

Supplementary Materials

Application Potential of Cyanide Hydratase from *Exidia Glandulosa*: Free Cyanide Removal from Simulated Industrial Effluents

Anastasia Sedova ^{1,2}, Lenka Rucká ¹, Pavla Bojarová ^{1,2}, Michaela Glozlová ^{1,2}, Petr Novotný ¹, Barbora Kříštková ^{1,3}, Miroslav Pátek ¹ and Ludmila Martínková ^{1,*}

¹ Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-142 20 Prague, Czech Republic; sedova_aa@mail.ru (A.S.); rucka@biomed.cas.cz (L.R.); bojarova@biomed.cas.cz (P.B.); m.glozlova@gmail.com (M.G.); petr.novotny@biomed.cas.cz (P.N.); barbora.kristkova@biomed.cas.cz (B.K.); patek@biomed.cas.cz (M.P.)

² Department of Health Care Disciplines and Population Protection, Faculty of Biomedical Engineering, Czech Technical University in Prague, Nám. Sítná 3105, CZ-272 01 Kladno, Czech Republic

³ Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Technická 5, CZ-166 28 Prague, Czech Republic

* Correspondence: martinko@biomed.cas.cz; Tel.: +420-296-442-569

Contents:

Figure S1. Optimized sequence of the gene encoding NitEg.

Figure S2. (A) SDS-PAGE of purified NitEg. (B) Determination of enzyme molecular mass.

Figure S3. Shelf life of NitEg at pH 8.0 and 4 °C.

Figure S4. Multiple sequence alignment of NitEg (UniProtKB: A0A165HZS1) and its closest characterized homologue from *Neurospora crassa* (UniProtKB: Q7RVT0).

Table S1. Specific activities of cyanide hydratases.

Table S2. Purification of NitEg from 200 mL of culture.

Table S3. Performance of cyanide hydratase and cyanide dehydratase in model mixtures and effluents.

