catalysts

MDPI

Review

Nitrile-Converting Enzymes: Industrial Perspective, Challenges and Emerging Strategies

Binuraj R. K. Menon [1,*], James David Philpin [1], Joe James Scaife [1,2] and Thomas Chua [1]

[1] Centre for Enzyme Innovation, School of the Environment and Life Sciences, University of Portsmouth, Portsmouth PO1 2DT, UK; joe.scaife@myport.ac.uk (J.J.S.); thomas.chua@myport.ac.uk (T.C.)
[2] Defence Science and Technology Laboratory, Porton Down, Salisbury SP4 0JQ, UK
* Correspondence: binuraj.menon@port.ac.uk

Abstract

Nitrile-containing compounds are integral to pharmaceuticals, agrochemicals and polymer industries, yet their environmental persistence and toxicity pose major challenges. Biocatalytic approaches using nitrile-converting enzymes—particularly nitrilases and nitrile hydratases—offer sustainable alternatives to conventional hydrolysis, enabling the selective transformation of nitriles into amides and acids under mild conditions. This review presents an industrial perspective on nitrile-converting enzymes, summarising their catalytic potential, current limitations, and emerging strategies for stability, activity and performance enhancement. Advances in protein engineering, metagenomic discovery and biocatalytic optimisation have already expanded their wider applicability, while synthetic biology and protein design tools are accelerating the development of tailored biocatalysts. The integration of these enzymes into cascades and chemoenzymatic processes supports scalable and innovative solutions to green manufacturing. Collectively, these emerging strategies position nitrile-converting enzymes as versatile tools for sustainable catalysis, with growing relevance in fine chemical synthesis, waste remediation, and bio-based synthetic platforms.

Keywords: biocatalysis; nitrilase; nitrile hydratase; catalysis; cyano groups; enzymes; chemocatalysis; cascades

check for updates

Academic Editor: Evangelos Topakas

Received: 7 August 2025
Revised: 14 September 2025
Accepted: 25 September 2025
Published: 1 October 2025

Citation: Menon, B.R.K.; Philpin, J.D.; Scaife, J.J.; Chua, T. Nitrile-Converting Enzymes: Industrial Perspective, Challenges and Emerging Strategies. *Catalysts* **2025**, *15*, 939. https://doi.org/10.3390/catal15100939

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Cyano- or nitrile (–C≡N)-containing molecules are widely distributed in nature, present as inorganic cyanides and organic carbonitriles [1]. They originate primarily from microbial and plant biosynthetic pathways, as well as from anthropogenic chemicals. Many abiotic processes, such as combustion and volcanic emissions, could also indirectly lead to the formation of nitrile-containing molecules [2]. The nitrile group is highly versatile, contributing to an enhanced binding affinity, metabolic stability, and favourable pharmacokinetic properties, which have inspired its strategic incorporation into a broad range of pharmaceuticals and agrochemicals [3–5]. The presence of nitrile functionalities in polymers confers an improved thermal stability, chemical resistance, and mechanical strength. Additionally, the nitrile group serves as a reactive handle for the post-synthetic modifications of many intermediates, facilitating the design of novel macromolecules with tailored properties and expanded application potentials [6].

Despite their utility, nitrile-containing compounds pose high levels of environmental and health concerns [7,8]. Organic carbonitriles and nitrile-based polymers are known for

their persistence in the environment due to their resistance to degradation, contributing to long-term environmental pollution. The release of cyanide derivatives and hydrogen cyanide from these materials can cause significant health risks, underscoring the need for the sustainable design of nitrile-based materials and the development of enzyme-based efficient biodegradation and bioremediation methods.

Conversely, the biological efficacy of nitrile bioactive molecules used in pharma and agrochemical applications is often linked to the presence of a specific stereoisomer, and the biological activity varies significantly among stereoisomers [5,9]. The selective isolation of the active form is essential for optimising therapeutic and agricultural outcomes. Thus, nitrile-converting enzymes—comprising nitrilases, nitrile hydratases (NHase), cyanide dihydratases and cyanide hydratases—hold great promise not only for environmentally sustainable waste reduction, but also for the product refinement and resolution of the most biologically active isomers. Consequently, nitrile-converting enzymes have gathered significant attention in recent years due to their potential to replace the harsh chemical processes traditionally used in nitrile hydrolysis [10–12].

There are several review articles and studies published in the last few decades focusing on various aspects of nitrile-converting enzymes, including their classification [13,14], mechanisms [15,16], catalytic properties, biotechnological applications [17,18] and advantages [19–21]. In this review, our aim is to summarise the advances in nitrile-converting enzyme catalysis in reference to industrial applications and fine chemical synthesis. Drawing upon the relevant literature, this review will discuss the strategies for addressing the inherent limitations of two nitrile-converting enzymes—nitrilases and NHases. More emphasis is placed on their identification, screening and enhancement of catalytic performance and stability via protein engineering, along with a summary of progress to date, current developments and future perspectives.

2. Nitrilases and NHases

Nitrilase and NHase are two downstream enzyme groups that act on nitrile molecules produced via the aldoxime–nitrile catabolic pathway (Figure 1). Aldoximes, which are derived from amino acids, serve as biochemical precursors for different biomolecules, such as cyanogenic glucosides and glucosinolates, which are important in plant defence mechanisms. Aldoxime dehydratases mediate the conversion of aldoximes to nitriles in the aldoxime–nitrile catabolic pathway. Microorganisms, notably certain bacterial and fungal taxa involved in plant ecological networks or mutualisms, utilise nitriles as substrates for carbon and nitrogen assimilation. The metabolic degradation of nitriles to carboxylic acids in these organisms proceeds via two enzymatic routes: (1) direct hydrolysis catalysed by nitrilases and (2) a sequential pathway employing NHases and amidases (Figure 1).

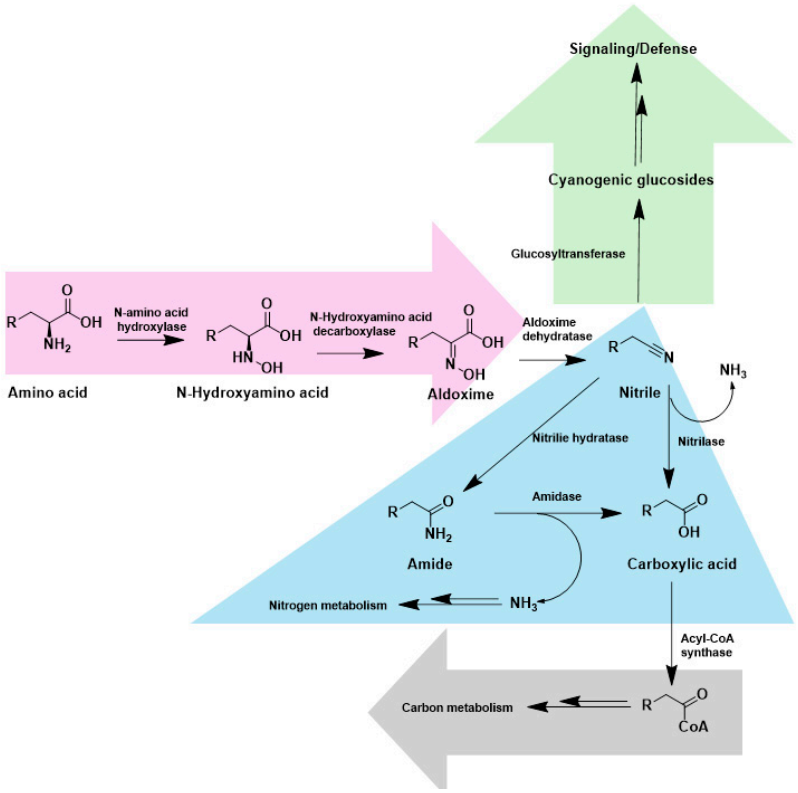


Figure 1. Aldoxime–nitrile pathway in microbes and plants, and the downstream metabolism of nitriles. Nitrilase directly hydrolyses nitriles produced in this pathway to carboxylic acids and ammonia. NHase catalyses the hydration of nitriles to amides, which are subsequently converted to carboxylic acids and ammonia by an amidase enzyme.

3. Nitrilases and Controlled Nitrile Hydrolysis via the Catalytic Triad

The nitrilase superfamily comprises various structurally related enzymes that catalyse diverse hydrolytic and condensation reactions on nitriles, as well as the detoxification of hydrogen cyanide. While early annotations grouped several enzymes including amidases under the nitrilase superfamily label, the contemporary evidence indicates that bona fide nitrilase activity is confined only to a branch of enzymes that act on organic cyanides and HCN [13,19]. The earlier classification groups may have originated because nitrilase-related domains are also present in other enzymes involved in cellular signalling and ammonia utilisation pathways, reflecting their divergent catalytic adaptations. In this review, nitrilase enzymes that hydrolyse non-peptide –C≡N bonds to form carboxylic acids are only considered for further discussion.

The nitrilase enzymes typically feature a conserved Glu-Lys-Cys catalytic triad and adopt a characteristic four-layered α-β-β-α sandwich fold (Figure 2A,B). The residues present in the α-β-β-α fold often play a role in protein interactions by forming dimers, which in turn assemble into larger oligomeric structures (Figure 2C) [22,23]. Cyanide-converting enzymes such as cyanide dihydratase and cyanide hydratase, detoxify HCN into formate and formamide, respectively, also belonging to the nitrilase superfamily as well. They share a conserved Glu–Lys–Cys catalytic triad, along with additional conserved flanking residues that support their classification as specialised nitrilase variants acting on free cyanide [18]. Nitrilases uniquely catalyse the direct hydrolysis of nitriles, without producing intermediate amides, via a covalent thiol-mediated catalysis. However, the promiscuous amide formation activity was also reported in nitrilases towards benzylic nitriles and other aromatic nitriles substituted with electron-withdrawing groups (Figure 2D) [24,25]. This moonlighting activity was further improved by the site-directed

mutagenesis of non-polar amino acid residues that stabilise the native tetrahedral intermediate during catalysis [24,26]. The native nitrilase reaction mechanism, which involves sequential water additions and the formation of distinct geometric intermediates, sets them apart from other family enzymes that lack such a conformational flexibility in catalysis. Mechanistically, most nitrilases, except cyanide hydratases, exhibit a preference for sterically demanding cyano-group-containing substrates, suggesting that geometric distortion facilitates the thioimidation of the nitrile substrate [27]. Cyanide hydratase is a mechanistically distinct outlier here, and, by catalysing the reaction with a single hydration event, it bypasses the conventional hydrolytic pathway leading to carboxylic acid formation in nitrilases, requiring less substrate-specific adaptations [28].

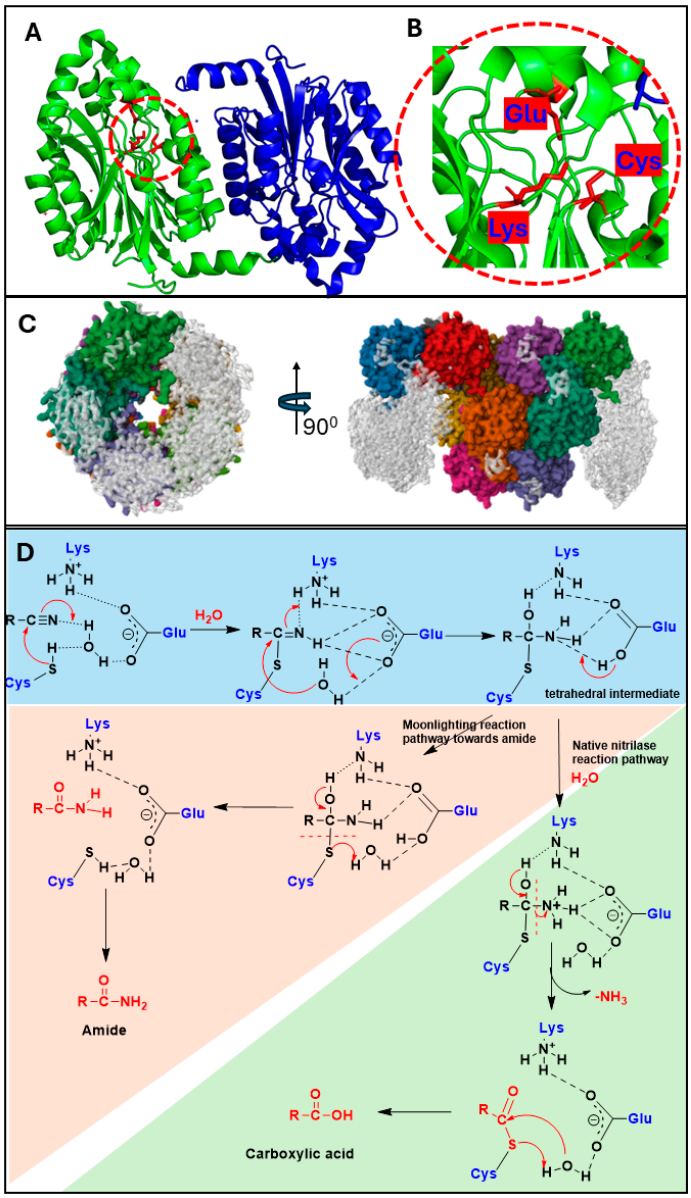


Figure 2. (**A**) The crystal structure of nitrilase from *Synechocystis* sp. PCC6803 (Pdb 3WUY). The catalytic triad (Glu-Lys-Cys) in the active site is shown as red sticks. Individual monomers are represented in green and blue cartoons. (**B**) The active site and catalytic triad. (**C**) Cryo-EM structure of an active helical nitrilase, the engineered nitrilase 4 from *Arabidopsis thaliana* (EMD-0320), showing higher order helical twist complex. (**D**) The proposed reaction mechanisms for the nitrilase-catalysed reaction. The native reaction pathway to the formation of carboxylic acid and the mechanism for moonlighting reaction that forms amide products are also shown.

