Received: 4 June 2022 | Accepted: 4 June 2022

DOI: 10.1111/1462-2920.16097

BURNING QUESTIONS

environmental microbiology sfam

What is the fate of the biofilm matrix?

Joy M. Muthami[1] | **Laura Fernández-García**[1,2] | **María Tomás**[2] | **Thomas K. Wood**[1]

[1]Department of Chemical Engineering, Pennsylvania State University, University Park, Pennsylvania, USA

[2]Microbiology Translational and Multidisciplinary (MicroTM)-Research Institute Biomedical A Coruña (INIBIC) and Microbiology Department of Hospital A Coruña (CHUAC), University of A Coruña (UDC), A Coruña, Spain

Correspondence
Thomas K. Wood, Department of Chemical Engineering, Pennsylvania State University, University Park, PA 16802-4400, USA.
Email: tuw14@psu.edu

Funding information
Fulbright Association; Instituto de Salud Carlos III FEDER; ISCIII-Deputy General Directorate for Evaluation and Promotion of Research - European Regional Development Fund; State Plan for R + D + I 2013–2016, Grant/Award Number: PI19/00878; Xunta de Galicia; Bunton-Waller Assistantship; Pennsylvania State University

Since biofilms clearly are not accumulating, in this opinion piece we hypothesize that the biofilms are used primarily as nutrients; hence, the biofilm matrix does not accumulate in the environment because it provides the carbon, nitrogen, and phosphate building blocks for hungry microorganisms. Given that there are little data to support this hypothesis at present, we propose four scenarios for biofilm matrix recycling.

The biofilm homes of bacteria and archaeal strains cover the Earth and have an economic significance of $5000 billion per year (Cámara et al., 2022); hence, it behoves us to ponder their fate. The building blocks for these homes are known as the matrix, which encompasses over 90% of the dry mass, and consists primarily of extracellular DNA, polysaccharides and protein (Flemming & Wingender, 2010). These homes are formed by free-living microorganisms that adhere to a surface (or one another), form a colony by propagating and by cementing themselves in place, and un-cement themselves to disperse and conquer new territory. But after dispersal, where does the bulk of the biofilm go, i.e. what is the fate of the matrix (Figure 1).

UBIQUITY OF BIOFILMS

Wherever water is in the liquid state, microorganisms will form biofilms (Wood et al., 2011); hence, biofilms literally are everywhere. Biofilm formation was recognized as early as the 1930s (Henrici, 1933), and as much as 80% of the microbial world exists as biofilms (Flemming & Wuertz, 2019). Moreover, biofilm formation in Bacteria and Archaea is an ancient adaptation that occurred at least 3 billion years ago (Hall-Stoodley et al., 2004). The prevalence of biofilms is explained by the recognition that microorganisms are frequently under stress (Song & Wood, 2021) and make biofilms as a response to this stress (Jefferson, 2004; Zhang et al., 2007). Compared to the planktonic lifestyle, their biofilm homes provide protection from predation, phage and environmental insults as well as provide greater opportunities for food and sex (Visnapuu et al., 2022).

GENETICS OF BIOFILM FORMATION

Biofilm formation is elegantly regulated rather than primarily a response to physical phenomena like fluid flow (O'Toole et al., 2000). Hence, gene expression and protein production govern initial attachment, microcolony development and dispersal. For example, a wide range of microorganisms increase the level of the internal signal cyclic diguanylate (c-di-GMP) via diguanylate cyclases to reduce motility as they increase biofilm formation (Boyd & O'Toole, 2012) and decrease c-di-GMP via phosphodiesterases to initiate dispersal (Rumbaugh & Sauer, 2020). Since c-di-GMP levels are enhanced upon surface recognition by cell appendages (Kimkes & Heinemann, 2019), this second messenger is involved at all stages of biofilm formation. Therefore, c-di-GMP concentrations may be manipulated by proteins like BdcA from *Escherichia coli* which triggers biofilm dispersal by decreasing c-di-GMP

