the alignment of three trans-isoprene motifs, a structure that intricately interlaces and elongates to encompass thousands of chains of cis-isoprene units (de Witt *et al.*, 2020). Among nature's skilled chemists, trees such as Palaquium gutta and Crotalaria chicle stand out as virtuoso producers of trans-poly isoprene rubber, colloquially known as Chicle Gum. This exceptional substance finds its habitat predominantly in the rich tapestry of South America, where towering trees, soaring to heights of 32-34 meters, bestow upon us this valuable resource. Remarkably, Chicle Gum emerges from the elongated chains of trans-poly isoprene units intricately woven by these trees (Birke and Jendrossek, 2014).

On a global scale, the production of NR reaches staggering proportions, driven by key players such as China, contributing a substantial 5.7 million metric tons (mmt), Indonesia following suit with 3.12 mmt, and India contributing 1.24 mmt. This collective effort yields a tapestry of NR that spans continents and underpins numerous industries (Mollea and Bosco, 2020). NR is widely used in industries like automotive, construction, and medical industries. In the automotive industry, NR is used in the production of tires, belts, hoses, and other parts (Zhang *et al.*, 2023a). It has been investigated that the effect of incorporating natural rubber into tire tread compounds leads to improving their performance and sustainability. In the construction industry, it is used in the production of resin systems, sealants, and waterproofing (Yong *et al.*, 2019). The potential of using NR-based coatings for corrosion protection of metal surfaces. In the medical industry, NR is used in the production of gloves, catheters, and other medical devices (Bendahou *et al.*, 2010). In the consumer goods industry, NR is used in the production of toys, footwear, and other products. The potential of using NR-based bioplastics are used as a sustainable alternative to petroleum-based plastics (Singha *et al.*, 2019).

As a consequence of its burgeoning demand, NR finds itself woven into a multitude of everyday applications, ranging from latex products and adhesives to toys, sports equipment, and industrial goods. However, this widespread utilization has given rise to a challenge: the disposal of NR-containing products, leading to their accumulation at dumping sites. Herein lies an environmental concern, as the persistence of NR in these sites engenders multifaceted impacts on the surrounding ecosystem (Fernandez *et al.*, 2016). It also reduces soil fertility by altering soil nutrient availability and nutrient cycling processes (Nguyen *et al.*, 2018). It changes the soil structure and reduces soil porosity, which can affect water infiltration and drainage (Mendis *et al.*, 2021). As a result, it disturbs the terrestrial ecosystem. Waste rubber can be disposed of via several techniques. Due to their negative environmental effects on the mechanical qualities of NR, such as oxidative degradation and photo-degradation, pyrolysis, junkyards, and recycling have become obsolete (Barana *et al.*, 2016). We delve into the realm of microbial alteration and breakdown of hazardous and industrial substances. This realm not only holds significant value in the realm of research but also offers intriguing possibilities for harnessing biotechnology to restore and purify contaminated areas (Gu, 2021b). Supportability efforts to manage waste products of rubber derivatives should be Carried out through microorganisms by bio-degradation processes that are self-mediated at dumping sites (Clark, 2015). This method is favorable because it is environment friendly, low cost, and high efficiency (Li *et al.*, 2021). It helps in the management of organic waste, such as rubber waste, food waste, agricultural residues, and municipal solid waste. By breaking down this waste, biodegradation has the potential to minimize the quantity of trash disposed in landfills and also limit the environmental consequences of garbage disposal (Nanda and Berruti, 2021). It can also degrade a wide range of organic pollutants, including pesticides, petroleum hydrocarbons, and polychlorinated biphenyls (PCBs), thereby reducing their toxicity and restoring ecosystem health through biodegradation (Dua *et al.*, 2002).

Nestled within the captivating realm of mycology, the genus Aspergillus emerges as a constellation of filamentous fungi, its delicate threads weaving through the intricate tapestry of soil ecosystems and decaying organic matter. This genus boasts an astonishing diversity, encompassing more than 300 distinct species, each an embodiment of nature's creative prowess (Najib *et al.*, 2009). Among this array, certain members stand out for their remarkable potential in the realms of medicine and biotechnology, forging connections between the microscopic world and human innovation. Remarkably, Aspergillus has shown a remarkable affinity for environments that lean slightly towards acidity, thriving within a pH range of 4.0 to 6.5 (Kamali, 2001). Beyond its temperature and pH preferences, this genus also stands as a resilient champion in the face of adversity. Its tolerance to elevated levels of heavy metals and other pollutants serves as a testament to its prowess as a bioremediation agent. This unique capacity positions *Aspergillus* as a potential ally in the restoration of contaminated soils and water bodies, hinting at its transformative potential in environmental stewardship. The Genome size of *A. niger* is 42.2 Mb, consisting of 14 chromosomes; ~14,000 protein-coding genes; genomic analysis has revealed the presence of multiple gene clusters involved in the biosynthesis of secondary metabolites (Machida *et al.*, 2005; Pel *et al.*, 2007). It has also been isolated from foods such as grapes, coffee beans, and other fruits (Borchers *et al.*, 2010). It has a black color due to the production of melanin, which helps protect it from UV radiation, and grows on a variety of materials, including fruits, vegetables, and cereals (Begum *et al.*, 2009). The carbon sources for the growth of *A. niger* are glucose, fructose, maltose, and sucrose. It requires a source of nitrogen for growth, which can be provided by inorganic nitrogen sources such as ammonium sulfate, or organic nitrogen sources such as peptone and yeast extract and grow in aerobic conditions. Good aeration is important for optimal growth and productivity. *A. niger* requires a certain level of moisture in order to grow (Bankar *et al.*, 2009). It produces a wide range of extracellular enzymes that can degrade complex organic compounds, including rubber oxidases and latex clearing which helps in the oxidation of isoprene and glucose units (Gurung *et al.*, 2013; Pathak, 2017).