4. NHases and Metal-Catalysed Nitrile Hydrolysis

NHases are a widely distributed class of metalloenzymes, identified from both prokaryotic and eukaryotic organisms in nature. NHases are composed of α- and β-subunits, with a conserved **VC(T/S)LCSC(Y/T)** motif in the α-subunit serving as a metal-binding catalytic domain, and are classified as Fe- or Co-dependent based on their active-site metal ions (Figure 3A). Though the majority of known NHases coordinate with either low-spin trivalent Fe or Co metal ions in the active site, NHase variants bound with Mn^{2+}, Ni^{2+} or multi-metal centres (such as Co, Cu, Zn) were also reported [29,30]. The specific role and involvement of metal-specific activators, subunit dynamics and metallochaperones were proposed for the metallocentre assembly and activation of NHases. A major difference between Fe-NHase and other metal NHase is photo regulation—a well-characterised phenomenon present in Fe-NHases by which the enzyme could toggle between active and inactive states via the light-induced displacement of a nitric oxide (NO) from the iron centre [31–34].

Despite the high sequence similarity between Fe-type and Co-type NHases, experimental evidence suggests their distinct assembly mechanisms guided by metal selectivity and separate activator proteins. Beyond metal incorporation, the activator's role is also implicated in catalytic cysteine oxidation and in the formation of post-translational modified sulfonate—a prerequisite for metal binding and enzyme activity (Figure 3B,C). Around six different types of NHase gene organisations are currently reported, with a different gene order for α, β and activator (or γ) subunits. These include NHases lacking an activator unit, which consist of either a fused α and β unit or a solo α unit that retains activity [14,35–37]. The fused α and β unit NHase from the eukaryotic organism *Monosiga brevicollis* exhibits a unique architecture, indicating an unconventional maturation pathway which differs from all canonical bacterial NHases, possibly acquired via horizontal gene transfer. In this fused β-α NHase, the β unit is fused into a single polypeptide chain with the α-subunit via a histidine-rich linker—typically denoted as a His_{17} region. Notably, the histidine linker region was found to play no active role in NHase metal ion incorporation [36]. This subunit fusion strategy was also explored to improve the catalytic activity in normal NHases that lack a His_{17} region [38].

Regardless of extensive investigations, the catalytic mechanism of NHases remains highly debated, with competing distinct catalytic models supported by spectroscopic, structural, site-directed mutational and quantum mechanical studies. Central to all these mechanisms is the redox-active metal centre in a low-spin state, coordinated by highly conserved cysteine residues—often oxidised to sulfenic or sulfinic acid—which mediate substrate activation and catalysis. The inner-sphere mechanism proposes that the nitrile substrate binds directly to the active site metal ion, which is often coordinated by a unique ligand environment, including oxidised cysteine residues, through its cyano group [39,40]. This facilitates the polarisation and activation of the nitrile carbon, promoting a subsequent nucleophilic attack by a water-derived species. The outer-sphere mechanism—one of the widely accepted models—proposes the indirect substrate activation. In this model, the metal ion activates water molecules or hydroxide ions bound within its coordination sphere, which then attack the nitrile carbon from the periphery, without direct substrate–metal binding, as proposed in the inner-sphere mechanism (Figure 3D) [41,42]. An alternative mechanism for NHase is the formation of a cyclic intermediate via the direct attack of an activated sulfenate or post-translationally sulfonated cysteine residue acting as a nucleophile. This mechanism is currently well recognised, with further validations and evidence collected via computational and mechanistic studies [40,43–45]. This direct attack leads to either the formation of a disulfide intermediate or a water-mediated conversion to imidic acid (Figure 3E). The isotopic oxygen exchange, time-resolved crystallography and FTIR

studies also supported mechanisms that involve a post-translationally modified sulfenato group in the catalysis [46,47]. This nucleophile could activate a water molecule, which in turn transiently retains a labelled oxygen through the catalytic cycle within the protein, as observed during the isotopic exchange analysis using native high-resolution protein mass spectrometry [43].

The cyclic intermediate model is reinforced by high-resolution structures of NHase active sites showing sulfenated cysteines adjacent to the metal centre, further supporting it as the most chemically and structurally plausible pathway for NHase catalysis. In contrast, the inner-sphere mechanism, which posits the direct coordination of the nitrile to the metal ion, lacks structural evidence for stable substrate–metal complexes and fails to explain activity retention in the mutants of metal-binding residues [40]. Similarly, the outer-sphere activation model is undermined by the absence of a suitably positioned water molecule in crystal structures and by inconsistent kinetic data which does not support a water-mediated attack [46,47]. Together, these mechanisms elucidate a highly coordinated catalytic framework in NHases, wherein metal ion coordination, site-specific post-translational modifications and strategically positioned amino acid residues collectively govern substrate activation and product formation. This convergence of structural and chemical features confers exceptional specificity, turnover efficiency and mechanistic adaptability to the NHase active site, enabling the precise metal-assisted hydrolysis of diverse nitrile substrates under mild conditions.

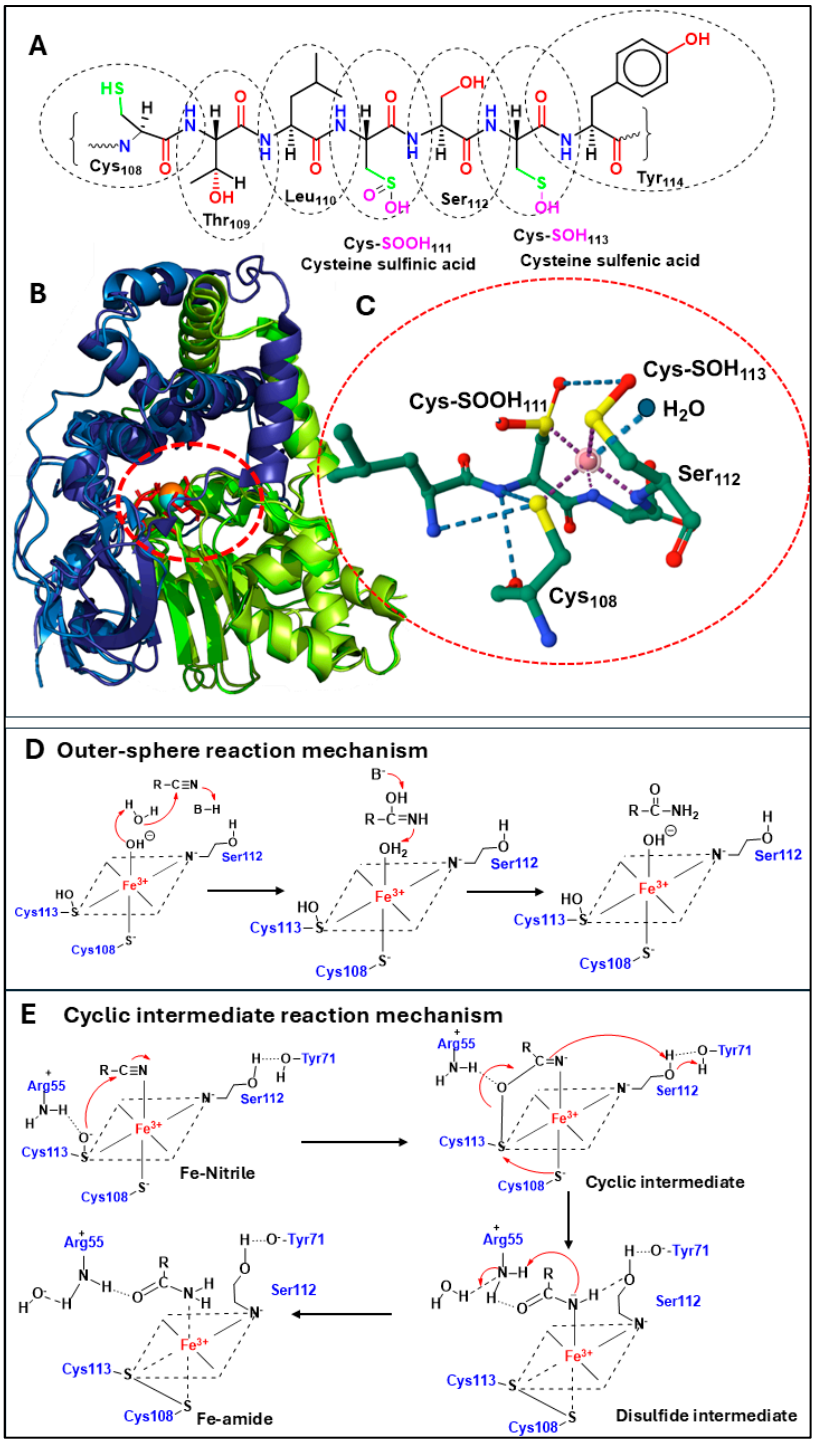


Figure 3. (**A**) Highly conserved metal binding **VC(T/S)LCSC(Y/T)** motif present in Fe- and Co-NHase enzymes. (**B**) The overlaid crystal structures for Co- and Fe-NHase enzymes. α and β subunits are shown in blue and green colour shades. The metal cofactor is shown as a small sphere, whereas the corresponding residues that interact with the metal ion are shown as sticks. (**C**) Metal coordination in the active site of Co-NHases. Crystal structure of Co-NHase from *Pseudomonas thermophila* (Pdb 8I6N) and Fe-NHase from *Comamonas testosterone* (Pdb 4FM4) were used as the structure template for the images. (**D**,**E**) show the proposed outer-sphere and cyclic intermediate reaction mechanisms, respectively, for NHase reaction [16,44,48].

5. Metagenomics and Identification of Nitrilases and NHases

The scientific exploration of nitrile-hydrolysing enzymes began in the early 1960s, driven by various studies on the formation and transport of the plant hormone auxin, as well as the identification of other microbial and plant-based associated pathways [49]. The capability of certain plant-associated fungi to hydrolyse 3-indoleacetonitrile was initially reported, followed later by the partial purification of nitrilase enzymes from *Pseudomonas* species and barley leaves [49,50]. The genus *Rhodococcus* subsequently emerged as a prolific source of nitrilases with broad substrate specificity, including those that exhibit activity toward aromatic and aliphatic nitriles [51]. The first NHase was also identified from *Rhodococcus*, followed by an industrial-scale production of acrylamide in 1996 [52,53]. Recent advances in metagenomics and computational database mining have revolutionised the discovery and functional annotation of nitrile-converting enzymes. These approaches allow the identification of novel nitrilases and NHases from environmental and genomic data without the need of traditional culture-based methods that were often used a decade ago.

Previously, 137 distinct nitrilase enzymes were identified using microbial culture-independent metagenomic approaches. This was achieved via the high-throughput screening and functional assays of selected enzymes of over 600 biotope-specific environmental DNA libraries [54]. This pioneering work has spurred further exploration into various untapped ecological niches, resulting in the isolation of nitrilases from diverse microbial communities with enhanced properties and substrate scopes [55–59]. The isolation of specific nitrilase types from environmental samples was also made possible by designing Polymerase chain reaction (PCR) primers for different nitrilase clades. These approaches soon became highly relevant for expanding the enantioselective profiles of nitrile-converting enzymes, which are often determined by their sequence patterns and identity with a particular nitrilase clade [60]. Additionally, linking nitrilase gene BLAST searches with the specific gene operons and neighbouring genes present in diverse nitrilase biosynthetic clusters has proven advantageous for identifying novel nitrilases, such as arylacetonitrilases and nitrilases, with a high specificity for dinitriles [61,62]. The data mining approaches for nitrilases often rely on the identification of conserved Glu-Lys-Glu-Cys motifs and other conserved structural regions, including α-β-β-α sandwich motifs [63].

Similarly to nitrilases, the early screenings for NHase gene identification from environmental samples were heavily based on NHase-specific PCR primers for gene amplification. Further advancement has also led to the use of primer pairs that can distinguish between cobalt and iron NHase genes [64]. The bioinformatics-based identification of NHase enzymes from genome deposits relies on the presence of NHases operons, which are typically encoded by genes for α- and β-subunits, along with an activator. The motif search algorithms were employed to identify the characteristic Cys–Ser–Cys triad and metal-binding residues—often featuring post-translationally oxidised cysteines [65,66]. Increasingly, the proteomic, functional and transcriptomic profiling of microbial consortia exposed to nitrile pollutants has revealed inducible NHase expression and regulatory pathways, opening new avenues for various synthetic biology applications [67–69].

Recent advancements in metagenomic data mining using computational and AI-based tools have markedly improved the ability to identify novel nitrile-converting enzymes from environmental samples [70–72]. In a recent study, metagenomic datasets derived from coal-rich environments were processed using MEGA-HIT and SqueezeMeta, enabling the efficient annotation of microbial genomes. The structure prediction via AlphaFold2 and molecular docking analysis led to the identification of a previously uncharacterised *Alphaproteobacteria* nitrilase that exhibits a high affinity toward environmental nitriles [71]. Complementarily, functional metagenomic screening of the same ecosystem also revealed different nitrile-hydrolysing enzymes, including putative NHases. This workflow inte-

grated InterProScan-based motif identification, NetSurfP secondary structure prediction, and CASTp active site mapping to prioritise enzyme candidates for further biophysical studies. Collectively, these studies exemplify how advanced and predictive metagenomic mining strategies, when combined with computational modelling, could facilitate the accelerated discovery of robust nitrile-converting biocatalysts.