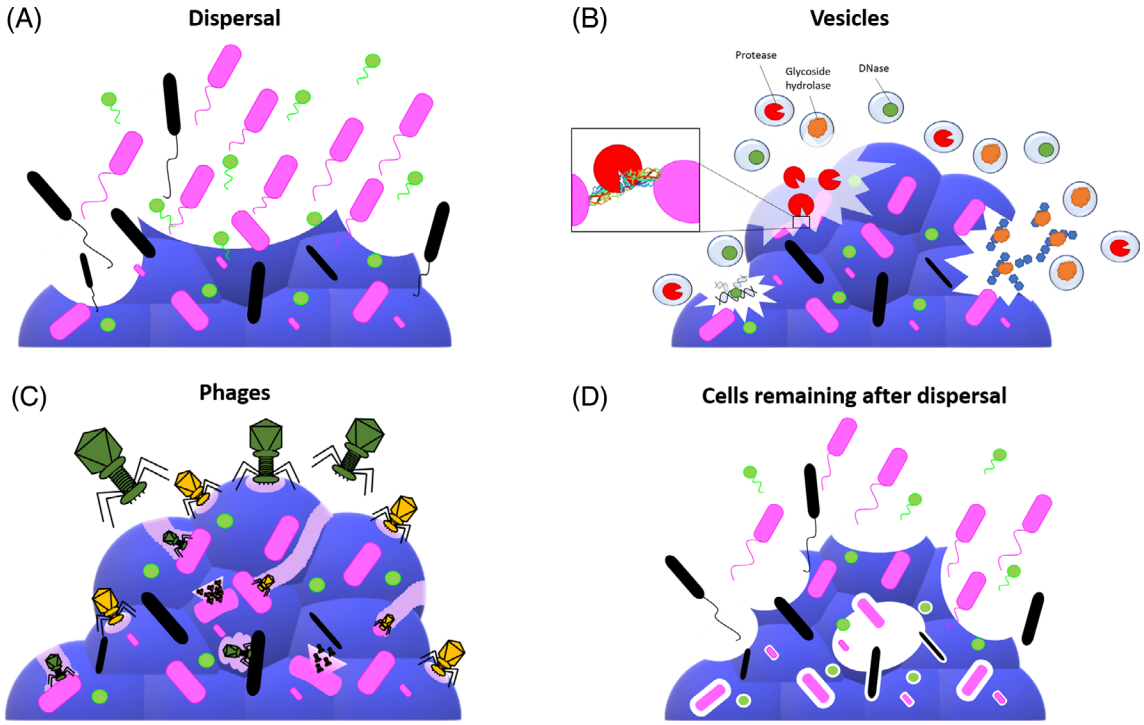

FIGURE 1 Schematic of matrix recycling possibilities. (A) Dispersal destroys the matrix. (B) Vesicles containing matrix-degrading enzymes (e.g. protease, glycoside hydrolase, DNase) recycle biofilm matrices. Representative biofilm protein is FimA from *Escherichia coli* (Protein Data Bank 2M5G), polysaccharides are indicated by hexagon chains, and extracellular DNA is shown as a double helix. (C) Phages recycle biofilm matrices. (D) Biofilm cells digest the matrix that remains after partial dispersal. White indicates areas of where the matrix has been removed and purple indicates remaining matrix. For simplicity, dispersing cells are indicated in pink, black, and green with polar flagella whereas the biofilm cells are shown without flagella

in a wide range of bacteria including *E. coli*, *Pseudomonas aeruginosa* and *Rhizobium meliloti* (Hong et al., 2012; Ma et al., 2011a; Ma et al., 2011b). Most recently, the genetic basis of biofilm formation has been shown to include biofilm maintenance (Katharios-Lanwermeyer et al., 2022), by enzymes such as the tyrosine phosphatase TpbA/diguanylate cyclase TpbB system of *P. aeruginosa* (Pu & Wood, 2010; Ueda & Wood, 2009). Based on this intricate regulation, microorganisms frequently use different matrix components, and the same species may use different ratios and different matrix components depending on its environment (Visnapuu et al., 2022).

DISASSEMBLY OF BIOFILMS

Since there is a genetic basis for biofilm formation, it stands to reason then that there is a genetic basis for biofilm disassembly. Therefore, there should be enzymes used by cells to degrade each matrix building block used by different microorganisms so residents can disperse from their homes. Dispersal is necessary and occurs due to both feast (to expand territory) and famine (to forage) conditions as well as due to environmental stress (e.g. oxygen depletion) (Petrova & Sauer, 2016). Dispersal also occurs as a result of environmental cues, like nitric oxide that activates phosphodiesterases to reduce c-di-GMP (Barraud et al., 2009), rhamnolipids that reduce adhesion between biofilm cells (Zezzi do Valle Gomes & Nitschke, 2012) and trigger a genetic response in sulfate-reducing bacteria (Wood et al., 2018), and *cis*-decenoic acid (Davies & Marques, 2009) that increases motility and metabolism (Rahmani-Badi et al., 2015). Since biofilms are frequently composed of polysaccharides, analogues of polysaccharides may also be used for dispersal (Poosarla et al., 2017).

The enzymes known to degrade biofilm matrices for dispersal include DNases, polysaccharide-degrading enzymes and proteases (Petrova & Sauer, 2016). For degrading matrix extracellular DNA during dispersal, DNases are prevalent as they are found in ocean sediments (Corinaldesi et al., 2007) for both Bacteria and Archaea (Wasmund et al., 2021). For degrading matrix polysaccharides during dispersal, enzymes like dispersin B (β-*N*-acetylglucosaminidase from *Actinobacillus actinomycetemcomitans*) (Kaplan et al., 2003) and the glycoside hydrolase PslG from *P. aeruginosa* (Yu et al., 2015) are widespread in bacterial genomes. For example, the glycoside hydrolase *N*-acetyl-β-D-hexosaminidase DisH was identified as encoded in the genome of the sulfate-reducing bacterium *Desulfovibrio vulgaris* and

then used to disperse its biofilms by degrading the *N*-acetyl β-D-glucosamine in its matrix (Poosarla et al., 2017; Zhu et al., 2018). For degrading matrix proteins during dispersal, specific biofilm proteases like LapG of *Pseudomonas fluorescens* degrade the adhesion LapA (Newell et al., 2011). In conclusion, enzymes to disassemble biofilms are readily available and should exist for every biofilm matrix component, to facilitate biofilm dispersal.