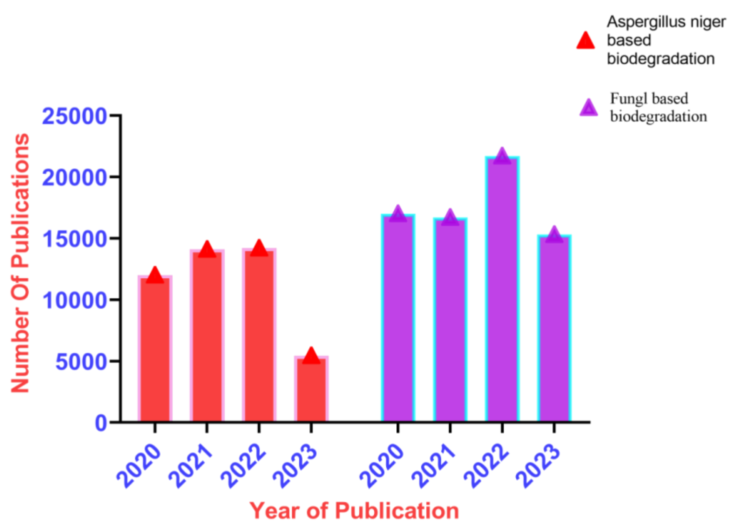

Fig. 1. Number of publications on *A.niger*-based biodegradation and fungal-based biodegradation.

Scientists have discovered up to 100 different types of rubber-degrading fungi and bacteria from different habitats (Basik *et al.*, 2021). *Penicillium, A. niger, Emericellanidulans, A. fumigatus,* and *A. flavus* are capable of degrading the NR with the most efficiency (Bezuneh, 2016).

The cultivation of *A. niger* demands a judicious selection of growth mediums, each contributing a unique backdrop for its flourishing. Notable options include Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt Yeast Agar (MYA), Minimal Media, and Czapek Dox Agar (CDA). Within this array, the pinnacle of efficiency emerges in the context of PDA media, serving as the stage upon which *A. niger* displays its robust growth and intricate metabolic dance (Ikechi-Nwogu and Elenwo, 2012).

The presence of *A. niger* triggers the secretion of cutinase, a potent enzyme that orchestrates the hydrolysis of the rubber's uppermost layer. Within this intricate choreography, a pivotal participant emerges rubber oxygenase, an enzyme with a penchant for oxygen molecules. As this enzyme converges with the rubber polymer, its transformative power becomes evident (Kum *et al.*, 2015). It masterfully cleaves both the cis- and trans-isoprene units, setting forth a cascade of reactions that culminate in the emergence of oxidized rubber products. This symphony of enzymatic interactions sheds light on the remarkable capacity of *A. niger* to engage with and transform complex substrates, offering a glimpse into the intricate interplay of biology and chemistry at the microbial scale (Rose and Steinbuchel, 2005). The beta-oxidation pathway emerges as a pivotal player, orchestrating the intricate dance of breakdown. This biochemical journey finds its maestros in the form of LCPK30, RoxA, and RoxB, regulatory entities that guide the harmonious progression of reactions. This pathway is marked by a sequence of transformations, a meticulous choreography that ultimately achieves the complete degradation of NR. The essence of this process extends beyond mere degradation, as the beta-oxidation pathway carries an additional dimension of significance. It carves a path for the utilization of NR as an energy source, illuminating the intricate tapestry of biological assimilation (Kasai, 2020).

Rubber Wastes and their management

Rubber waste, which encompasses discarded or unused rubber materials from various industrial sources such as tire manufacturing, automobile maintenance, and other processing, poses significant environmental challenges if not properly managed. Large piles of waste tires, for example, tower into the skies resembling modern Stonehenges in junkyards. However, these rubber stockpiles leach harmful chemicals like heavy metals into the surrounding soil and waterways over time, contaminating the environment and posing risks to human and ecosystem health (Ramachandra, 2006).

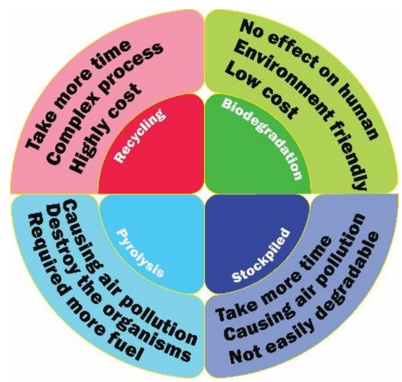

Fig. 2. Various methods used for the biodegradation of NR.

Methods involved in rubber waste management are recycling, incineration, or pyrolysis. Recycled rubber can be used in various applications, such as playground surfaces, athletic fields, and road construction. Additionally, rubber waste can be converted into energy through incineration or pyrolysis, which involves heating the rubber waste to break it down into usable fuels such as oil and gas (Sarkawi *et al.*, 2016). But these are obsolete now due to their adverse environmental drawbacks such as oxidative degradation and photo-degradation as they affect the mechanical properties (Martins *et al.*, 2008).

Table 1. Limitations and approaches to enhance NR degradation.

Limitation of NR Degradation	Approaches to Enhance NR Degradation
Due to the complex polymeric structure of NR, the rate of biodegradation is slow (Tyagi and Kumar, 2021)	The use of mixed cultures of NR-degrading fungi and bacteria is known as microbial consortia (Krishnaswamy and Ahongsangbam, 2017)
The degradation mechanism of rubber by fungi is not fully understood because of the Complex degradation mechanism (Chittella *et al.*, 2021)	To understand the mechanism of rubber degradation by fungi at the molecular level through the use of omics technologies such as genomics, transcriptomics, and proteomics (Ijoma *et al.*, 2021)
Availability of appropriate fungal strain for NR biodegradation (Tsuchii, 1995)	Discovery and Isolation of new fungal strain for the Use of genetic engineering techniques (Chittella *et al.*, 2021)
Rubber biodegradation by fungi is performed at laboratory level and scaling up the process to an industrial level is challenging (Chittella *et al.*, 2021)	Develop suitable bioreactor systems such as Continuous culture systems and immobilization of fungal (Islam, 2015)

Supportability efforts to manage waste products of rubber derivatives could be managed through microorganisms by bio-degradation processes that are self-mediated at dumping sites (Clark, 2015). This method is favorable because it is environment friendly, low cost, and high efficiency (Raymond *et al.*, 2001). It helps in the management of organic waste, such as rubber waste, food waste, agricultural residues, and municipal solid waste. By breaking down this waste, biodegradation has the potential to minimize the quantity of trash disposed in landfills and also limit the environmental consequences of garbage disposal (Albright III and Chai, 2021). It can also degrade a wide range of organic pollutants, including pesticides, petroleum hydrocarbons, and polychlorinated biphenyls (PCBs), thereby reducing their toxicity and restoring ecosystem health through biodegradation (Dua *et al.*, 2002).