6. Screening and Assay Development for Nitrilases and NHases

Screening assays for nitrilase and NHase activity span from basic cell-based selection assays to more complex enzymatic coupled assays with purified nitrilases or NHase enzymes, as well as high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR)-based assays [73,74]. Over the past five decades, numerous high-throughput screening strategies have been developed to facilitate the identification of nitrile-hydrolysing enzymes from microbial and engineered libraries. The early developed colorimetric techniques—which are efficient in ammonia detection via various mechanisms—are still in use. For NHases, coupled amidase enzymatic assays were reported which indirectly quantify the hydratase activity from the amount of ammonia liberated via amide hydrolysis [75]. Alternatively, the amide products could also be detected via initially converting them into hydroxyamic acid through a reaction with hydroxyl amine. An amidase enzyme able to catalyse the acyl transfer from a variety of amides onto hydroxylamine—such as *Rhodococcus erythropolis* amidases—could complement this reaction in a bienzymatic assay route [76].

The well-established ammonia detection assays for nitrile-converting enzymes include the use of pH indicators like phenol red that detect pH changes associated with ammonia release; Nessler's reagent assays that exploit the reaction of potassium mercury (II) iodide with ammonia; and the Berthelot (or indophenol) reaction in which ammonia reacts with hypochlorite to form a monochloramine [77–79]. For nitrile substrates that strongly absorb at the far UV region, such as acrylamide and aromatic nitriles, spectroscopic measurements were reported [77,80,81]. An alternative approach with a glutamate dehydrogenase (GDH) enzyme was also reported by spectrophotometrically monitoring the formation of nicotinamide adenine dinucleotide (NADH). In this method, the biproduct ammonia will react with α-ketoglutarate in the presence of a GDH enzyme [82]. While sensitive, these methods often suffer from buffer instability and a limited compatibility with complex biological matrices.

Metal ion-based colorimetric assays, such as the complexation of ammonia with externally introduced $CoCl_2$, also showed limitations due to poor sensitivity, with a <5 mM detection threshold and interference from proteinaceous media [83]. An alternative ferric hydroxamate-based spectrophotometric assay was reported for high-throughput readouts [84]. In this method, carboxylic acids formed via the enzymatic hydrolysis of nitriles are first converted to hydroxamic acids through a coupling reaction with hydroxylamine perchlorate and dicyclohexylcarbodiimide, which activates the acid group. The hydroxamic acids then react with acidic ferric perchlorate to form a purple ferric hydroxamate complex, allowing for a quantification via absorbance measurements [84].

More refined approaches, such as using time-resolved luminescence and fluorescent probes, though offering an improved sensitivity by minimising background noise, are restricted to a narrow range of substrates, specifically o-hydroxy-benzonitrile derivatives [74]. In one of these developed assays, o-hydroxy-benzonitrile derivates were converted into salicylic acid derivatives by the nitrilase reaction, which in turn bind with metal ions (e.g., Tb^{3+}), producing characteristic fluorescence emission spectra via metal chelation with the acid products. Another reported fluorescence-based approach utilised a modified Roth's fluorimetric method, in which ammonia derivatisation with o-phthaldialdehyde/N-

acetylcysteine (OPA/NAC) was performed to create isoinoldoles that are detectable and quantifiable under specific fluorescence measurement conditions [27,85,86]. A milder version of this assay was also reported for the activity screening of nitrilases from *Rhodococcus rhodochrous* species. In this method, the ammonia liberated was allowed to react with o-phthaldialdehyde and 2-mercaptoethanol to form a fluorochrome [87].

Though mass spectrometry-based assays, such as MALDI-TOF MS and nano-DESI, have revolutionised metabolite profiling directly from microbial colonies, they remain underutilised for nitrile-converting enzymes due to the nature of their reaction products [88,89]. Amides and carboxylic acids lack distinctive fragmentation patterns or ionisation efficiency under conventional mass spectrometry (MS) settings, due to their low volatility. Additionally, nitrile conversion often occurs intracellularly or in complex media, complicating the direct MS analysis from colonies. However, some of the emerging and above-mentioned strategies could bridge this gap: for example, coupling MS imaging with derivatisation protocols (e.g., hydroxamate tagging), integrating NHase activity screens with metabolomic fingerprinting or employing ambient ionisation techniques such as DESI-MS combined with functional assays. Beyond MS, other scalable platforms—such as colorimetric ferric hydroxamate assays, time-resolved fluorescence probes and pH-shift indicators—could also offer advantages for the high-throughput screening of nitrile-converting enzymes in diverse biological matrices, especially when adapted for microfluidic or droplet-based formats.

7. Industrial Substrate Scope and Current Limitations

The acrylonitrile hydrolysis of nitrile-converting enzymes is amongst the earliest examples of a well-established and economically leveraged enzyme-mediated process. Acrylamide is a key monomer ingredient in the synthesis of various industrially useful polymers, including polyacrylamide and its copolymers, which are used across industrial sectors. Polyacrylamide polymers exhibit a broad acceptance for different applications across various sectors: from enhanced oil recovery to the production of textile fibres, paints, adhesives and cosmetics. Whole-cell NHase biocatalysis conversions using *Rhodococcus rhodochrous, Nocardia spp*. and *Pseudonocardia thermophila* were reported for their industrial adaptability to produce acrylamide [90–92]. The strains that are capable of producing acrylic acid via nitrilase activity were also identified and exploited widely for industrial applications, including the surface modification of acrylic fibres, and for extensive use in polyacrylamide copolymer synthesis [93–96]. Their isolation and identification were often guided by the bioremediation potential of the isolated microbial strains' ability to grow in nitrile- and petroleum-contaminated sludge environments [96]. The industrial deployment of nitrilases and NHases has demonstrated remarkable titres and conversions, yielding above 400 g/L acrylamide and acrylic acid; and, in most cases, a near completion was reported under batch or fermenter reaction set-ups [91,97]. The advent of metabolic engineering and CRISPR/Cas9-based genome editing tools for various bacterial species, also reflected in the bio-acrylamide research, has contributed to improving product titres [98]. Researchers have further developed *Rhodococcus* strains as biocatalysts for producing acrylic acid and diverse acrylic monomers using adaptive laboratory evolution and engineering amide metabolism [99]. Recently, a CRISPR/Cas9-mediated gene knockout was performed on the carotenoid biosynthetic gene cluster to address the low purification yields of acrylamide from reaction cultures due to the high inherent pigment contaminations in *Rhodococcus ruber* species [100].

Nitrile-converting enzymes are also widely used for the synthesis of nicotinic acid, nicotinamide and their derivatives, which are key precursors in pharmaceutical intermediates, and specifically for the synthesis vitamin B_3 active forms. NHases such as variant

NHT-120, with an engineered lower level of cyanopyridine inhibition, exhibited higher catalytic activities for the biotransformation of cyanopyridine [101]. A genetically modified *Rhodococcus rhodochrous* strain was reported to yield complete NHase substrate conversion for cyanopyridine, with titres exceeding 850 g/L under optimised fermentation conditions [101]. Although the biocatalytic conversion of 4-cyanopyridine to isonicotinic acid using nitrilases has been less extensively studied, certain strains of *Pseudomonas putida* and *Nocardia globerula* have shown significant levels of performance, with titres exceeding 120 g/L [102,103]. The substrate and product inhibition during NHase and nitrilase scale-up reactions were evident, particularly during prolonged fermentation in fed-batch operations. Enzyme cascade reactions for 3-cyanopyridine bioconversion were developed using NHase–amidase coupled reactions to circumvent these issues in large-scale bioreactors [104].

The NHase-based conversion of adiponitrile to 5-cyanovaleramide—a key intermediate to produce nylon 6, azafenidin and caprolactam—is a biocatalytic alternative to the traditional high-pressure, high-temperature, chemical-based manufacturing route. Whole-cell catalysts of *Pseudomonas* and *Rhodococcus ruber* and *Rhodococcus erythropolis* strains were excellent for this reaction, yielding a complete conversion under optimised conditions, with minimal traces of unwanted side products such as adipamide [105,106]. This indicates that NHase is a promising catalyst for the creation of different regioselective pathways for single-nitrile hydrolysed products.

(R)-Mandelic acid and (R)-Mandelamide are chiral pharmaceutical intermediates for various pharmaceutically important molecules—such as cefamandole, clopidogrel, cyclandelate, dexmethylphenidate and pemoline—and are also widely used in textile dyeing processes. Whole-cell and recombinant *Alcaligenes* nitrilases have demonstrated excellent mandelonitrile conversion rates. In certain cases, to address product inhibition and low reusability, the recombinant strain was immobilised with tris(hydroxymethyl)phosphine or by glutaraldehyde cross-linking [107]. Purified NHase from *Rhodococcus rhodochrous*, which was crossed linked in a poly(vinyl alcohol)/chitosan-glutaraldehyde matrix, displayed a near completion with over 81% enantiomeric excess [108].

Nitrile-converting enzymes have also demonstrated significant potential in industrial and environmental waste remediation. For instance, *Rhodococcus rhodochrous* J1 has been employed to degrade toxic nitrile herbicides such as dichlobenil and bromoxynil, converting them into less harmful amide or acid derivatives suitable for further microbial breakdown [109]. Similarly, NHases from *Microbacterium imperiale* and *Pseudonocardia thermophila* have been used to detoxify acrylonitrile and cyanopyridines containing industrial effluents, which are common by-products in polymer and pharmaceutical manufacturing [110–112]. Another example is the nitrilases from *Rhodococcus erythropolis*, which were employed to hydrolyse benzonitrile and acetonitrile in petrochemical wastewater, reducing the chemical oxygen demand and toxicity levels [113].

Though nitrile-converting enzymes are capable of the industrial production of the above-mentioned chemicals and various other small-molecule structural analogues (Table 1), their wider industrial adaptability is often hindered by a limited substrate scope, their poor stability to withstand organic solvent and reaction conditions, product inhibition and the slow progress in protein engineering approaches to improve activity and stereoselectivity for the synthesis of fine chemicals. The poor substrate scope of NHases and nitrilases is often linked to their intrinsic structural and mechanistic constraints. The enzyme's active site is typically optimised for small, linear or mildly branched nitriles, limiting their ability to accommodate bulky, rigid or highly functionalised substrates. This specificity arises from the geometry of the substrate-binding pocket and access tunnel, which restricts the entry and proper orientation of diverse nitrile compounds. Additionally, the

catalytic mechanism—relying on a conserved cysteine residue in nitrilases for nucleophilic attack or post-translationally modified cysteine in NHases for metal binding—could be sensitive to steric hindrance and electronic effects, further narrowing the range of compatible substrates. Another contributing factor is the evolutionary origin of most characterised nitrilases, which are derived from soil bacteria, fungi and plants adapted to metabolise naturally occurring nitriles [114]. As a result, these enzymes have not evolved to process synthetic or structurally complex industrial nitriles.

Table 1. Selected examples showing the recent progresses reported in NHase- and nitrilase-catalysed reactions.

(A) NHase-Catalysed Reactions					
Origin/ Engineering strategy	**Substrates**	**Purpose**	**Conversion rate**	**Main product**	**References**
NHase from *Rhodococcus* sp. (integrated cascade with amidase, NHase, nitrilase, amide bond synthetase, decarboxylase, metal/ photocatalysts)	Aromatic nitriles	Regioselectivity and stereoselectivity	>90%	Enantioenriched amides	Torri et al., 2025 [115]
NHase βM40A mutant (substrate channel engineered for selective monocyanamide synthesis)	Malononitrile	Regioselectivity (monocyanamide enrichment)	~92%	Cyanoacetamide	Wang et al., 2025 [116]
Tunnel engineered NHase from *Carbonactinospora thermoautotrophicus*	4-cyanopyridine	Product yield	100%	Isonicotinamide	Guo et al., 2023 [117]
NHase variant with tunnel mutations	Phenylacet onitrile/ benzonitriles	Product yield	85–95%	Corresponding amides	Ma et al., 2022 [118]
Rhodococcus erythropolis CCM2595	Adiponitrile	Product yield	>95%	5-cyanovaleramide	Wang et al., 2020 [106]
Rhodococcus rhodochrous J1	Adiponitrile, malononitrile, terephthalonitrile, phthalonitrile	Product yield	>98%	Adipamide Malonamide Terephthalamide Phthalamide	Cheng et al., 2018 [119,120]
Pseudomonas putida NRRL-18668	Adiponitrile/ Malononitrile	Product yield	>99%	5-cyanovaleramide/ Cyanoacetamide	Cheng et al., 2018 [121]
βL37 mutants of *Pseudomonas putida* NRRL-18668 and *C. testosteroni*	Adiponitrile/ Malononitrile α,ω-dinitriles	Product yield/ Diamide synthesis	>98%	Adipoamide/ Malonamide α,ω-diamides	Cheng et al., 2016 [121]
Rhodococcus aetherivorans ZJB1208	1-cyanocycloh exaneacetonitrile	Regioselectivity/ Product yield	100%	1-cyanocyclohex aneacetamide	Zheng et al., 2016 [122]
Rhodococcus ruber CGMCC3090	Adiponitrile	Product yield	100%	5-cyanov aleramide	Shen et al., 2012 [105]
Rhodopseudomonas palustris HaA2	2-phenylpr opionitrile/ 2-phenylb utyronitrile	Stereoselectivity	—	(S)-2-phenylpro pionamide/ (S)-2-phenyl butyramide	van Pelt et al., 2011 [123,124]
Rhodococcus rhodochrous DSM43269	2-phenylp ropionitrile	Stereoselectivity	E-value >100	(S)-2-phenylp ropionamide	van Pelt et al., 2011 [123,124]