1.	M	P	I	T	K	Y	K	A	A	A	V	T	S	E	P	G	W	F	D	L	E	G	
70.	CAT	ATGCCGAT	ACCAAGT	ACAAGGCCGCTG	CTGTCACCTCTG	AGGCCAGGATGGT	TGACCTCGAAGGC																
70.	G	V	G	K	T	I	N	F	I	N	E	A	G	G	A	G	C	K	L	V	A	F	P
70.	GGCGTT	CAGAAGACGAT	CAACTTCAT	CAACGAAGCTGGCC	AAGCAGGCTG	CAAGCTTG	AGCTTAGG	CCTTCCCC															
139.	E	V	W	I	P	G	Y	P	Y	W	M	W	K	V	N	Y	G	Q	S	L	P	M	L
139.	GAAGT	CTGGATCCCAGG	CTATCCGTACT	GGATGTG	GAAGGTCA	ACTATCAGCAGT	CCCTTCCC	ATGCTG															
208.	K	K	Y	R	E	N	S	L	G	V	N	T	E	E	M	R	R	I	R	R	A	A	R
208.	AAGAAGT	ATCGCGAGAA	CTCCCTCGGAG	CTAACACGGAGG	AAATGAGACG	CATCCGCCGCG	CGCGCGCG																
277.	D	N	G	I	Y	V	S	M	G	F	S	E	I	D	H	A	T	L	Y	L	A	Q	V
277.	GACAACCAGA	CTACGTCTCGAT	GGGCTTCTCGAGA	TCGACCACCG	CGACGTTG	ACCTAGCGCAGG	TG																
346.	L	I	S	P	T	G	E	V	I	N	H	R	R	K	I	K	P	T	H	V	E	K	L
346.	CTCAT	CTCTCGGACGGG	CGAGGTG	ATCAACCACAG	ACGCAAGA	TCAGCCGACGC	ACGTCGAGAA	ACTC															
415.	V	Y	G	D	G	A	G	D	T	F	L	S	V	T	E	T	D	I	G	R	L	G	Q
415.	GTCTACGGCGACGGCG	AGGGCACCTT	CCCTCTCGCT	CACGAAACCG	ACATCGGAC	GGCTCGGGCAG																	
484.	L	N	C	W	E	N	M	N	P	F	L	K	A	L	N	V	S	A	G	E	Q	V	H
484.	CTGAACT	GCTGGGAGAACAT	GAACCCGTTCTCA	AGGGCCCTG	AACGTCTCG	CCGCCGGAGAGCAGG	TG	CAC															
553.	V	A	A	W	P	V	Y	P	G	K	E	T	L	K	Y	P	D	P	A	T	N	V	A
553.	GTGCGCCG	TGCGCCGGT	TACCCCTGGCA	AGGAGACG	GCTCAAGT	TACCCCGACCC	CGGACG	ACGTGGCC															
622.	E	P	A	S	D	L	V	T	P	A	Y	A	I	E	T	G	T	W	T	L	A	P	F
622.	GAGCCCGCGTCCG	ACCTCGTTACG	CCCGCTTATCG	GATTGAGAC	CCGGAC	CTGCTGGACT	CTCGCGCCG	TTCTC															
691.	Q	R	L	S	K	E	G	L	K	K	N	T	P	E	G	V	E	P	E	T	D	P	S
691.	CAGCGCCCTGAGTA	GGAGTAAGGAGGG	CTTGAGAAAGAAC	ACGCCC	GAGGGAGTC	GAACCTGAGAC	GGAT	CCACG															
760.	T	Y	N	G	H	A	R	I	F	A	P	D	G	T	L	L	V	K	P	D	K	D	F
760.	ACGTACAACGGCCAC	GCACCGCGCAT	CTTCGCGCCCG	ACGGTACG	GCTCGTCA	AGCCGGACAAGG	ACTTC																
829.	D	G	L	L	F	V	N	I	D	L	N	E	C	H	L	T	K	A	L	A	D	F	G
829.	GACGGGCTGCTTCG	TCACATCGAC	CTCAACG	CTAACGAGT	GCTCCAC	TACTAAGG	GCTCGT	GACTTCGGC															
898.	G	H	Y	M	R	P	D	L	I	R	L	L	V	D	T	R	R	K	E	L	V	T	E
898.	GGCCACTATATGCGT	CCGGACCTCATCCG	CTGCTGTTG	CGACACGCC	CGCAAGGA	ACTCG	GACAGAA																
967.	A	D	P	D	G	G	I	A	T	Y	T	T	R	E	R	L	G	L	N	L	P	L	A
967.	GC GGACCC	CAGACGGCGG	CATTGCC	ACCTACACCAC	GGCG	GAACGG	CTGGC	CTGA	ACTTG	CCATTGGC													
1036.	E	K	E	E	K	K	G	G	S	S	T	K	K	H	D	G	K	K	A	G	D	L	
1036.	GAGAAGGAGGAGA	AGAAGGGTGGGAG	CAGCACCAAGA	AGCACG	ATGGGA	AGAAGCTGG	CGACCTC																
1105.	GAG																						

Figure S1. Optimized sequence of *cynH* gene from *Exidia glandulosa*. Gene optimization in terms of *E. coli* codon usage and optimized gene synthesis were carried out by GeneArt (ThermoFisher), Regensburg, Germany.