Table 2. Various types of rubber waste and their management.

Rubber Waste	Management Methods	Advantages	Disadvantages	References
Scrap Tires	Recycling, Pyrolysis, Landfilling, Civil Engineering Applications	Resource conservation and energy savings Reduction of landfill space	Limited recycling infrastructure Require high capital investment	(Tsang, 2013)
Rubber Conveyor Belts	Recycling, Repurposing, Landfilling	Recycling conserves resources and reduces waste Landfilling provides a disposal option	Limited recycling options for complex rubber components Long degradation time for rubber in landfills	(Peng *et al.*, 1997)
Rubber Powder	Reclaimed Rubber Production, Rubberized Asphalt, Sports Surfaces	Reduction of waste and environmental impact Enhanced safety and performance in sports surfaces	Cost of production and limited markets for reclaimed rubber Challenges in blending rubber powder	(Diekmann *et al.*, 2019)

Rubber Mulch	Landscaping, Playground Surfaces, Equestrian Surfaces	Aesthetic appeal and durability in Landscaping Enhanced safety and cushioning in playgrounds	Leaching of chemicals and potential environmental impact Limited biodegradability and accumulation in the environment	(Pelkki *et al.*, 1996)
Rubberized Concrete	Construction Applications, Noise Reduction	Improved impact resistance and durability Reduced noise pollution and vibration	Increased cost compared to conventional concrete Challenges in achieving a consistent rubber-to-concrete ratio	(Eldin and Senouci, 1994)

Biodegradation of NR

Currently, biodegradation continues to be a vibrant subject of research, particularly gaining momentum within developing nations. However, the prevailing outcomes presented in existing reports exhibit certain irregularities and noticeable vulnerabilities (Gao and Gu, 2021). In this process, microbes such as bacteria, fungi, and enzymes, break down and convert complex organic compounds (NR) into simpler compounds, which are then used by the organisms as nutrients (Khatoon *et al.*, 2017). This process is important for the recycling of nutrients in ecosystems, as well as for the degradation of pollutants in the environment. Fungi decompose a wide range of organic substances., including lignin, cellulose, and chitin, which are found in plant and animal tissues (Gupta *et al.*, 2017).

Table 3. Different research conducted on *A. niger* base biodegradation.

Topic of Research	Rubber degraded (%)	Time (Days)	Reference
Degradation of Natural Rubber by Fungi: *Aspergillus niger* and *Penicillium sp*	28.3	40	(Nayanashree and Thippeswamy, 2013)
Biodegradation of Waste Natural Rubber by *Aspergillus niger*	15.6	236	(Bosco and Mollea, 2021)
Aspergillus niger Van Tieghem Degradation of Different Types of Rubber Waste	22	49	(Sharma *et al.*, 2022)
Rubber gloves biodegradation by a consortium, mixed culture and pure culture isolated from soil samples	18	30	(Nawong *et al.*, 2018)
Biodegradation of natural and synthetic rubbers	40	91	(Ali Shah *et al.*, 2013)

One of the key mechanisms by which fungi degrade organic matter is through the production of extracellular enzymes. These enzymes are secreted by the fungi and act to break down complex molecules into smaller, more easily metabolizable compounds The biodegradation of NR can occur through two main pathways: aerobic and anaerobic. Aerobic degradation occurs in the presence of oxygen, while anaerobic degradation occurs in the absence of oxygen (Bosco and Mollea, 2021). Various enzymes, such as oxygenases, hydratases, and rubber-cleaving enzymes, play a pivotal role in degrading rubber's polymer chains. As a result of aerobic rubber biodegradation, the ultimate byproducts are carbon dioxide and water (Mollea and Bosco, 2020).

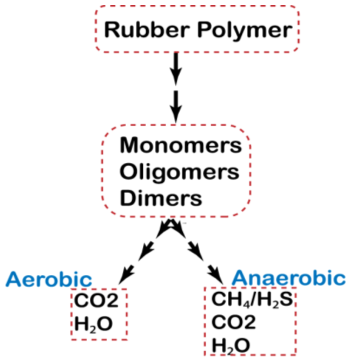

Fig. 3. The degradation of rubber polymers can occur under both aerobic and anaerobic conditions redrawn from (Gu, 2003).

The anaerobic biodegradation of rubber is a comparatively slower process that occurs through the activity of bacteria in environments lacking oxygen and is primarily involved in the production of methane and carbon dioxide (Samešová *et al.*, 2023). *Myceliophthora thermophila, A. niger, Bacillus sp,* and *Pseudomonas aeruginosa* are also able to degrade NR that produce a variety of extracellular. *A. niger* secretes Rubber Oxidases (RO) and peroxidase, which are responsible for breaking down the rubber polymer (Mohamed *et al.*, 2017a). RO cleaves the double bonds within the rubber polymer, that are metabolized by *A. niger*. *Penicillium chrysogenum* secretes laccase and peroxidase for NR degradation (Mollea and Bosco, 2020). *Rhizopus stolonifer* can degrade both natural and synthetic rubber that secretes RO, peroxidase, and laccase (Jain *et al.*, 2017).

Table 4. Various methods were used in the biodegradation of NR by *A. niger* along with research methods and key findings.