Table 1. *Cont.*

(B) Nitrilase-Catalysed Reactions					
Origin/Engineering strategy	**Substrate**	**Regio-/stereoselectivity**	**Conversion rate**	**Main product**	**References**
Engineered nitrilase with photoredox catalysts (integrated reaction)	Aromatic nitriles	Regioselectivity	~80%	Fluorinated acids	Angiolini et al., 2025 [125]
Substrate channel engineered mutants of *PpNit* nitrilase gene from *Pseudomonas putida*	3-chloropro pionitrile and various aliphatic nitriles	Product yield	~100%	Corresponding aliphatic carboxylic acids	Bian et al., 2025 [126]
Nit6803 nitrilase homologue from *Pseudomonas fluorescens* NCIMB 11764	Succinonitrile/ fumaronitrile/ Sebaconitrile	Dicarboxylic acid synthesis	~90–20%	Dicarboxylic acids	Jones et al., 2021 [127]
Variovorax boronicumulans J1 nitrilase (arylacetonitrilase Nit09)	Phenylace tonitrile	Product yield	~90%	Phenylacetic acid	Egelkamp et al. 2020 [82]
Alcaligenes faecalis MTCC 12629	4-hydroxyphe nylacetonitrile	Product yield	90%	4-hydroxyph enylacetic acid	Thakur et al., 2018 [128]
Recombinant *E. coli* JM109 cells harbouring nitrilase gene from *Alcaligenes faecalis* MTCC 126	3-cyanopyridine	Product yield	98%	Nicotinic acid	Pai et al. 2014 [129]
Pseudomonas sp Strain UW4	Indole-3-acetonitrile	Product yield	—	Indole-3-acetic acid	Duca et al., 2014 [130]
Nocardia globerula NHB-2	4-cyanopyridine Aliphatic nitriles	Product yield	100%	Isonicotinic acid Corresponding acids	Sharma et al., 2012 [103]
Pseudomonas fluorescens DSM 50106	Mandelonitrile	Stereoselectivity	95%	(R)-Mandelic acid	Layh et al., 1998 [131]
Rhodococcus rhodochrous ATCC BAA-870	2-phenylp ropionitrile	Stereoselectivity	E-value >100	(S)-2-phenylpr opionic acid	Gilligan et al., 1993 [132]

8. Improving the Efficiency of Nitrile-Converting Enzyme for Industrial Applications and Future Perspectives

Attempts to broaden the substrate scope of nitrile-converting enzymes through protein engineering have met with limited success, as mutations in the active site or tunnel regions often compromise enzyme stability or activity. Moreover, the high-throughput screening of nitrilase libraries remains labour-intensive and technically challenging, slowing the discovery of broadly active variants [27,133]. Despite all of these limitations, many successful outcomes were reported in addressing such issues [25,119,134]. Engineering the substrate channel of nitrilase enzymes has emerged as a common theme to expand substrate specificity and enhance catalytic efficiency. The residues around substrate channels in nitrilases and NHases were modified via site-specific, rational and semi-rational approaches (Figure 4A–D). A recent study utilised an active binding pocket remodelling strategy to identify the key residues in the substrate binding pocket of nitrilases; generating mutants such as V198L/W170G showed up to 26-fold activity toward aromatic nitriles [135]. These mutations introduced favourable π-alkyl interactions and expanded the substrate cavity volume from 225.66 $Å^3$ to 307.58 $Å^3$, facilitating a better substrate access. Similarly, by targeting residues such as W166G and F202L in the pocket, activity toward aliphatic nitriles was significantly improved [126]. Targeting key distal residues via site-directed saturation mutagenesis and combinatorial methods also displayed promising results in improving the catalytic activity of nitrilases [134]. Following a similar rational mutation

strategy for NHase resulted in variants with expanded channel geometry activity toward aromatic and branched substrates [118]. Structural analysis revealed that these mutations facilitated substrate access by enlarging the tunnel and introducing favourable dynamic movements around the active site. Similarly, semi-rational engineering of the substrate channel of NHase was used as a strategy for the selective synthesis of cyanoacetamide [116]. The βM40A mutant thus created exhibited an improved regioselectivity by narrowing the channel and increasing the metal ion–substrate distance, thereby reducing unwanted side reactions [126]. These findings underscore the importance of tunnel flexibility and residue polarity in modulating substrate preferences.

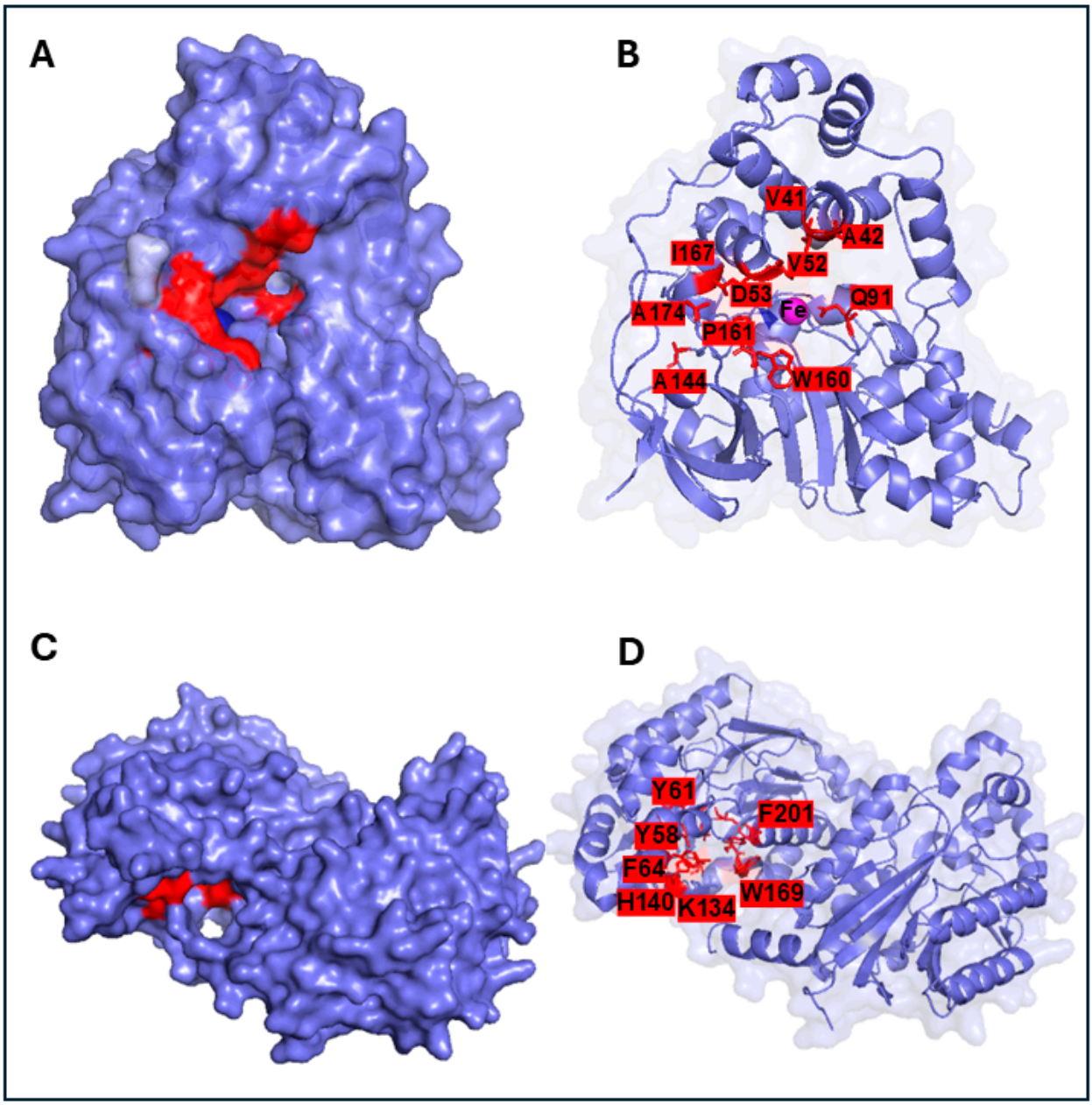


Figure 4. (**A**) The entrance to substrate binding channels in NHase enzymes. (**B**) The amino acid residues present at the entrance of substrate binding channel are shown as red sticks. Crystal structure of Fe-NHase from *Rhodococcus* sp. *R312* (Pdb 1AHJ) was used as a structure template for the images A and B. (**C**) The entrance to substrate binding channel in nitrilase enzymes (in dimeric form). (**D**) The amino acid residues present at the entrance of the substrate binding channel and around catalytic triads are shown as red sticks. Crystal structure of nitrilase from *Synechocystis* sp. PCC6803 (Pdb 3WUY) was used as a structure template for the images (**C**,**D**).

Integrating computational and AI-assisted structural prediction and enzyme engineering tools have significantly accelerated the rational design of nitrile-converting biocatalysts [136]. One notable example is the development of the ASSMD (Ancestral Sequence–Structure–Molecular Dynamics) strategy, which enabled the reconstruction and engineering of an extreme thermophilic ancestral nitrilase which is active at 100 °C [137]. The mutant ASR135-M4 exhibited an enhanced thermostability and catalytic activity, further verified by molecular dynamics simulations and structural rigidity analysis. Complementing ancestral reconstruction, a semi-rational design workflow combining substrate channel modelling and molecular docking to redesign nitrilases led to the improved activity toward aliphatic nitriles [126]. In another study, virtual screening, AI-assisted residue selection and targeted mutagenesis were employed to enhance the biosynthesis of 4-cyanobenzoic acid from terephthalonitrile using a nitrilase from *Acidovorax facilis*. A triple mutant, F168V/T201N/S192F, thus identified exhibited a higher activity and better conversion at high substrate loading, further validating the predictive power of computational design for industrial scale biocatalysis [138]. The computational docking and molecular dynamics simulations were also used to redesign NHases. By incorporating ten non-catalytic residue substitutions in NHase from *Pseudonocardia thermophila* JCM3095 through this approach, a mutant M10 was generated with an improved thermostability and activity [139]. A recent study reported advanced NHase design strategies by modelling coevolutionary residue networks and post-translational modifications. This approach could reveal stabilising interactions in protein structure that are essential for catalytic resilience and robustness under industrial conditions [140].

The limited thermal stability of the nitrile-converting enzyme often restricts its industrial applications. For example, the activity of many industrially used NHases drastically reduces above 20°C, necessitating energy-intensive cooling systems for reactions [94,130]. The stabilisation of inter-subunit salt bridges present in thermophilic NHases inspired efforts to incorporate such salt bridges into the *Rhodococcus ruber* NHase sequence via mutagenesis, which significantly improved its thermostability and structural rigidity [141]. Several other innovative strategies are as follows: designing chimeric NHases via the fragment swapping of NHase thermolabile regions with thermostable fragments [141]; designing fusion NHases with amphipathic self-assembling peptides—EAK16 and ELK16 — to promote inclusion body formation [142]; and creating a single-chain fusion construct with fused α and β subunits of NHases [143–145]. These approaches collectively offer different scalable solutions for stabilising NHases under industrial conditions. While most native nitrilases operate optimally at moderate temperatures, recent discoveries from extremophiles have revealed several variants with remarkable heat tolerance. For instance, a nitrilase from *Pyrococcus abyssi* retained activity after 6 h at 90°C, demonstrating its potential for high-temperature bioprocessing [58]. Similarly, a nitrilase isolated from the Atlantis II brine pool in the Red Sea exhibited a robust activity at 68°C and resistance to heavy metals [56]. The immobilisation of nitrile active enzymes—either as whole cells or as purified enzymes from native host or recombinant sources—has also emerged as a key strategy to enhance the operational stability, reusability and substrate versatility of biocatalytic systems. The encapsulation of *Pseudonocardia thermophila* NHase in silica-derived sol–gel matrices, immobilisation of *Geobacillus pallidus* NHase in different support materials and NHase ES-NHT-118 immobilisation in biological metal–organic frameworks were the noteworthy examples of this [123,146,147]. In addition, various conventional approaches, such as immobilisation using sodium alginate beads and preparing crosslinking enzyme aggregates (CLEA) of nitrile-converting enzymes, were also reported over the years [148,149].

Regio- and stereoselectivity are the defining features for industrial enzymatic biotransformation reactions, particularly for synthesising chiral amides and carboxylic acids, which act as chiral auxiliaries for asymmetric synthesis. NHases have shown promising regioselective behaviour towards the hydrolysis of the aliphatic or aromatic dinitriles, a selectivity not reported with traditional organocatalysis [122]. NHases isolated from several *Rhodococcus* and *Pseudomonas* species showed enantioselectivity. For example, PVA/chitosan-glutaraldehyde cross-linked NHase from *Rhodococcus rhodochrous* ATCC BAA-870 catalysed the hydrolysis of R-mandelonitrile with 81% enantiomeric excess [150]. Recently, it was also reported that NHases are capable of recognising a planar element of chirality during reactions with nitrile ferrocene derivatives [151]. Despite these successes, engineered NHases with high enantioselectivity remains rare, though earlier studies revealed that tunnel-lining residues play a critical role in substrate orientation and enantiofacial discrimination [119,152]. More recently, it was demonstrated that the steric bulk and electrophilicity of nitrile substrates influence both binding and reaction rates, reinforcing the importance of tunnel geometry and active-site polarity in stereoselective outcomes [153]. Parallel developments were also reported in nitrilase research. Nitrilases have been explored for the enantioselective synthesis of α-substituted acids, including mandelic and phenylacetic acid derivatives [154]. Directed evolution and active-site mutagenesis have been used to improve chiral discrimination of nitrilases, though success has been limited by the enzyme's rigid substrate channel and sensitivity to steric hindrance [155,156].