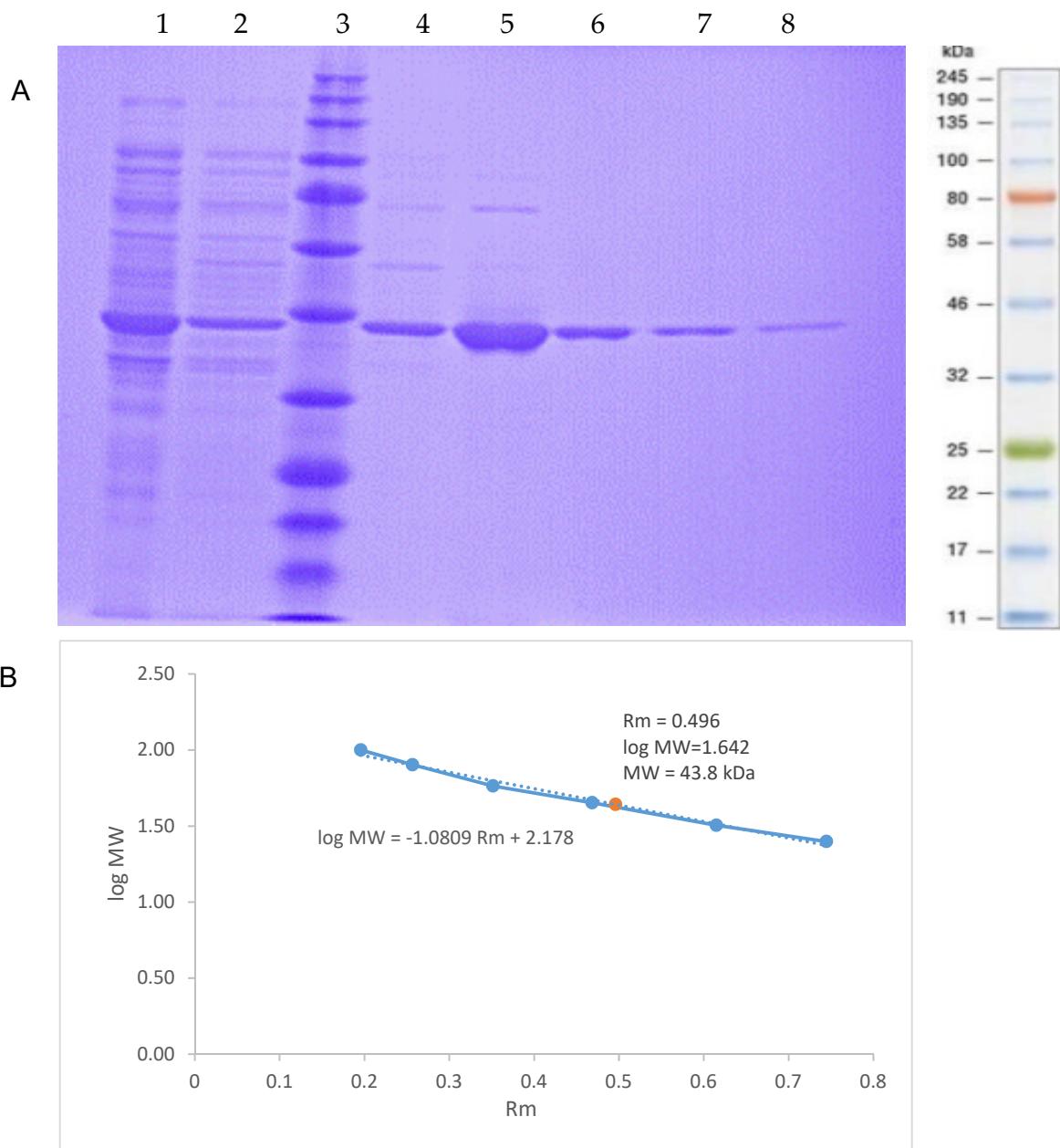


Figure S2. (A) Left, SDS-PAGE of NitEg in 10% polyacrylamide gels. (1) Cell-free extract, (2) column wash, (3) marker, (4-8) fractions from cobalt-affinity chromatography; Right, prestained marker; (B) Determination of molecular weight of NitEg ($\log MW = 1.642$, $MW = 43.85$ kDa); MW = molecular weight; Rm, relative mobility = distance traveled by the protein/distance traveled by the dye front). The calibration curve (blue) was constructed for standard proteins within MW range of 25-100 kDa; NitEg in orange.