Research Methods	Conclusion	Requirements	References
Solid fermentation and submerged fermentation	*A. niger* effectively degrades natural rubber within 30 days	Initial latex concentration, Nitrogen source, Carbon source, pH, Temperature	(Dutt and Kumar, 2014)
NMR spectroscopy, HPLC and electron microscopy	*A. niger* causes surface erosion of natural rubber and breaks down the polymer	Surface erosion, Hydrolysis, Oxidation, pH, temperature	(Arutchelvi *et al.*, 2008)
Rubber biodegradation under different conditions	*A. niger* demonstrates significant rubber biodegradation under varying conditions	pH, Temperature, Agitation speed, Carbon source, Nitrogen source	(Arimoro, 2009)
Rubber-degrading potential using dye-based germination test	*A. niger* exhibits substantial natural rubber biodegradation capabilities, providing sustainable waste management options	Triphenyl tetrazolium chloride (TTC) reduction, ,Carbon source, Nitrogen source	(Song *et al.*, 2009)
Solid-state fermentation and enzyme characterization	*A. niger* isolated from soil demonstrates significant natural rubber biodegradation	Isolation method, Strain identification, Enzyme assay	(Bosco and Mollea, 2021)

Factor affecting the rate of biodegradation

The rate of biodegradation can be influenced by several factors, including temperature, moisture, oxygen availability, pH, and the presence of microorganisms.

1. Temperature: Higher temperatures generally increase the rate of biodegradation, as microorganisms grow and metabolize more quickly at higher temperatures. For example, bacteria in soil can degrade organic matter up to 30% faster at 25°C than at 15°C (Haider *et al.*, 2019).

2. Moisture: Microorganisms require water to carry out their metabolic processes, so adequate moisture is essential for biodegradation. In general, higher moisture levels lead to faster biodegradation rates (Abatenh *et al.*, 2017).
3. Oxygen availability: Many microorganisms require oxygen to carry out their metabolic processes, so the availability of oxygen can impact the rate of biodegradation. In aerobic environments, where oxygen is present, biodegradation tends to occur faster than in anaerobic environments (Bouwer and Zehnder, 1993).
4. pH: The pH of the environment can also influence the rate of biodegradation. Most microorganisms prefer a narrow range of pH values, typically between 6 and 8, and changes in pH outside this range can affect their growth and activity (Grima *et al.*, 2000).
5. Presence of microorganisms: The presence of microorganisms, such as bacteria, fungi, and protozoa, can significantly impact the rate of biodegradation. These organisms play a crucial role in breaking down organic matter through various mechanisms, including hydrolysis, fermentation, and respiration (Doukani *et al.*, 2023; Leahy and Colwell, 1990).

Fungi involved in the Biodegradation of NR

There are various fungi that are involved in the biodegradation of NR. Different types of fungi degrade different types of NR. While the degradation by fungi is a more complex mechanism in the scientific world. It has been investigated *that A. niger* can degrade both Natural Rubber and Artificial Rubber (Mohamed *et al.*, 2017b). Several studies have investigated the biodegradation of NR by *A. niger* and compared its effectiveness to other fungi. One study published in the Journal of Applied Polymer Science found that *A. niger* was capable of degrading NR within 28 days under optimized conditions, while other fungi tested were only able to degrade up to 20-30% of the rubber within the same timeframe.

Table 5. List of Fungus involved in Biodegradation of NR.

Fungal Strain	Source	Weight loss (%)	References
Aspergillus niger	Dumping sites	28.30	(Mohamed *et al.*, 2017c)
Aspergillus tubingensis	Dumping sites	91.30	(Cho *et al.*, 2016)
Aspergillus terreus	Trunk of *Ficus elastica*	31.04	(Ismail *et al.*, 2013)
Myceliophthora thermophila	Trunk of *Ficus elastica*	23.47	(Ismail *et al.*, 2013)
Aspergillus flavus	Trunk of *Ficus elastica*	28.73	(Ismail *et al.*, 2013)
Penicillium chrysogenum	Dumping sites	64.30	(Mohamed *et al.*, 2017c)
Rhodotorula mucilaginosa	Soil	5.60	(Bosco *et al.*, 2018)
Phlebia radiate	Woody area	27.70	(Joseph *et al.*, 2022)
Penicillium variable	Permafrost	vary	(Joseph *et al.*, 2022)
Paecilomyces variotii	Soil	35.50	(Joseph *et al.*, 2022)
Rhizopus oryzae	Decaying plant	28.30	(Abdullah *et al.*, 2015)
Paecilomyces lilacinus	Plant Debris	20	(Joseph *et al.*, 2022)
Cladosporium cladosporioides	Woods	10	(Joseph *et al.*, 2022)
Aspergillus versicolor	Soil	30	(Jain *et al.*, 2017)

Another study published in the International Biodeterioration & Biodegradation journal compared the biodegradation of natural rubber by three different fungi: *A. niger*, *Penicillium chrysogenum,* and *Fusarium solani*. The results showed that *A. niger* was the most effective at degrading natural rubber, with a degradation rate of up to 60% after 28 days. It is performed at a laboratory in various steps. *A. niger* secrete enzymes to the extracellular medium. The fungus strain mycelium diffuses into dextrose, and agarose and degrades the rubber polymer into its monomer or microparticles. While, large molecular isoprene products are used up by themselves (Röther *et al.*, 2016). By using an overlay procedure, it was possible to create latex-coated agarose by letting the lower layer of agar, which contains mineral salts, harden in a growth or Petri plate. Then it was coated by adding Agar supplemented with 20 (g/ml) pure

Latex. It assists in separating the bacteria and fungus responsible for degrading rubber (Aguilar-Bolados *et al.*, 2023). The second group does not produce semi-transparent aurora for degradation. Therefore, they must interact directly with the rubber strain to degrade them (Tsuchii and Takeda, 1990).

Table 6. Physiological parameters and growth conditions for *Aspergillus niger* during the degradation of NR.