Enzyme cascade reactions involving nitrile-converting enzymes could also streamline complex stereo- and regiospecific syntheses by linking multiple biocatalytic steps, minimising purification needs and improving reaction efficiency. This approach was exploited to synthesise enantiomerically pure β-amino acids from β-keto nitriles using nitrilase and ω-transaminase enzymes [157]. A nitriliase (known as NitBJ) and its site-directed mutants from *Bradyrhizobium japonicum* were utilised to hydrolyse β-keto nitriles to β-keto acids, followed by ω-transaminase to aminate β-keto acids, producing chiral (S)-β-amino acids. Similarly, integrating chemical and enzymatic nitrile transformations could offer a broader substrate compatibility and fine-tuned selectivity, creating more sustainable and scalable pathways to high-value industrial compounds. A one-pot method for synthesising functionalised amides was achieved by integrating *E. coli* over expressed NHase enzymes with copper-catalysed Ullmann-type N-arylation [158]. Recently, bacterial nitrilases were integrated with photoredox catalysts capable of decarboxylative fluorination, creating a one-pot chemo–bio cascade for the transformation of organic nitriles into fluorinated, trifluoromethylated and pentafluoroethylated products [125]. Nitrile-converting enzymes could also be utilised for regioselective C−H bond functionalisation by integrating them with chemo catalysts. A pioneering example is the integration of the regioselective flavin-dependent halogenase enzyme with palladium-catalysed cyanation, which site-specifically incorporates nitrile groups in bigger aryl molecules. This will be followed by a NHase- or nitrilase-dependent biocatalytic conversion of generated nitriles to amides or carboxylic acids [159]. A recent report described a multi-component enzymatic one-pot biocatalytic cascade that converts racemic nitriles into amide products by integrating nitrilase, NHase, amidase and amide bond synthetase [115]. This cascade accommodates the photochemical cyanation of arenes to generate nitrile precursors in situ, enabling formal C–H bond amidation without isolating intermediates. Collectively, these methodologies underscore the versatility of nitrile-converting enzymes in enabling the design of novel biocatalytic cascades and hybrid chemoenzymatic processes. Such integrated approaches hold significant promise for advancing sustainable and selective chemical synthesis, particularly through the development of innovative reaction pathways and enhanced functional group transformations. In addition, the discovery and design of stereoselective nitrile-converting

enzymes could be potentially accelerated by integrating advanced computational tools to analyse and design novel nitrile-converting enzyme structures combined with high-throughput screening, metagenomic mining and a further understanding of the role that tunnel-lining residues play in substrate orientation. Such advances hold great promises for producing more enantiopure pharmaceuticals, agrochemicals and fine chemicals via green biocatalysis in the near future.

9. Conclusions and Future Perspectives

Over the last two decades, the rapid progress and increased awareness about the industrial potential of nitrile-converting enzymes—particularly about nitrilases and NHases—have revolutionised the biocatalytic research field. Advances in enzyme discovery, mechanistic elucidation, genetic and metabolic engineering and targeted modification have transformed these biocatalysts into strategic tools for amide and acid production across pharmaceutical, agrochemical, material and textile sectors. The functional expression, purification and characterisation of enzymes from diverse origins—including metagenomic and extremophilic sources—have yielded a growing portfolio of catalytic candidates, some of which are now integrated into the commercial-scale production of compounds like acrylamide, nicotinamide and (R)-mandelic acid. Despite their promises, natural nitrile-converting enzymes often lack the robustness required for large-scale deployment. Catalytic limitations, thermal and pH instability and unintended byproduct formation remain common hurdles. However, contemporary molecular and cellular biology strategies—ranging from genetic engineering and site-directed mutagenesis to domain truncation and protein technologies—have substantially improved enzyme performance. Bioinformatics and gene mining have also empowered the identification of tailored nitrile-converting enzymes with enhanced substrate affinity and activity. The convergence of synthetic biology, AI-driven structure prediction and next-generation sequencing is poised to accelerate the rational design of nitrile-converting enzymes. In parallel, immobilisation strategies and co-expression platforms offer scalable routes for incorporating these enzymes into cascade and integrated chemoenzymatic processes, minimising intermediate handling and maximising yield and selectivity. The continuing expansion of metagenomic libraries and structure–function databases will likely yield bespoke nitrile-converting biocatalysts tailored for specific industrial challenges. Collectively, these developments position nitrile-converting enzymes as an indispensable agent in green chemistry and bio-based manufacturing, with untapped potential in the synthesis of advanced intermediates, specialty chemicals and sustainable polymers.

Author Contributions: Conceptualization and resources: B.R.K.M.; original draft preparation: B.R.K.M.; review and editing: B.R.K.M., J.D.P., J.J.S. and T.C. All authors have read and agreed to the published version of the manuscript.

Funding: JJC's MRes studentship is supported by a scholarship awarded by the Defence Science and Technology Laboratory (DSTL). TC's PhD scholarship is funded through the PGR Faculty Bursary Scheme, Faculty of Science and Health, University of Portsmouth.

Data Availability Statement: The data will be available upon request.

Acknowledgments: We gratefully acknowledge the scientific and technical support provided by the Centre for Enzyme Innovation (CEI) and the School of the Environment and Life Sciences. We also thank Andy Pickford and Joy Watts for their valuable scientific discussions on nitrile-acting enzymes.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Scotti, C.; Barlow, J.W. Natural Products Containing the Nitrile Functional Group and Their Biological Activities. *Nat. Prod. Commun.* **2022**, *17*, 1934578X221099973. [CrossRef]
2. Rimmer, P.B.; Shorttle, O. A Surface Hydrothermal Source of Nitriles and Isonitriles. *Life* **2024**, *14*, 498. [CrossRef]
3. Wang, X.; Wang, Y.; Li, X.; Yu, Z.; Song, C.; Du, Y. Nitrile-Containing Pharmaceuticals: Target, Mechanism of Action, and Their SAR Studies. *RSC Med. Chem.* **2021**, *12*, 1650–1671. [CrossRef] [PubMed]
4. Sruthi, P.R.; Anas, S. An Overview of Synthetic Modification of Nitrile Group in Polymers and Applications. *J. Polym. Sci.* **2020**, *58*, 1039–1061. [CrossRef]
5. Fleming, F.F.; Yao, L.; Ravikumar, P.C.; Funk, L.; Shook, B.C. Nitrile-Containing Pharmaceuticals: Efficacious Roles of the Nitrile Pharmacophore. *J. Med. Chem.* **2010**, *53*, 7902–7917. [CrossRef] [PubMed]
6. Nakao, Y. Metal–mediated C–CN Bond Activation in Organic Synthesis. *Chem. Rev.* **2020**, *121*, 327–344. [CrossRef]
7. Peter, J.K.; Singh, R.; Yadav, A.K.; Kothari, R.; Mehta, P.K. Toxicity of Nitriles/Amides-Based Products in the Environment and Their Enzymatic Bioremediation. *J. Hazard. Mater. Adv.* **2023**, *13*, 100389. [CrossRef]
8. Egelkamp, R.; Zimmermann, T.; Schneider, D.; Hertel, R.; Daniel, R. Impact of Nitriles on Bacterial Communities. *Front. Environ. Sci.* **2019**, *7*, 103. [CrossRef]
9. Jeschke, P. Current Status of Chirality in Agrochemicals. *Pest. Manag. Sci.* **2018**, *74*, 2389–2404. [CrossRef]
10. Martínková, L. Nitrile-Converting Enzymes and their Synthetic Applications. In *Green Biocatalysis*; Wiley: Hoboken, NJ, USA, 2016; pp. 331–349. [CrossRef]
11. Yamaguchi, T.; Asano, Y. Nitrile-Synthesizing Enzymes and Biocatalytic Synthesis of Volatile Nitrile Compounds: A Review. *J. Biotechnol.* **2024**, *384*, 20–28. [CrossRef]
12. Sun, S.; Zhou, J.; Jiang, J.; Dai, Y.; Sheng, M. Nitrile Hydratases: From Industrial Application to Acetamiprid and Thiacloprid Degradation. *J. Agric. Food Chem.* **2021**, *69*, 10440–10449. [CrossRef]
13. Pace, H.C.; Brenner, C. The Nitrilase Superfamily: Classification, Structure and Function. *Genome Biol.* **2001**, *2*, 1–9. [CrossRef]
14. Feng, C.; Chen, J.; Ye, W.; Wang, Z. Nitrile Hydratase as a Promising Biocatalyst: Recent Advances and Future Prospects. *Biotechnol. Lett.* **2024**, *46*, 1171–1185. [CrossRef] [PubMed]
15. O'Reilly, C.; Turner, P.D. The Nitrilase Family of CN Hydrolysing Enzymes-a Comparative Study. *J. Appl. Microbiol.* **2003**, *95*, 1161–1174. [CrossRef] [PubMed]
16. Cheng, Z.; Xia, Y.; Zhou, Z. Recent Advances and Promises in Nitrile Hydratase: From Mechanism to Industrial Applications. *Front. Bioeng. Biotechnol.* **2020**, *8*, 352. [CrossRef] [PubMed]
17. Gong, J.-S.; Shi, J.-S.; Lu, Z.-M.; Li, H.; Zhou, Z.-M.; Xu, Z.-H. Nitrile-Converting Enzymes as a Tool to Improve Biocatalysis in Organic Synthesis: Recent Insights and Promises. *Crit. Rev. Biotechnol.* **2015**, *37*, 69–81. [CrossRef]
18. Bhatt, A.; Prajapati, D.; Gupte, A. Current Status and Future of Nitrile Catalysis Using Key Nitrilases Enzymes and Their Biotechnological Impact. *Open Biotechnol. J.* **2021**, *15*, 71–81. [CrossRef]
19. Martínková, L.; Kulik, N.; Sedova, A.; Křístková, B.; Bojarová, P. Recent Progress in the Production of Cya-nide-Converting Nitrilases—Comparison with Nitrile-Hydrolyzing Enzymes. *Catalysts* **2023**, *13*, 500. [CrossRef]
20. Martínková, L.; Rucká, L.; Nešvera, J.; Pátek, M. Recent Advances and Challenges in the Heterologous Production of Microbial Nitrilases for Biocatalytic Applications. *World J. Microbiol. Biotechnol.* **2016**, *33*, 1–11. [CrossRef]
21. Martínková, L.; Vejvoda, V.; Kaplan, O.; Kubáč, D.; Malandra, A.; Cantarella, M.; Bezouška, K.; Křen, V. Fungal Nitrilases as Biocatalysts: Recent Developments. *Biotechnol. Adv.* **2009**, *27*, 661–670. [CrossRef]
22. Woodward, J.D.; Trompetter, I.; Sewell, B.T.; Piotrowski, M. Substrate Specificity of Plant Nitrilase Complexes Is Affected by Their Helical Twist. *Commun. Biol.* **2018**, *1*, 186. [CrossRef]
23. Aguirre-Sampieri, S.; Casañal, A.; Emsley, P.; Garza-Ramos, G. Cryo-EM Structure of Bacterial Nitrilase Reveals Insight into Oligomerization, Substrate Recognition, and Catalysis. *J. Struct. Biol.* **2024**, *216*, 108093. [CrossRef] [PubMed]
24. Wang, L.; Jiang, S.; Sun, Y.; Yang, Z.; Chen, Z.; Wang, H.; Wei, D. Switching the Secondary and Natural Activity of Nitrilase from Acidovorax Facilis 72 W for the Efficient Production of 2-Picolinamide. *Biotechnol. Lett.* **2021**, *43*, 1617–1624. [CrossRef] [PubMed]
25. Gong, J.-S.; Li, H.; Lu, Z.-M.; Zhang, X.-J.; Zhang, Q.; Yu, J.-H.; Zhou, Z.-M.; Shi, J.-S.; Xu, Z.-H. Engineering of a Fungal Nitrilase for Improving Catalytic Activity and Reducing By-Product Formation in the Absence of Structural Infor-mation. *Catal. Sci. Technol.* **2016**, *6*, 4134–4141. [CrossRef]
26. Jiang, S.; Zhang, L.; Yao, Z.; Gao, B.; Wang, H.; Mao, X.; Wei, D. Switching a Nitrilase from *Syechocystis* Sp. PCC6803 to a Nitrile Hydratase by Rationally Regulating Reaction Pathways. *Catal. Sci. Technol.* **2017**, *7*, 1122–1128. [CrossRef]
27. Black, G.W.; Brown, N.L.; Perry, J.J.B.; Randall, P.D.; Turnbull, G.; Zhang, M. A High-Throughput Screening Method for Determining the Substrate Scope of Nitrilases. *Chem. Commun.* **2014**, *51*, 2660–2662. [CrossRef]
28. Kushwaha, M.; Kumar, V.; Mahajan, R.; Bhalla, T.C.; Chatterjee, S.; Akhter, Y. Molecular Insights into the Activity and Mechanism of Cyanide Hydratase Enzyme Associated with Cyanide Biodegradation by Serratia Marcescens. *Arch. Microbiol.* **2018**, *200*, 971–977. [CrossRef]