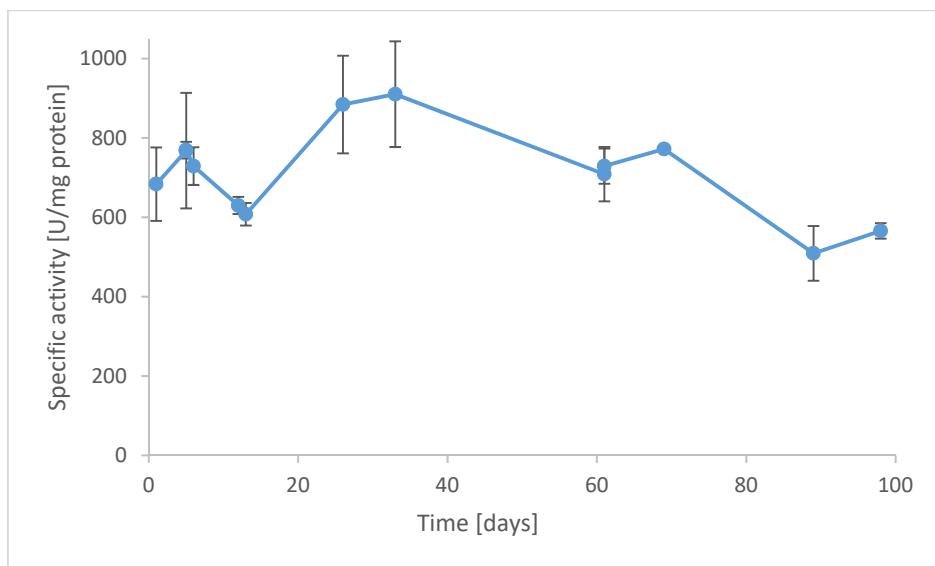


Figure S3. Shelf life of NitEg at pH 8.0 and 4 °C.

A0A165HZA1	MPITKYKAAAVTSEPGWFDLEGGVQKTINFINEAGQAGCKLVAFPEVWI PGYPYWMWKVN	60
Q7RVT0	MVLTKYKAAAVTSEPCWFDFLEGGVRKTIDFINEAGQAGCKLVAFPEVWI PGYPYWMWKVT	60
	* :*****:*****:*****:*****:*****:*****:*****:*****.	
A0A165HZA1	YQQSLPMLKKYRENGLGVNTEEMRRIRRAARDNQIYVSMGFSEIDHATLYLAQVLISPTG	120
Q7RVT0	YQQSLPMLKKYRENAMAVDSDEFRRIRRAARDNQIYVSLGFAEIDHATLYLAQALIDPTG	120
	*****:*****:*****:*****:*****:*****:*****:*****.**.***	
A0A165HZA1	EVINHRRKIKPHTHEVKEVLYVGDGAGDTFLSVTETDIGRLGQLNCWENMNPFLKALNVSAE	180
Q7RVT0	EVINHRRKIKPHTHEVKEVLYVGDGAGDTFMSVTPTEIGRLGQLNCWENMNPFLKSLNVSMGE	180
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****	
A0A165HZA1	QVHVAAWPVYPGKETLKYPDPATNVAAEPASDLVTPAYAIETGTWT LAPFQRLSKEGLKKN	240
Q7RVT0	QIHIAAWPIYPGKETLKYPDPATNVADPASDLVTPAYAIETGTWT LAPFQRLSVEGLKKN	240
	*:*****:*****:*****:*****:*****:*****:*****:*****	
A0A165HZA1	TPEGVEPETDPSTYNGHARI FAPDGTLVVKPDKD FDGLLFVNIDLNECHLT KALADFGGH	300
Q7RVT0	TPEGVEPETDPSTYNGHARI YRPDGSLVVRPDKD FDGLLFVDIDLNECHLT KALADFAGH	300
	*****:*****:*****:*****:*****:*****:*****:*****.**	
A0A165HZA1	YMRPDLIRLLVDTRRKELVTEADPDGGIATYTT RERLGLNLPLAEKEKKGGSSTKKHKG	360
Q7RVT0	YMRPDLIRLLVDTSRKELVTEVDRN GIVQYSTRERLGLNTPLENDKEGKK-----	351
	*****:*****:*****. * :***. * :*****:*****:*****:*****:*****	
A0A165HZA1	KKAGDL 366	
Q7RVT0	-----	

Figure S4. Multiple sequence alignment of NitEg (UniProtKB: A0A165HZA1) and its closest characterized homologue from *Neurospora crassa* (UniProtKB: Q7RVT0) using UniProt Align (<https://www.uniprot.org/align/>). The catalytic residue is Cys (highlighted in red); *identical amino acids; : similar amino acids; . less similar amino acids.