Parameter	Value/Ranges	Citation/Reference
Temperature	25-37°C (optimal: 30-35°C)	(Selouane *et al.*, 2009)
pH	2-7 (optimal: 5-6)	(Selouane *et al.*, 2009)
Carbon Source	Rubber	(Dutt and Kumar, 2014)
Nitrogen Source	Ammonium sulfate, peptone	(Iram *et al.*, 2022)
Moisture Content	50-70% (optimal: 60-65%)	(Pal and Khanum, 2010)
Oxygen Requirement	Aerobic	(Hou and Bao, 2018)
Agitation/Shaking Speed	150-200 rpm (optimal)	(Dlamini *et al.*, 2019)
Incubation Period	3-7 days	(Fernandez Vergano *et al.*, 1996; Nisyak and Wardani, 2023)
Light Requirement	Dark or low-light conditions	(Al Hallak *et al.*, 2023)
Inoculum Size	1-5% (v/v) of total medium volume	(Zhang *et al.*, 2023b)
Growth Medium Composition	Potato Dextrose Agar (PDA) or defined media	(Patil and Pangrikar, 2023)

Media used for the growth of *Aspergillus niger*

We have compared different media used for the growth of *A. niger.* Among all the media the growth and rate of degradation of *A. niger* were more efficient in PDA. It contains potato extract and dextrose that degrades rubber upto 40-90% at the rate of 5.2mm/day in 5.6 pH at 7 days (HAMAD *et al.*, 2014). Selection of media for the degradation of NR, depending on the specific requirements of the strain being cultured and the purpose of the growth. Therefore, it is recommended to consult the literature and the supplier's recommendations when selecting a growth medium for *A. niger*. Here is the list of commonly used media for the growth of *A. niger*.

Table 7. Characteristics of PDA, SDA, MYA, Minimal media, and CDA that are used for the growth of *A. niger*.

Media	Composition	pH	Rate of NR Degradation	Growth rate (mm/day)	Incubation time
PDA	Potato infusion, dextrose, Agar	5.6	40-90%	5.2	3-7 days
MYA	Malt extract, peptone, Agar	5.5	61.7%	7	7 days
SDA	Peptone, dextrose, Agar	5.6	60%	5.2	3-7 days
Minimal Media	Asparagine, glucose, trace elements	6.0	75%	3.6	7 days
CDA	Sodium nitrate, Dipotassium phosphate Agar	6.5	36%	6.9	7 days

Mass balance and stoichiometry study

It is the study of the quantitative relationships between reactants and products in chemical reactions. It is used to track the movement of matter into and out of a body which is helpful to identify any potential bottlenecks in the biodegradation process and to optimize the conditions for biodegradation (Hidayat *et al.*, 2023). To calculate the amount of product that can be produced from a given amount of reactant and to identify any potential limiting reactants (Ghosh *et al.*, 2003). It helps to identify the optimal conditions for biodegradation and efficiency of NR biodegradation processes.

To conduct these studies, you would need to collect data on various parameters:

In this experimental process, a range of conditions, including initial NR concentration, temperature, pH, and inoculum size, are set up. *A. niger* culture is grown under these conditions, with periodic sampling to track changes over time. Collected samples are then analyzed to determine NR, biomass, oxygen, and nutrient quantities, and this data is used to calculate mass balance and stoichiometry. To understand how different factors affect the biodegradation process, the steps are repeated with various experimental conditions. In your example, if you start with 100 grams of NR and after 10 days you have biodegraded 50 grams of NR, along with producing 20 grams of biomass, it means that 30 grams of NR have been converted into biomass and other products, including carbon dioxide and water.

We can use the mass balance equation to calculate the amount of oxygen consumed by the microorganisms:

Mass of oxygen consumed = Mass of NR biodegraded - Mass of biomass produced - Mass of other products

A. niger consumed a total of 30 grams of oxygen, which we determined by subtracting the mass of biodegraded NR from the combined mass of biomass and other byproducts. This information is quite valuable in helping us understand the metabolic processes involved in rubber biodegradation by *A. niger*, shedding light on the stoichiometry of the process. We can also use these stoichiometric calculations to figure out the amount of nutrients that the microorganisms consumed. For instance, if the microorganisms need 1 gram of nitrogen for every 2 grams of NR biodegraded, then they would have used up 2.5 grams of nitrogen during this experiment. Conducting mass balance and stoichiometry studies allows us to gain a deeper understanding of how *A. niger* degrades NR, and this knowledge can guide us in developing more efficient and environmentally friendly rubber biodegradation processes (Ghosh *et al.*, 2003).

Biochemical pathway of NR degradation

A. niger can degrade rubber efficiently, however, they do so in order to use the polymer as an energy and carbon source for growth. Hence, there is a proposed mechanism for rubber biodegradation (Ibrahim *et al.*, 2006). Firstly, *A. niger* produces an extracellular enzyme called cutinase, which hydrolyzes the surface layer of the rubber material and enables the fungus to attach and colonize the surface. Rubber oxygenase and rubber glucanase are enzymes that break down the rubber polymer into smaller molecules. Rubber oxygenase cleaves both cis and trans-isoprene units in the rubber polymer by introducing oxygen molecules into the polymer, resulting in the formation of oxidized products (Röther *et al.*, 2016). Rubber hydratase then hydrates the oxidized products, resulting in the formation of keto acids. Finally, rubber dioxygenase cleaves the carbon-carbon bonds in the keto acids, resulting in the formation of shorter chain acids and aldehydes that can be utilized by the microorganism. The endo-β-1,4-glucanases cleave the rubber polymer chains at random points, producing smaller oligomers (Birke and Jendrossek, 2014). LCP cleaves rubber into multiple products corresponding mainly to oligo(cis-1,4-isoprene) molecules containing from four up to seven isoprene units (Andler, 2020). Rubber oxygenase and rubber glucanase are capable of breaking down the rubber polymer into smaller molecules that cleave both cis- and trans-isoprene units in the rubber polymer (Chandra and Rustgi, 1998). It involves the introduction of oxygen molecules into the rubber polymer, resulting in the formation of oxidized products. Rubber hydratase is involved in the hydration of the oxidized products, resulting in the formation of keto acids (Hiessl *et al.*, 2012). Finally, rubber dioxygenase cleaves the carbon-carbon bonds in the keto acids, resulting in the formation of shorter chain acids and aldehydes that can be utilized by the microorganism (Abu Hassan *et al.*, 2023; Leja and Lewandowicz, 2010). The endo-β-1,4-glucanases cleave the rubber polymer chains at random points, producing smaller oligomers. LCP cleaves rubber into multiple products corresponding mainly to oligo(cis-1,4-isoprene) molecules containing from four up to seven isoprene units (Highley, 1997). Whereas oxi A and oxi B are located at the downstream of LCP operon and help in degradation (Basik *et al.*, 2021). Gene oxi A is smaller in size, which is crucial for iron-sulfur centers to respond with their genes. Oxi B is bigger and is coupled with a dinucleotide cofactor. Both oxi A and oxi B genes are released into the media and aid in metabolism via the beta-oxidation pathway (Awakawa *et al.*, 2023).