29. Lipowicz, B.; Hanekop, N.; Schmitt, L.; Proksch, P. An Aeroplysinin-1 Specific Nitrile Hydratase Isolated from the Marine Sponge Aplysina Cavernicola. *Mar. Drugs* **2013**, *11*, 3046–3067. [CrossRef]
30. Okamoto, S.; Eltis, L.D. Purification and Characterization of a Novel Nitrile Hydratase from *Rhodococcus* Sp. RHA1. *Mol. Microbiol.* **2007**, *65*, 828–838. [CrossRef]
31. Noguchi, T.; Honda, J.; Nagamune, T.; Sasabe, H.; Inoue, Y.; Endo, I. Photosensitive nitrile hydratase intrinsically possesses nitric oxide bound to the non-heme iron center: Evidence by Fourier transform infrared spectroscopy. *FEBS Lett.* **1995**, *358*, 9–12. [CrossRef]
32. Rose, M.J.; Betterley, N.M.; Oliver, A.G.; Mascharak, P.K. Binding of Nitric Oxide to a Synthetic Model of Iron-Containing Nitrile Hydratase (Fe-NHase) and Its Photorelease: Relevance to Photoregulation of Fe-NHase by NO. *Inorg. Chem.* **2010**, *49*, 1854–1864. [CrossRef] [PubMed]
33. Nagamune, T.; Kurata, H.; Hirata, M.; Honda, J.; Hirata, A.; Endo, I. Photosensitive Phenomena of Nitrile Hydratase of *Rhodococcus* sp. N-771. *Photochem. Photobiol.* **1990**, *51*, 87–90. [CrossRef]
34. Kobayashi, M.; Shimizu, S. Metalloenzyme nitrile hydratase: Structure, regulation, and application to biotechnology. *Nat. Biotechnol.* **1998**, *16*, 733–736. [CrossRef] [PubMed]
35. Guo, J.; Cheng, Z.; Zhou, Z. An Archaeal Nitrile Hydratase from the Halophilic Archaeon A07HB70 Exhibits High Tolerance to 3-Cyanopyridine and Nicotinamide. *Protein Expr. Purif.* **2023**, *214*, 106390. [CrossRef]
36. Yang, X.; Bennett, B.; Holz, R.C. Analyzing the function of the insert region found between the and-subunits in the eukaryotic nitrile hydratase from *Monosiga brevicollis*. *Arch. Biochem. Biophys.* **2018**, *657*, 1–7. [CrossRef]
37. Hussain, A.; Ogawa, T.; Saito, M.; Sekine, T.; Nameki, M.; Matsushita, Y.; Hayashi, T.; Katayama, Y. Cloning and expression of a gene encoding a novel thermostable thiocyanate-degrading enzyme from a mesophilic alphaproteobacteria strain THI201. *Microbiology* **2013**, *159*, 2294–2302. [CrossRef]
38. Guo, J.; Cheng, Z.; Berdychowska, J.; Zhu, X.; Wang, L.; Peplowski, L.; Zhou, Z. Effect and Mechanism Analysis of Different Linkers on Efficient Catalysis of Subunit-Fused Nitrile Hydratase. *Int. J. Biol. Macromol.* **2021**, *181*, 444–451. [CrossRef]
39. Wu, Y.; Chang, C.; Wang, C.; Hsieh, C.; Horng, Y. C=N Bond Activation and Hydration by an Iron (III) Complex with Asymmetric Sulfur Oxygenation. *Eur. J. Inorg. Chem.* **2017**, *2017*, 840–843. [CrossRef]
40. Light, K.M.; Yamanaka, Y.; Odaka, M.; Solomon, E.I. Spectroscopic and Computational Studies of Nitrile Hydratase: Insights into Geometric and Electronic Structure and the Mechanism of Amide Synthesis. *Chem. Sci.* **2015**, *6*, 6280–6294. [CrossRef]
41. Hopmann, K.H.; Himo, F. Theoretical Investigation of the Second-Shell Mechanism of Nitrile Hydratase. *Eur. J. Inorg. Chem.* **2008**, *2008*, 1406–1412. [CrossRef]
42. MacDonald, C.A.; Boyd, R.J. Competing Nitrile Hydratase Catalytic Mechanisms: Is Cysteine-Sulfenic Acid Acting as a Nucleophile? *Comput. Theor. Chem.* **2015**, *1070*, 48–54. [CrossRef]
43. Nelp, M.T.; Song, Y.; Wysocki, V.H.; Bandarian, V. A Protein-Derived Oxygen Is the Source of the Amide Oxygen of Nitrile Hydratases. *J. Biol. Chem.* **2016**, *291*, 7822–7829. [CrossRef] [PubMed]
44. Hopmann, K.H. Full Reaction Mechanism of Nitrile Hydratase: A Cyclic Intermediate and an Unexpected Disulfide Switch. *Inorg. Chem.* **2014**, *53*, 2760–2762. [CrossRef] [PubMed]
45. Kayanuma, M.; Shoji, M.; Yohda, M.; Odaka, M.; Shigeta, Y. Catalytic Mechanism of Nitrile Hydratase Subsequent to Cyclic Intermediate Formation: A QM/MM Study. *J. Phys. Chem. B* **2016**, *120*, 3259–3266. [CrossRef]
46. Yamanaka, Y.; Kato, Y.; Hashimoto, K.; Iida, K.; Nagasawa, K.; Nakayama, H.; Dohmae, N.; Noguchi, K.; Noguchi, T.; Yohda, M.; et al. Time-Resolved Crystallography of the Reaction Intermediate of Nitrile Hydratase: Revealing a Role for the Cysteinesulfenic Acid Ligand as a Catalytic Nucleophile. *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 10763–10767. [CrossRef]
47. Hashimoto, K.; Suzuki, H.; Taniguchi, K.; Noguchi, T.; Yohda, M.; Odaka, M. Catalytic Mechanism of Nitrile Hydratase Proposed by Time-Resolved X-Ray Crystallography Using a Novel Substrate, Tert-Butylisonitrile. *J. Biol. Chem.* **2008**, *283*, 36617–36623. [CrossRef]
48. Yu, H.; Liu, J.; Shen, Z. Modeling Catalytic Mechanism of Nitrile Hydratase by Semi-Empirical Quantum Mechanical Calculation. *J. Mol. Graph. Model.* **2008**, *27*, 522–528. [CrossRef]
49. Thimann, K.V.; Mahadevan, S. Nitrilase. *Arch. Biochem. Biophys.* **1964**, *105*, 133–141. [CrossRef]
50. Hook, R.H.; Robinson, W.G. Ricinine Nitrilase. *J. Biol. Chem.* **1964**, *239*, 4263–4267. [CrossRef]
51. Kobayashi, M.; Shimizu, S. Versatile Nitrilases: Nitrile-Hydrolysing Enzymes. *FEMS Microbiol. Lett.* **1994**, *120*, 217–223. [CrossRef]
52. Asano, Y.; Tani, Y.; Yamada, H. A new enzyme "Nitrile hydratase" which degrades acetonitrile in combination with amidase. *Agric. Biol. Chem.* **1980**, *44*, 2251–2252. [CrossRef]
53. Yamada, H.; Kobayashi, M. Nitrile Hydratase and Its Application to Industrial Production of Acrylamide. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1391–1400. [CrossRef] [PubMed]
54. Robertson, D.E.; Chaplin, J.A.; DeSantis, G.; Podar, M.; Madden, M.; Chi, E.; Richardson, T.; Milan, A.; Miller, M.; Weiner, D.P.; et al. Exploring Nitrilase Sequence Space for Enantioselective Catalysis. *Appl. Environ. Microbiol.* **2004**, *70*, 2429–2436. [CrossRef] [PubMed]

55. Bayer, S.; Birkemeyer, C.; Ballschmiter, M. A Nitrilase from a Metagenomic Library Acts Regioselectively on Aliphatic Dinitriles. *Appl. Microbiol. Biotechnol.* **2010**, *89*, 91–98. [CrossRef]
56. Sonbol, S.A.; Ferreira, A.J.S.; Siam, R. Red Sea Atlantis II Brine Pool Nitrilase with Unique Thermostability Profile and Heavy Metal Tolerance. *BMC Biotechnol* **2016**, *16*, 1–13. [CrossRef]
57. Dennett, G.V.; Blamey, J.M. A New Thermophilic Nitrilase from an Antarctic Hyperthermophilic Microorganism. *Front. Bioeng. Biotechnol.* **2016**, *4*, 5. [CrossRef]
58. Liu, Z.-Q.; Baker, P.J.; Cheng, F.; Xue, Y.-P.; Zheng, Y.-G.; Shen, Y.-C. Screening and Improving the Recombinant Nitrilases and Application in Biotransformation of Iminodiacetonitrile to Iminodiacetic Acid. *PLoS ONE* **2013**, *8*, e67197. [CrossRef]
59. Riffiani, R.; Sulistinah, N.; Sunarko, B. Gene Encoding Nitrilase from Soil Sample of Lombok Gold Mine Industry Using Metagenomics Approach. *KnE Life Sci.* **2017**, *201*, 201–207. [CrossRef]
60. Dooley-Cullinane, T.; O'REilly, C.; Aslam, B.; Weiner, D.P.; O'NEill, D.; Owens, E.; O'MEara, D.; Coffey, L. The use of clade-specific PCR assays to identify novel nitrilase genes from environmental isolates. *Microbiologyopen* **2018**, *8*, e00700. [CrossRef]
61. Veselá, A.B.; Rucká, L.; Kaplan, O.; Pelantová, H.; Nešvera, J.; Pátek, M.; Martínková, L. Bringing Nitrilase Sequences from Databases to Life: The Search for Novel Substrate Specificities with a Focus on Dinitriles. *Appl. Microbiol. Biotechnol.* **2015**, *100*, 2193–2202. [CrossRef]
62. Zhu, D.; Mukherjee, C.; Biehl, E.R.; Hua, L. Discovery of a Mandelonitrile Hydrolase from Bradyrhizobium Japonicum USDA110 by Rational Genome Mining. *J. Biotechnol.* **2007**, *129*, 645–650. [CrossRef]
63. Seffernick, J.L.; Samanta, S.K.; Louie, T.M.; Wackett, L.P.; Subramanian, M. Investigative Mining of Sequence Data for Novel Enzymes: A Case Study with Nitrilases. *J. Biotechnol.* **2009**, *143*, 17–26. [CrossRef]
64. Precigou, S.; Goulas, P.; Duran, R. Rapid and Specific Identification of Nitrile Hydratase (NHase)-Encoding Genes in Soil Samples by Polymerase Chain Reaction. *FEMS Microbiol. Lett.* **2001**, *204*, 155–161. [CrossRef]
65. Marron, A.O.; Akam, M.; Walker, G. Nitrile Hydratase Genes Are Present in Multiple Eukaryotic Supergroups. *PLoS ONE* **2012**, *7*, e32867. [CrossRef]
66. Zhao, Y.-X.; Yang, W.-L.; Guo, L.; Jiang, H.-Y.; Cheng, X.; Dai, Y.-J. Bioinformatics of a Novel Nitrile Hydratase Gene Cluster of the N 2-Fixing Bacterium Microvirga Flocculans CGMCC 1. 16731 and Characterization of the Enzyme. *J. Agric. Food Chem.* **2020**, *68*, 9299–9307. [CrossRef] [PubMed]
67. Bragança, S.C.; Dooley-Cullinane, T.; O'Reilly, C.; Coffey, L. Applying Functional Metagenomics to Search for Novel Nitrile-Hydrolyzing Enzymes Using Environmental Samples. *Biomaterials Tissue Technol.* **2017**, *1*, 10–15761.
68. An, X.; Cheng, Y.; Miao, L.; Chen, X.; Zang, H.; Li, C. Characterization and Genome Functional Analysis of an Efficient Nitrile-Degrading Bacterium, Rhodococcus Rhodochrous BX2, to Lay the Foundation for Potential Bioaugmentation for Remediation of Nitrile-Contaminated Environments. *J. Hazard. Mater.* **2020**, *389*, 121906. [CrossRef] [PubMed]
69. Roldán, M.D.; Olaya-Abril, A.; Sáez, L.P.; Cabello, P.; Luque-Almagro, V.M.; Moreno-Vivián, C. Bioremediation of cyanide-containing wastes: The potential of systems and synthetic biology for cleaning up the toxic leftovers from mining. *EMBO Rep.* **2021**, *22*, e53720. [CrossRef]
70. Amrutha, M.; Nampoothiri, K.M. In Silico Analysis of Nitrilase-3 Protein from Corynebacterium Glutamicum for Bioremediation of Nitrile Herbicides. *J. Genet. Eng. Biotechnol.* **2022**, *20*, 51. [CrossRef]
71. Achudhan, A.B.; Kannan, P.; Saleena, L.M. Functional Metagenomics Uncovers Nitrile-Hydrolysing Enzymes in a Coal Metagenome. *Front. Mol. Biosci.* **2023**, *10*, 1123902. [CrossRef]
72. Kumari, P.; Poddar, R. A comparative multivariate analysis of nitrilase enzymes: An ensemble based computational approach. *Comput. Biol. Chem.* **2019**, *83*, 107095. [CrossRef] [PubMed]
73. Jallageas, J.C.; Arnaud, A.; Galzy, P. Nitrilases and Amidases: Determination of Activity by Proton Magnetic Resonance Spectrometry. *Anal. Biochem.* **1979**, *95*, 436–443. [CrossRef] [PubMed]
74. Zhu, Q.; Fan, A.; Wang, Y.; Zhu, X.; Wang, Z.; Wu, M.; Zheng, Y. Novel Sensitive High-Throughput Screening Strategy for Nitrilase-Producing Strains. *Appl. Environ. Microbiol.* **2007**, *73*, 6053–6057. [CrossRef] [PubMed]
75. Angelini, L.M.L.; Da Silva, A.R.M.; Rocco, L.d.F.C.; Milagre, C.D.d.F. A High-Throughput Screening Assay for Distinguishing Nitrile Hydratases from Nitrilases. *Braz. J. Microbiol.* **2015**, *46*, 113–116. [CrossRef]
76. Lin, Z.-J.; Zheng, R.-C.; Lei, L.-H.; Zheng, Y.-G.; Shen, Y.-C. Ferrous and Ferric Ions-Based High-Throughput Screening Strategy for Nitrile Hydratase and Amidase. *J. Microbiol. Methods* **2011**, *85*, 214–220. [CrossRef]
77. Sahu, R.; Meghavarnam, A.K.; Janakiraman, S. A Simple, Efficient and Rapid Screening Technique for Differentiating Nitrile Hydratase and Nitrilase Producing Bacteria. *Biotechnol. Rep.* **2019**, *24*, e00396. [CrossRef]
78. Nichols, M.L.; Willits, C.O. Reactions of Nessler's Solution1. *J. Am. Chem. Soc.* **1934**, *56*, 769–774. [CrossRef]
79. Banerjee, A.; Kaul, P.; Sharma, R.; Banerjee, U.C. A High-Throughput Amenable Colorimetric Assay for Enantioselective Screening of Nitrilase-Producing Microorganisms Using pH Sensitive Indicators. *Slas Discov. Adv. Sci. Drug Discov.* **2003**, *8*, 559–565. [CrossRef]