Table S1. Specific activities of cyanide hydratases

Organism	Heterologous expression ¹	Specific activity ²			Reference ³	
		[U/mg dcw]	[U/mg protein]	Cell-free extract	Purified enzyme	
<i>Gloeocercospora sorghi</i>	-	0.044	23.3	555	[22]	
<i>Fusarium lateritium</i>	-	102.5	55.9	1109	[23]	
	+	≈ 600	n.d.	n.d.	[29]	
<i>Fusarium solani</i>	-	1.4	4.6	128	[24]	
<i>Fusarium oxysporum</i>	-	n.d.	85.0	840	[25]	
<i>Pyrenophora tritici</i>	+	n.d.	n.d.	185	[31]	
<i>Botryotinia fuckeliana</i>	+	n.d.	n.d.	100	[31]	
<i>Stereum hirsutum</i>	+	153	n.d.	n.d.	[32]	
<i>Aspergillus niger</i>	+	385	736	1324	[33]	

¹in *Escherichia coli*²substrate KCN³see main text for references

dcw = dry cell weight; n.d. = not determined

Table S2. Purification of NitEg from 200 mL of culture

Sample	Volume [mL]	Protein [mg/ml]	Total protein [mg]	Specific activity [U/mg protein]	Total activity [U]	Purification (fold)	Yield [%]
Cell-free extract	23	9.3±0.1	214±2	280±14	≈ 59,920	-	-
Purified enzyme	1.2	10.3±0.3	12.4±0.4	697±95 ^a	≈ 8,643	≈ 2.5	≈14.4

Activities were determined by picric acid method.

Table S3. Performance of cyanide hydratase and cyanide dihydratase in model mixtures and real effluents

Reaction mixture, fCN concentration, pH	Biocatalyst	Removal (Time)	Reference ¹
Buffer, 0.6 mM, pH 9.0	NitEg ²	100% (10 min)	This work
Buffer, 4.5 mM, pH 9.0		100% (30 min)	
Buffer, 25 mM, pH 9.0		100% (20 min)	
Buffer, 25 mM, pH 9.5		100% (45 min)	
Buffer, 25 mM, pH 10.0		83% (1 h)	
Buffer, 25 mM, pH 10.5		22% (1 h)	
Buffer, 100 mM, pH 9.0		98% (2 h)	
Buffer, 100 mM, pH 8.0	CynH (<i>N. crassa</i>) ²	100% (1 h)	[3]
Buffer, 10 mM, pH 8.0	CynH (<i>A. niger</i>) ²	100% (5 min)	[4]
Coking effluent, simulated, 0.6 mM, pH 9.0	NitEg ^b	100% (1.5 h)	This work
Mine effluent, 528 mM, pH 11	CynD (<i>B. pumilus</i>) immobilized cells	43% (4 h) 98% (4 h)	[2]
Mine effluent, 17.6 mM, -			
Petrochemical effluent, simulated, 4.6 mM, pH 9.1	NitEg ²	96% (45 min)	This work
Cu-plating effluent, simulated, 100 mM, pH 9.0		96% (2 h)	
Ag-plating effluent, simulated, 100 mM, pH 9.0		98 (2 h)	
Cu-plating effluent, diluted, 100 mM, pH 8.0	CynH (<i>N. crassa</i>) ²	≈ 65% (12 h)	[3]
Ag-plating effluent, diluted, 100 mM, pH 8.0		≈ 90 (2 h)	[3]

¹ see main text for references

² purified