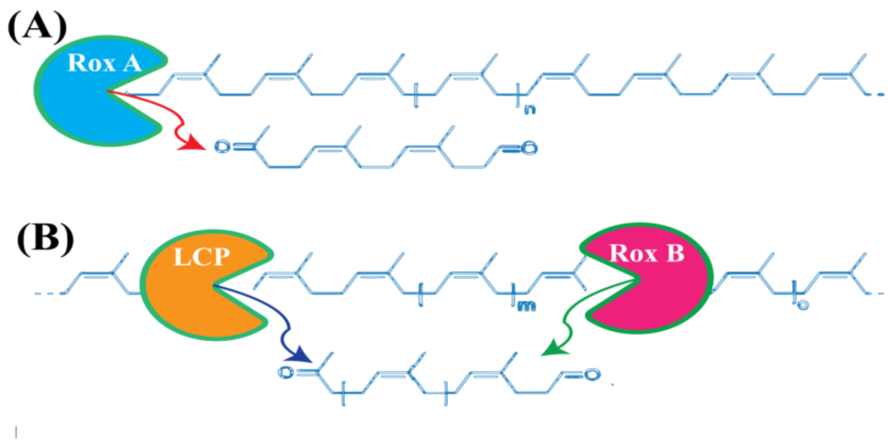

Fig. 4. Cleavage activity of Rox A, Rox B, and LCP. **(A)** Rox A break the isoprene bonds from the fixed directional side and form ODTD(12-oxo-4,8-dimethyl-trideca-4,8-diene-1-al) **(B)** Rox B and LCP cleave the bonds from any point or direction. As a result, it degraded the isoprene units redrawn from (Soares and Steinbüchel, 2022).

Enzymes are the most important for the degradation of NR through *Aspergillus* (Genus). Because they lack growth regulators and media for their cultivation. When polyisoprene is treated with these enzymes it makes holes in the rubber and degrades in a few days (3-8) (Nikolaivits *et al.*, 2021). If both enzymes (rubber oxygenase and regulatory systems) are combined, they develop a new chemical reaction in which there is no need for a mediator like fatty acid (Rose and Steinbuchel, 2005). *Rox* facilitates the oxidation of cis double bonds in polyisoprene (polymer backbone), and the product generated is carbonyl and aldehyde functional groups in this type of degradation (Rovaletti *et al.*, 2023). LCP catalyzes the breakdown of the C=C double bond found in polymers (cis-1,4-isoprene). During this process, oxygen is added between the double bonds, resulting in the development of an operon with three genes, LCP, OxiA, and OxiB. LCP catalyzes the breakdown of the C=C double bond found in polymers (cis-1,4-isoprene). OxiA is required for the iron-sulfur cluster core, whereas OxiB is a large subunit that may bind the molybdopterin cytosine dinucleotide cofactor (Nanthini *et al.*, 2017). OxiA is a molybdenum-dependent hydroxylase that is discharged from the cell. It is possible for cells to absorb and digest the low molecular weight early breakdown products via oxidation once they have been transformed by oxidation into necessary molecules like carboxyl. Therefore, rubber fragmentation through an enzymatic process is a much more attractive methodology to produce fine chemicals from NR. By analyzing the enzymes present in the clearing zones two major types of rubber-degrading enzymes have been identified so far (Watcharakul *et al.*, 2016). The LCP Operon pathway has three kinds of genes that aid in the cleavage of double bonds in polyisoprene with the presence of oxygen (Hiessl *et al.*, 2014).