80. Singh, R.; Pandey, D.; Dhariwal, S.; Sood, P.; Chand, D. Bioconversion of Acrylonitrile Using Nitrile Hydratase Activity of *Bacillus* Sp. APB-6. *3 Biotech* **2018**, *8*, 225. [CrossRef]
81. Hjort, C.M.; Godtfredsen, S.E.; Emborg, C. Isolation and Characterization of a Nitrile Hydratase from a *Rhodococcus* Sp. *J. Chem. Technol. Biotechnol.* **1990**, *48*, 217–226. [CrossRef]
82. Egelkamp, R.; Friedrich, I.; Hertel, R.; Daniel, R. From Sequence to Function: A New Workflow for Nitrilase Identification. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 4957–4970. [CrossRef]
83. Yazbeck, D.R.; Durao, P.J.; Xie, Z.; Tao, J. A Metal Ion-Based Method for the Screening of Nitrilases. *J. Mol. Catal. B Enzym.* **2006**, *39*, 156–159. [CrossRef]
84. He, Y.-C.; Ma, C.-L.; Xu, J.-H.; Zhou, L. A High-Throughput Screening Strategy for Nitrile-Hydrolyzing Enzymes Based on Ferric Hydroxamate Spectrophotometry. *Appl. Microbiol. Biotechnol.* **2010**, *89*, 817–823. [CrossRef] [PubMed]
85. Roth, M. Fluorimetric methods in a laboratory of clinical chemistry. *Ann. Dell'istituto Super. Sanità* **1971**, *7*, 199–207.
86. Lloret, S.; Andres, J.; Legua, C.; Falco, P. Determination of ammonia and primary amine compounds and Kjeldahl nitrogen in water samples with a modified Roth's fluorimetric method. *Talanta* **2005**, *65*, 869–875. [CrossRef]
87. Banerjee, A.; Sharma, R.; Banerjee, U.C. A Rapid and Sensitive Fluorometric Assay Method for the Determination of Nitrilase Activity. *Biotechnol. Appl. Biochem.* **2003**, *37*, 289–293. [CrossRef]
88. Lasch, P.; Beyer, W.; Bosch, A.; Borriss, R.; Drevinek, M.; Dupke, S.; Ehling-Schulz, M.; Gao, X.; Grunow, R.; Jacob, D.; et al. A MALDI-ToF Mass Spectrometry Database for Identification and Classification of Highly Pathogenic Bacteria. *Sci. Data* **2025**, *12*, 187. [CrossRef]
89. Iqfath, M.; Wali, S.N.; Amer, S.; Hernly, E.; Laskin, J. Nanospray Desorption Electrospray Ionization Mass Spectrometry Imaging (Nano-DESI MSI): A Tutorial Review. *ACS Meas. Sci. Au* **2024**, *4*, 475–487. [CrossRef]
90. Martinez, S.; Kuhn, M.L.; Russell, J.T.; Holz, R.C.; Elgren, T.E. Acrylamide production using encapsulated nitrile hydratase from Pseudonocardia thermophila in a sol–gel matrix. *J. Mol. Catal. B Enzym.* **2014**, *100*, 19–24. [CrossRef]
91. Raj, J.; Prasad, S.; Bhalla, T.C. Rhodococcus Rhodochrous PA-34: A Potential Biocatalyst for Acrylamide Synthesis. *Process. Biochem.* **2006**, *41*, 1359–1363. [CrossRef]
92. Jiao, S.; Li, F.; Yu, H.; Shen, Z. Advances in Acrylamide Bioproduction Catalyzed with Rhodococcus Cells Harboring Nitrile Hydratase. *Appl. Microbiol. Biotechnol.* **2019**, *104*, 1001–1012. [CrossRef]
93. Luo, T.; Yu, S. Control and Communication Mechanisms of a SoftRouter. In Proceedings of the COIN-NGNCON 2006-The Joint International Conference on Optical Internet and Next Generation Network, Jeju, Republic of Korea, 13 September 2006. [CrossRef]
94. Nagasawa, T.; Nakamura, T.; Yamada, H. Production of Acrylic Acid and Methacrylic Acid Using Rhodococcus Rhodochrous J1 Nitrilase. *Appl. Microbiol. Biotechnol.* **1990**, *34*, 322–324. [CrossRef]
95. Bhatt, A.; Prajapati, D.; Gupte, A. Production, Purification and Characterization of Nitrilase from Bacillus Subtilis-AGAB-2 and Chemoenzymatic Synthesis of Acrylic Acid from Acrylonitrile. *Catal. Lett.* **2024**, *154*, 4302–4319. [CrossRef]
96. Kamal, A. Bioconversion of Acrylonitrile to Acrylic Acid by Rhodococcus Ruber Strain AKSH-84. *J. Microbiol. Biotechnol.* **2011**, *21*, 37–42. [CrossRef]
97. Wu, S.; Snajdrova, R.; Moore, J.C.; Baldenius, K.; Bornscheuer, U.T. Biocatalysis: Enzymatic Synthesis for Industrial Applications. *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 88–119. [CrossRef] [PubMed]
98. Liang, Y.; Jiao, S.; Wang, M.; Yu, H.; Shen, Z. A CRISPR/Cas9-Based Genome Editing System for Rhodococcus Ruber TH. *Metab. Eng.* **2020**, *57*, 13–22. [CrossRef] [PubMed]
99. Lavrov, K.V.; Shemyakina, A.O.; Grechishnikova, E.G.; Gerasimova, T.V.; Kalinina, T.I.; Novikov, A.D.; Leonova, T.E.; Ryabchenko, L.E.; Bayburdov, T.A.; Yanenko, A.S. A New Concept of Biocatalytic Synthesis of Acrylic Monomers for Obtaining Water-Soluble Acrylic Heteropolymers. *Metab. Eng. Commun.* **2023**, *18*, e00231. [CrossRef] [PubMed]
100. Liang, Y.; Zheng, Y.; Li, J.; Du, Y.; Wei, Y.; Yu, H. Engineering a Non-Pigmented Rhodococcus Ruber Strain for Enhanced Bio-Production of Acrylamide. *Chem. Eng. J.* **2025**, *508*, 160950. [CrossRef]
101. Prasad, S.; Raj, J.; Bhalla, T.C. Bench Scale Conversion of 3-Cyanopyidine to Nicotinamide Using Resting Cells of Rhodococcus Rhodochrous PA-34. *Indian J. Microbiol.* **2007**, *47*, 34–41. [CrossRef]
102. Zhu, X.-Y.; Gong, J.-S.; Li, H.; Lu, Z.-M.; Shi, J.-S.; Xu, Z.-H. Bench-Scale Biosynthesis of Isonicotinic Acid from 4-Cyanopyridine by Pseudomonas Putida. *Chem. Pap.* **2014**, *68*, 739–744. [CrossRef]
103. Sharma, N.N.; Sharma, M.; Bhalla, T.C. Nocardia Globerula NHB-2 Nitrilase Catalysed Biotransformation of 4-Cyanopyridine to Isonicotinic Acid. *AMB Express* **2012**, *2*, 25. [CrossRef]
104. Cantarella, L.; Gallifuoco, A.; Malandra, A.; Martínková, L.; Spera, A.; Cantarella, M. High–yield continuous production of nicotinic acid via nitrile hydratase–amidase cascade reactions using cascade CSMRs. *Enzym. Microb. Technol.* **2011**, *48*, 345–350. [CrossRef] [PubMed]
105. Shen, Y.; Wang, M.; Li, X.; Zhang, J.; Sun, H.; Luo, J. Highly efficient synthesis of 5-cyanovaleramide by Rhodococcus ruber CGMCC3090 resting cells. *J. Chem. Technol. Biotechnol.* **2012**, *87*, 1396–1400. [CrossRef]

106. Wang, L.; Liu, S.; Du, W.; Dou, T.; Liang, C. High Regioselectivity Production of 5-Cyanovaleramide from Adiponitrile by a Novel Nitrile Hydratase Derived from Rhodococcus Erythropolis CCM2595. *ACS Omega* **2020**, *5*, 18397–18402. [CrossRef] [PubMed]
107. Bhatia, S.K.; Mehta, P.K.; Bhatia, R.K.; Bhalla, T.C. Optimization of Arylacetonitrilase Production from *Alcaligenes* Sp. MTCC 10675 and Its Application in Mandelic Acid Synthesis. *Appl. Microbiol. Biotechnol.* **2013**, *98*, 83–94. [CrossRef]
108. Pawar, S.V.; Yadav, G.D. Enantioselective Enzymatic Hydrolysis of rac–Mandelonitrile to R–Mandelamide by Nitrile Hydratase Immobilized on Poly(vinyl alcohol)/Chitosan–Glutaraldehyde Support. *Ind. Eng. Chem. Res.* **2014**, *53*, 7986–7991. [CrossRef]
109. Yu, H.; Jiao, S.; Wang, M.; Liang, Y.; Tang, L. Biodegradation of Nitriles by Rhodococcus. In *Biology of Rhodococcus*; Springer: Cham, Switzerland, 2019; Volume 16, pp. 173–202. [CrossRef]
110. Li, M.; Ma, D.; Qiao, J.; Cheng, Z.; Wang, Q.; Zhou, Z.; Han, L. Development of High-Performance Nitrile Hydratase Whole-Cell Catalyst by Automated Structure-and Sequence-Based Design and Mechanism Insights. *Syst. Microbiol. Biomanufacturing* **2024**, *4*, 882–894. [CrossRef]
111. Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A. Nitrile Bioconversion by Microbacterium Imperiale CBS 498-74 Resting Cells in Batch and Ultrafiltration Membrane Bioreactors. *J. Ind. Microbiol. Biotechnol.* **2005**, *33*, 208–214. [CrossRef]
112. Srinivasan, K.; Hariharapura, R.C.; Mallikarjuna, S.V. Pharmaceutical Waste Management through Microbial Bioremediation. *Environ. Monit. Assess.* **2025**, *197*, 1–14. [CrossRef]
113. Langdahl, B.R.; Bisp, P.; Ingvorsen, K. Nitrile Hydrolysis by Rhodococcus Erythropolis BL1, an Acetonitrile-Tolerant Strain Isolated from a Marine Sediment. *Microbiology* **1996**, *142*, 145–154. [CrossRef]
114. Gong, J.-S.; Lu, Z.-M.; Li, H.; Shi, J.-S.; Zhou, Z.-M.; Xu, Z.-H. Nitrilases in Nitrile Biocatalysis: Recent Progress and Forthcoming Research. *Microb. Cell Factories* **2012**, *11*, 142. [CrossRef]
115. Torri, D.; Bering, L.; Yates, L.R.L.; Angiolini, S.M.; Xu, G.; Cuesta-Hoyos, S.; Shepherd, S.A.; Micklefield, J. Enzymatic Cascades for Stereoselective and Regioselective Amide Bond Assembly. *Angew. Chem. Int. Ed.* **2025**, *64*, e202422185. [CrossRef]
116. Wang, L.; Song, Y.; Guo, Y.; Chen, G.; Zhang, H.; Liang, C. Modification of Nitrile Hydratase by Substrate Channel Engineering Regulates Monocyanamide Synthesis. *Catal. Lett.* **2025**, *155*, 1–12. [CrossRef]
117. Guo, J.; Cheng, Z.; Peplowski, L.; Zhou, Z. Highly Efficient Biosynthesis of Isonicotinamide through a Substrate Access Tunnel Engineered Nitrile Hydratase from Carbonactinospora Thermoautotrophicus. *New J. Chem.* **2023**, *47*, 13279–13285. [CrossRef]
118. Ma, D.; Cheng, Z.; Peplowski, L.; Han, L.; Xia, Y.; Hou, X.; Guo, J.; Yin, D.; Rao, Y.; Zhou, Z. Insight into the Broadened Substrate Scope of Nitrile Hydratase by Static and Dynamic Structure Analysis. *Chem. Sci.* **2022**, *13*, 8417–8428. [CrossRef]
119. Cheng, Z.; Peplowski, L.; Cui, W.; Xia, Y.; Liu, Z.; Zhang, J.; Kobayashi, M.; Zhou, Z. Identification of key residues modulating the stereoselectivity of nitrile hydratase toward rac-mandelonitrile by semi-rational engineering. *Biotechnol. Bioeng.* **2017**, *115*, 524–535. [CrossRef] [PubMed]
120. Cheng, Z.; Cui, W.; Xia, Y.; Peplowski, L.; Kobayashi, M.; Zhou, Z. Modulation of Nitrile Hydratase Regioselectivity towards Dinitriles by Tailoring the Substrate Binding Pocket Residues. *ChemCatChem* **2018**, *10*, 449–458. [CrossRef]
121. Cheng, Z.; Cui, W.; Liu, Z.; Zhou, L.; Wang, M.; Kobayashi, M.; Zhou, Z. A switch in a substrate tunnel for directing regioselectivity of nitrile hydratases towards,-dinitriles. *Catal. Sci. Technol.* **2016**, *6*, 1292–1296. [CrossRef]
122. Zheng, R.-C.; Yin, X.-J.; Zheng, Y.-G. Highly Regioselective and Efficient Production of 1-Cyanocyclohexaneacetamide by Rhodococcus Aetherivorans ZJB1208 Nitrile Hydratase. *J. Chem. Technol. Biotechnol.* **2015**, *91*, 1314–1319. [CrossRef]
123. Van Pelt, S.; Quignard, S.; Kubáč, D.; Sorokin, D.Y.; Van Rantwijk, F.; Sheldon, R.A. Nitrile Hydratase CLEAs: The Immobilization and Stabilization of an Industrially Important Enzyme. *Green Chem.* **2007**, *10*, 395–400. [CrossRef]
124. Van Pelt, S.; Zhang, M.; Otten, L.G.; Holt, J.; Sorokin, D.Y.; Van Rantwijk, F.; Black, G.W.; Perry, J.J.; Sheldon, R.A. Probing the Enantioselectivity of a Diverse Group of Purified Cobalt-Centred Nitrile Hydratases. *Org. Biomol. Chem.* **2011**, *9*, 3011–3019. [CrossRef]
125. Angiolini, S.; Bruton, I.; Bering, L.; Thomas, S.S.; Thompson, J.; Shepherd, S.A.; Micklefield, J. Integrating Enzymes with Photoredox Catalysis for Conversion of Nitriles into Fluorinated Products. *ChemCatChem* **2025**, e202500240. [CrossRef]
126. Bian, S.-Q.; Wang, Z.; Gong, J.-S.; Su, C.; Li, H.; Xu, Z.-H.; Shi, J.-S. Enhancing the Substrate Specificity of Nitrilase toward Aliphatic Nitriles Based on Substrate Channel Design. *Acs Sustain. Chem. Eng.* **2025**, *13*, 5332–5344. [CrossRef]
127. Jones, L.B.; Wang, X.; Gullapalli, J.S.; Kunz, D.A. Characterization of the Nit6803 Nitrilase Homolog from the Cyanotroph Pseudomonas Fluorescens NCIMB 11764. *Biochem. Biophys. Rep.* **2021**, *25*, 100893. [CrossRef] [PubMed]
128. Thakur, N.; Kumar, V.; Thakur, S.; Sharma, N.; Sheetal; Bhalla, T.C. Biotransformation of 4-Hydroxyphenylacetonitrile to 4-Hydroxyphenylacetic Acid Using Whole Cell Arylacetonitrilase of Alcaligenes Faecalis MTCC 12629. *Process. Biochem.* **2018**, *73*, 117–123. [CrossRef]
129. Pai, O.; Banoth, L.; Ghosh, S.; Chisti, Y.; Banerjee, U.C. Biotransformation of 3-Cyanopyridine to Nicotinic Acid by Free and Immobilized Cells of Recombinant Escherichia Coli. *Process. Biochem.* **2014**, *49*, 655–659. [CrossRef]
130. Duca, D.; Rose, D.R.; Glick, B.R. Characterization of a Nitrilase and a Nitrile Hydratase from *Pseudomonas* Sp. Strain UW4 That Converts Indole-3-Acetonitrile to Indole-3-Acetic Acid. *Appl. Environ. Microbiol.* **2014**, *80*, 4640–4649. [CrossRef]