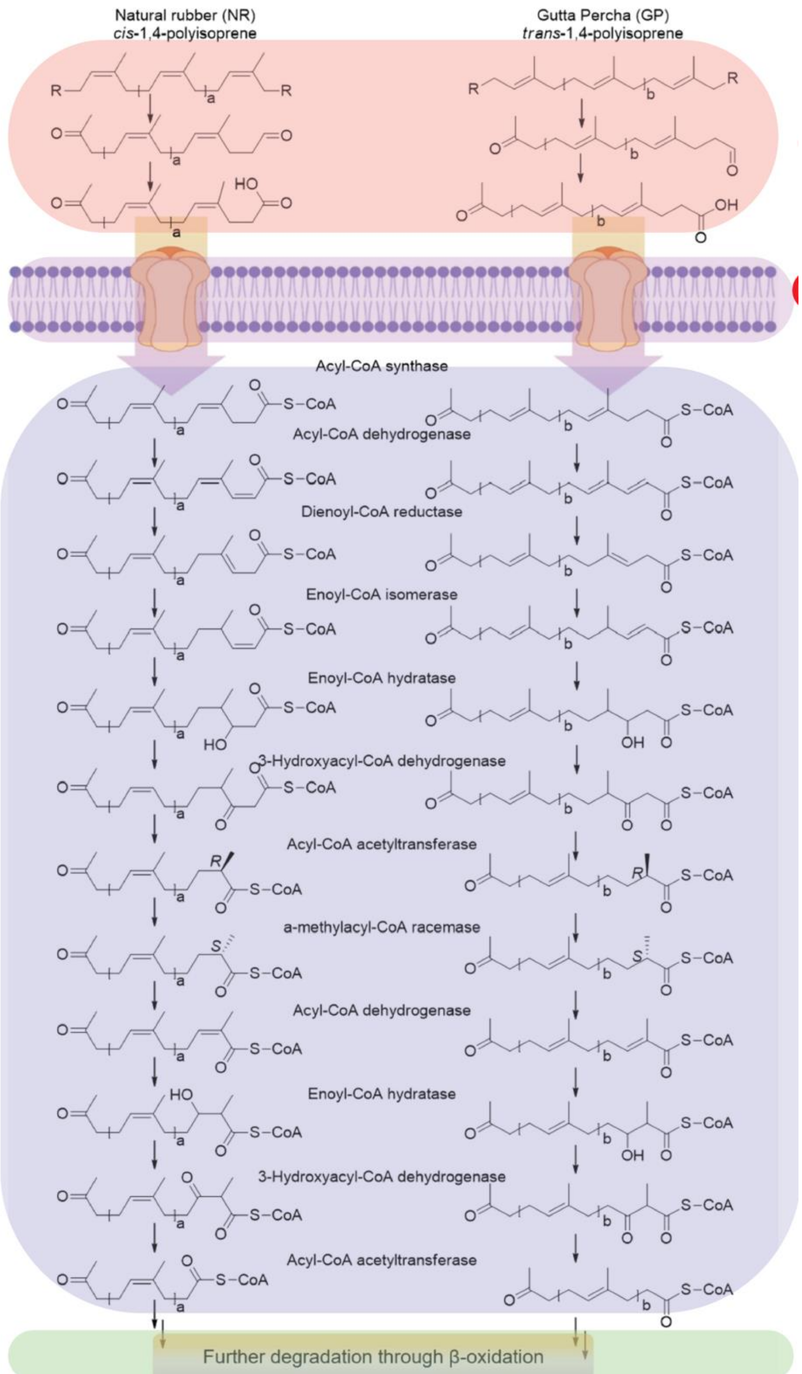

Fig. 5. Proposed mechanism **of** degradation of Cis-1,4-Polyisoprene and Trans-1,4-Polyisoprene **(A)** shows the conversion of polyisoprene by the action of LCP **(B)** Cell membrane with receptors that help in the transportation of Polyisoprene **(C)** Enzymatic activity involved in the degradation of polyisoprene by a series of reaction and enzymes **(D)** Beta oxidation of polyisoprene for further degradation redrawn from (Soares and Steinbüchel, 2022).

Beta oxidation of polyisoprene is a process by which the polymer is broken down into smaller molecules, starting with the removal of a 3-carbon unit from the end of the chain. The mechanism relies on a series of enzymes known as beta-oxidation enzymes. To kickstart the process, the initial enzyme, 3-hydroxyacyl-CoA dehydrogenase, extracts a hydrogen atom and a hydride ion from the 3-carbon atom of the polyisoprene molecule. This initiates the formation of a 3-hydroxy acyl-CoA intermediate. Subsequently, this intermediate undergoes oxidation, thanks to the action of the next enzyme in the pathway, 3-hydroxy acyl-CoA thiolase. This oxidation phase results in the creation of a 2-carbon acetyl-CoA molecule and a 5-carbon acyl-CoA molecule. The acyl-CoA molecule then proceeds through further oxidation, facilitated by subsequent enzymes in the pathway, ultimately breaking down the polyisoprene molecule into acetyl-CoA molecules. This process, known as the beta-oxidation of polyisoprene, plays a vital role in the metabolism of polyisoprene polymers like natural rubber and has applications in the production of synthetic rubbers like styrene-butadiene rubber (SBR) (Birke *et al.*, 2017). The rubber oxygenases produced by *A. niger* can further degrade the isoprene monomers into smaller molecules, such as 2-methyl-2,5-cyclohexadiene-1,4-dione (Chen *et al.*, 2020). A report has been published where they combine enzymes of LCPK30 and R*ox*A or R*ox*B and show a symbiotic effect on the rate of rubber degradation (Basik *et al.*, 2021).

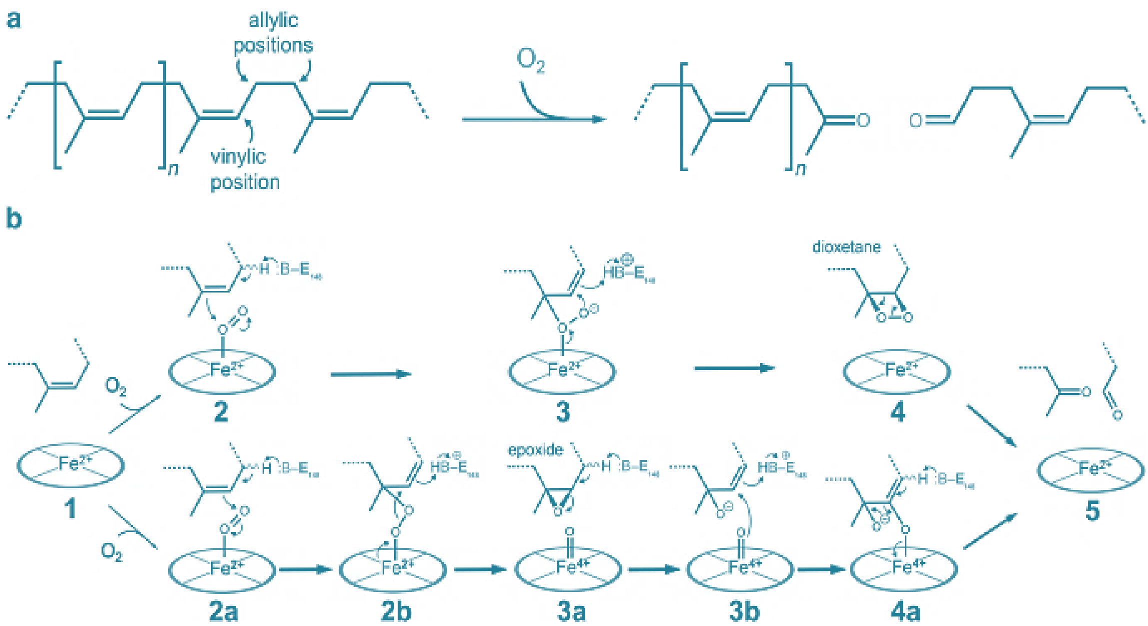

Fig. 6. Mechanistic models of oxidative polyisoprene cleavage by LcpK30 **(a).** LCPAK30 catalyzes isoprenoid cleavage by introducing both oxygen atoms from an O2 molecule. Mechanisms of possible response **(b).** Top: The surface polymer is inserted into the open network of LCPK30 (1). O_2 binds to the distal angular plane of haem iron, and a base in the channel, most likely Glu148, steals a proton from an allylic region, resulting in the creation of a bond with oxygen (2). The nearby carbon is attacked by the subsequent oxygen atom, which has a stronger nucleophilic nature (3). This results in the production of an unstable cyclic dioxetane intermediate (4) that dynamically reorganizes to the bisected precursor (5). Bottom: The haem-bound dioxygen can also be severed (2b) to produce a potting medium of epoxide and an oxy-ferryl intermediate Bottom: The haem-bound dioxygen can also be severed (2b) to produce a potting medium of epoxide and an oxy-ferryl intermediate (3a). A nucleophilic assault of the oxy-ferryl-oxygen on the epoxide carbon atom cleaves the epoxide bond (3bSeparation of the iron-oxygen link (4a) releases the haem group as well as the observable cleavage products (5) redrawn from (Jendrossek and Birke, 2019).

The mechanism of assimilation of isoprene units is not fully understood, but it is thought to involve several steps. The transport of isoprene units into the cell is an essential initial step. This can be achieved through passive diffusion or facilitated by specific transporters. Once these isoprene units are inside the cell, they can undergo various conversions, resulting in the production of a wide range of different compounds (Rasulov *et al.*, 2009). In specific cases, these isoprene units can be harnessed to produce rubber, a polymer comprised of long chains of isoprene units, found in various plants like rubber trees, dandelions, and goldenrods. Notably, rubber is also present in some insects, such as ladybugs and stink bugs. Conversely, isoprene units can be utilized to synthesize other compounds, including terpenes and steroids. Terpenes, a group of organic compounds formed from isoprene units, are abundant in a wide variety of plants, including conifers, citrus fruits, and mints. On the other hand, steroids, another class of organic compounds composed of isoprene units, are found in animals, plants, and fungi (Schwab *et al.*, 2013). *A. niger* successfully accumulated rubber from an NR latex solution. This rubber material was observed in the form of substantial aggregates, believed to result from the amalgamation of smaller rubber particles. Remarkably, these rubber aggregates exhibited toxicity towards various microorganisms, including bacteria like *Pseudomonas aeruginosa and Escherichia coli* (Dimapilis *et al.*, 2018). The field of rubber biodegradation offers vast opportunities for exploration and discovery. Gaining a comprehensive understanding of the entire metabolic process, uncovering new organisms with similar capabilities and investigating potential applications represent intriguing avenues for research and innovation.

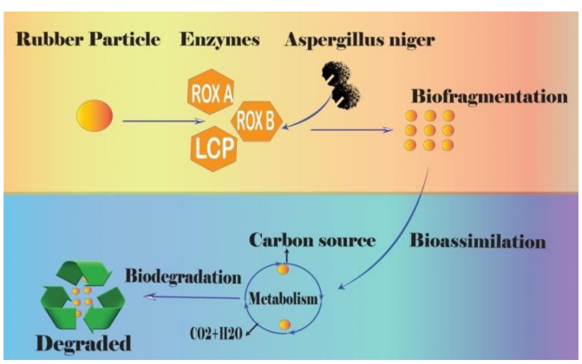

Fig. 7. Biofragmentation and Bioassimilation of NR.

This research holds significant implications, encompassing effective waste rubber management and the sustainable development of bio-based materials for diverse industries. The ongoing exploration of rubber bioassimilation presents a promising path toward environmental sustainability and industrial advancements.

FUTURE PERSPECTIVES

Science is an intricate and multifaceted discipline that has led to numerous groundbreaking discoveries and innovations (Gu, 2021a). A Path to a Sustainable Future The emergence of biodegradable NR offers a groundbreaking opportunity to transform the way we produce essential commodities. By leveraging the power of microbial consortia and genetically modified microorganisms, we can optimize rubber degradation and create a more sustainable future. Immobilizing rubber-degrading enzymes on supportive matrices like gels or membranes not only stabilizes them but also makes them more recyclable. Educating industry stakeholders about the benefits of biodegradation is crucial to drive demand for biodegradable products and encourage the adoption of sustainable practices. Parallels between rubber biodegradation and microbiology reveal a shared reality that extends beyond their respective domains.

By promoting achievements, fostering interdisciplinary partnerships, and organizing conferences, workshops, and symposiums dedicated to rubber biodegradation, we can create a more attractive field that attracts talented researchers and drives sustainable practices. Moreover, raising awareness about the environmental impact of conventional rubber production and investing in innovative protocols and methods specific to rubber biodegradation will accelerate progress in the field. Join us in unlocking the full potential of biodegradable natural rubber and shaping a brighter future for generations to come. By working together, industry associations, non-profit organizations, and government agencies can help propel the widespread adoption of biodegradation principles and pave the way for a greener future.

CONCLUSION

Aspergillus niger is a remarkable fungus with the potential to revolutionize natural rubber (NR) degradation. Through its rapid growth and diverse enzymatic toolkit*, A. niger* shows great promise in tackling the global rubber waste problem in an environmentally friendly manner. This hardy fungus can spread at an impressive rate of over 5 millimeters per day on agar plates, allowing it to efficiently colonize rubber substrates. Even more fascinating are its specialized enzymes that work in tandem to systematically break down rubber polymers. Rubber oxidases initiate the process by cleaving chemical bonds within the polymer chains. Latex-clearing proteins then help remove the initial tackiness so other enzymes can access the material. Pectinases further degrade the main building block, isoprene, into smaller units that can re-enter the carbon cycle. Not only can *A. niger* degrade natural rubber, but it has also demonstrated flexibility in attacking various synthetic rubbers as well. Through its ability to adapt to different polymer structures, this organism shows promise for recycling multiple types of post-consumer tires and hoses. With continued research, we may uncover even more enzymes in its toolkit that could be harnessed for new applications. By leveraging *A. niger's* natural abilities, the production of new rubber goods may one day rely more on recycled feedstocks rather than virgin materials. This could help reduce our environmental footprint while sustaining rubber industries. With further study of this remarkable fungus and refinement of degradation processes, we are moving closer to establishing a more circular rubber economy. *A. niger* could play a key role in shaping a greener future through its contributions to sustainable materials recovery.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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