131. Layh, N.; Parratt, J.; Willetts, A. Characterization and Partial Purification of an Enantioselective Arylacetonitrilase from Pseudomonas Fluorescens DSM 7155. *J. Mol. Catal. B Enzym.* **1998**, *5*, 467–474. [CrossRef]
132. Gilligan, T.; Yamada, H.; Nagasawa, T. Production of S-(+)-2-Phenylpropionic Acid from (R, S)-2-Phenylpropionitrile by the Combination of Nitrile Hydratase and Stereoselective Amidase in Rhodococcus Equi TG328. *Appl. Microbiol. Biotechnol.* **1993**, *39*, 720–725. [CrossRef]
133. Xue, Y.-P.; Yang, Y.-K.; Lv, S.-Z.; Liu, Z.-Q.; Zheng, Y.-G. High-Throughput Screening Methods for Nitrilases. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 3421–3432. [CrossRef]
134. Zheng, W.; Yang, L.; Meng, Y.; Yang, L.; Wu, J. Enhancing the Catalytic Efficiency of Nitrilase for Sterically Hindered Substrates by Distal Sites Engineering. *Green Synth. Catal.* **2024**. [CrossRef]
135. Wang, Z.-K.; Gong, J.-S.; Feng, D.-T.; Su, C.; Li, H.; Rao, Z.-M.; Lu, Z.-M.; Shi, J.-S.; Xu, Z.-H. Geometric Remodeling of Nitrilase Active Pocket Based on ALF-Scanning Strategy To Enhance Aromatic Nitrile Substrate Preference and Catalytic Efficiency. *Appl. Environ. Microbiol.* **2023**, *89*, e00220-23. [CrossRef]
136. Tang, X.-L.; Wen, P.-F.; Zheng, W.; Zhu, X.-Y.; Zhang, Y.; Diao, H.-J.; Zheng, R.-C.; Zheng, Y.-G. Bidirectional Regulation of Nitrilase Reaction Specificity by Tuning the Characteristic Distances between Key Residues and Substrate. *ACS Catal.* **2023**, *13*, 10282–10294. [CrossRef]
137. Wang, Z.-K.; Feng, D.-T.; Su, C.; Li, H.; Rao, Z.-M.; Rao, Y.-J.; Lu, Z.-M.; Shi, J.-S.; Xu, Z.-H.; Gong, J.-S. Designing ASSMD Strategy for Exploring and Engineering Extreme Thermophilic Ancestral Nitrilase for Nitriles Biocatalysis. *ACS Catal.* **2024**, *14*, 13825–13838. [CrossRef]
138. Jin, Y.; Shen, J.; Wang, Y.; Shao, H.; Xia, H.; Lin, J.; Liu, Z.; Zheng, Y. Modification of nitrilase based on computer screening and efficient biosynthesis of 4-cyanobenzoic acid. *Biotechnol. J.* **2024**, *19*, 2300706. [CrossRef] [PubMed]
139. Cheng, Z.; Lan, Y.; Guo, J.; Ma, D.; Jiang, S.; Lai, Q.; Zhou, Z.; Peplowski, L. Computational Design of Nitrile Hydratase from Pseudonocardia Thermophila JCM3095 for Improved Thermostability. *Molecules* **2020**, *25*, 4806. [CrossRef] [PubMed]
140. Song, J.; Qiao, J.; Cheng, Z.; Guo, J.; Wang, Q.; Zhou, Z.; Han, L. Computational Design of Coevolutionary Residues for Improved Stability and Activity of Nitrile Hydratase. *Biochem. Biophys. Res. Commun.* **2025**, *750*, 151400. [CrossRef]
141. Chen, J.; Yu, H.; Liu, C.; Liu, J.; Shen, Z. Improving Stability of Nitrile Hydratase by Bridging the Salt-Bridges in Specific Thermal-Sensitive Regions. *J. Biotechnol.* **2013**, *164*, 354–362. [CrossRef]
142. Liu, Y.; Cui, W.; Liu, Z.; Cui, Y.; Xia, Y.; Kobayashi, M.; Zhou, Z. Enhancement of Thermo-Stability and Product Tolerance of Pseudomonas Putida Nitrile Hydratase by Fusing with Self-Assembling Peptide. *J. Biosci. Bioeng.* **2014**, *118*, 249–252. [CrossRef]
143. Xia, Y.; Cui, W.; Liu, Z.; Zhou, L.; Cui, Y.; Kobayashi, M.; Zhou, Z. Construction of a Subunit-Fusion Nitrile Hydratase and Discovery of an Innovative Metal Ion Transfer Pattern. *Sci. Rep.* **2016**, *6*, 19183. [CrossRef]
144. Xia, Y.; Peplowski, L.; Cheng, Z.; Wang, T.; Liu, Z.; Cui, W.; Kobayashi, M.; Zhou, Z. Metallochaperone function of the self-subunit swapping chaperone involved in the maturation of subunit-fused cobalt-type nitrile hydratase. *Biotechnol. Bioeng.* **2018**, *116*, 481–489. [CrossRef]
145. Xia, Y.; Cui, W.; Cheng, Z.; Peplowski, L.; Liu, Z.; Kobayashi, M.; Zhou, Z. Improving the Thermostability and Catalytic Efficiency of the Subunit-Fused Nitrile Hydratase by Semi-Rational Engineering. *ChemCatChem* **2018**, *10*, 1370–1375. [CrossRef]
146. Wang, L.; Guan, S.; Bai, J.; Jiang, Y.; Song, Y.; Zheng, X.; Gao, J. Enzyme Immobilized in BioMOFs: Facile Synthesis and Improved Catalytic Performance. *Int. J. Biol. Macromol.* **2020**, *144*, 19–28. [CrossRef] [PubMed]
147. Chiyanzu, I.; Cowan, D.A.; Burton, S.G. Immobilization of Geobacillus Pallidus RAPc8 Nitrile Hydratase (NHase) Reduces Substrate Inhibition and Enhances Thermostability. *J. Mol. Catal. B Enzym.* **2010**, *63*, 109–115. [CrossRef]
148. Sheldon, R.A. Characteristic Features and Biotechnological Applications of Cross-Linked Enzyme Aggregates (CLEAs). *Appl. Microbiol. Biotechnol.* **2011**, *92*, 467–477. [CrossRef] [PubMed]
149. Kumar, S.; Mohan, U.; Kamble, A.L.; Pawar, S.; Banerjee, U.C. Cross-Linked Enzyme Aggregates of Recombinant Pseudomonas Putida Nitrilase for Enantioselective Nitrile Hydrolysis. *Bioresour. Technol.* **2010**, *101*, 6856–6858. [CrossRef]
150. Pawar, S.V.; Yadav, G.D. PVA/chitosan–glutaraldehyde cross–linked nitrile hydratase as reusable biocatalyst for conversion of nitriles to amides. *J. Mol. Catal. B Enzym.* **2014**, *101*, 115–121. [CrossRef]
151. D'ANtona, N.; Morrone, R.; Gambera, G.; Pedotti, S. Enantiorecognition of planar metallocenic chirality by a nitrile hydratase/amidase bienzymatic system. *Org. Biomol. Chem.* **2016**, *14*, 4393–4399. [CrossRef]
152. Peplowski, L.; Kubiak, K.; Nowak, W. Mechanical Aspects of Nitrile Hydratase Enzymatic Activity. Steered Molecular Dynamics Simulations of Pseudonocardia Thermophila JCM 3095. *Chem. Phys. Lett.* **2008**, *467*, 144–149. [CrossRef]
153. Mashweu, A.R.; Chhiba-Govindjee, V.P.; Bode, M.L.; Brady, D. Substrate Profiling of the Cobalt Nitrile Hydratase from Rhodococcus Rhodochrous ATCC BAA 870. *Molecules* **2020**, *25*, 238. [CrossRef]
154. Wang, M.-X. Enantioselective Biotransformations of Nitriles in Organic Synthesis. *Top. Catal.* **2005**, *35*, 117–130. [CrossRef]
155. Mulelu, A.E.; Kirykowicz, A.M.; Woodward, J.D. Cryo-EM and Directed Evolution Reveal How Arabidopsis Nitrilase Specificity Is Influenced by Its Quaternary Structure. *Commun. Biol.* **2019**, *2*, 1–11. [CrossRef]

156. Luo, H.; Ma, J.; Chang, Y.; Yu, H.; Shen, Z. Directed Evolution and Mutant Characterization of Nitrilase from Rhodococcus Rhodochrous Tg1-A6. *Appl. Biochem. Biotechnol.* **2015**, *178*, 1510–1521. [CrossRef] [PubMed]
157. Mathew, S.; Nadarajan, S.P.; Sundaramoorthy, U.; Jeon, H.; Chung, T.; Yun, H. Biotransformation of-keto nitriles to chiral (S)--amino acids using nitrilase and-transaminase. *Biotechnol. Lett.* **2016**, *39*, 535–543. [CrossRef]
158. Bering, L.; Craven, E.J.; Thomas, S.A.S.; Shepherd, S.A.; Micklefield, J. Merging enzymes with chemocatalysis for amide bond synthesis. *Nat. Commun.* **2022**, *13*, 1–10. [CrossRef]
159. Craven, E.J.; Latham, J.; Shepherd, S.A.; Khan, I.; Diaz-Rodriguez, A.; Greaney, M.F.; Micklefield, J. Programmable late-stage CH bond functionalization enabled by integration of enzymes with chemocatalysis. *Nat. Catal.* **2021**, *4*, 385–394. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.