POSSIBLE FATES OF BIOFILM MATRICES

Given that (i) biofilms are everywhere (Flemming & Wuertz, 2019), (ii) most cells in the environment are starving (Schmidt, 2012; Song & Wood, 2021), (iii) biofilms are potentially a good source of the nutrients carbon (e.g. polysaccharides), nitrogen (e.g. protein) and phosphorus (e.g. DNA), (iv) enzymes for matrix degradation are readily available to assist biofilm dispersal (above) and (v) biofilms do not accumulate long-term, it is reasonable to conclude that biofilms may be readily degraded as a source of nutrients. However, there are little data at present to support this hypothesis. Note that the biofilm matrix remains intact without cell activity, at least on the timescale of 1 day (Zrelli et al., 2013).

Cannibalization of the content of dead cells by viable cells that remain in the biofilm has been postulated to occur (Flemming et al., 2016), and cannibalization has been shown for *Bacillus subtilis* cells that produce the biofilm matrix components Eps and TasA (López et al., 2009). Hence, lysed cells may be used to build more biofilm mass, but at present there is little evidence for the use of the matrix itself as a nutrient by surviving or surrounding cells. Similarly, although DNA (10% phosphorus by weight) in oceans could supply 50% of the phosphorus required by procaryotes (Dell'Anno & Danovaro, 2005), and DNA has been shown to be utilized as a carbon and phosphorus source as well as for energy for species such as *Shewanella* spp. (Pinchuk et al., 2008), DNA in the biofilm matrix has not been shown to be directly utilized for nutrients.

If not directly degraded for nutrients, one possibility for the fate of the biofilm matrix is that the matrix is destroyed primarily upon dispersal; hence, there is little mystery as to the long-term fate of biofilms [Figure 1(A)]. For example, as much as 80% of *P. aeruginosa* biofilms may be dispersed by sudden nutrient addition (Sauer et al., 2004). Moreover, since *Pseudoalteromonas distincta* ANT/505 secretes vesicles with polysaccharide-degrading enzymes (Dürwald et al., 2021), another possibility is that vesicles from multiple species, each containing matrix-degrading enzymes, rapidly remove old biofilms [Figure 1(B)]. Also, since phage tail tip proteins, like that of the *Klebsiella pneumoniae* phage RAD2, encode depolymerases to degrade O-glycosidic bonds of polysaccharide components of the biofilm matrix (Dunstan et al., 2021; Visnapuu et al., 2022), and since phages outnumber bacteria 10:1 (Chibani-Chennoufi et al., 2004), phages may recycle biofilm matrices in their Sisyphean propagation that results in 10^{24} infections per second [Figure 1(C)]. Additionally, the biofilm matrix that remains after dispersal may be utilized for nutrients by the remaining cells in the biofilm after partial dispersal or by cells outside the biofilm, with the enzymes used for matrix degradation for nutrients distinct from those used for dispersal [Figure 1(D)], such as extracellular aminopeptidase PaAP from *P. aeruginosa* (Zhao et al., 2018). Also, dead biofilm cells may release polysaccharide matrix-degrading enzymes (Ma et al., 2009), like glycoside hydrolases (Zhao et al., 2018), to provide food for the remaining biofilm cells.

To experimentally test some of these possibilities, reference and environmentally relevant biofilms should be visualized non-destructively over significant timescales (e.g. weeks) to discern the fate of biofilms, perhaps by using lectin-based stains (Poosarla et al., 2017) or DNA-based stains like PicoGreen (Sanchez-Torres et al., 2010). To facilitate this analysis, some isotope-labelled precursors of matrix components could be fed during biofilm formation. In conjunction, cells could be lysed and their debris removed from the biofilm matrix (e.g. via ampicillin for *E. coli*), and the matrix could be tested as a carbon/nitrogen/phosphorus/energy source with various planktonic species. The importance of vesicles and phages for the fate of the biofilm matrix should perhaps be investigated by removing phages and vesicles by filtration. Myriad permutations on these basic themes are possible, of course, but the crux is determining the fate of biofilms is a compelling pursuit.

ACKNOWLEDGEMENTS

This work was supported by funds derived from the Biotechnology Endowed Professorship at the Pennsylvania State University for T.K.W., by a Bunton-Waller Assistantship for J.M.M., by a fellowship from Xunta de Galicia in collaboration with the Fulbright Organization for L.F.-G., by grant PI19/00878 awarded within the State Plan for R + D + I 2013–2016 (National Plan for Scientific Research, Technological Development and Innovation 2008–2011) co-financed by the ISCIII-Deputy General Directorate for Evaluation and Promotion of Research - European Regional Development Fund 'A way of Making Europe' for M.T., and by the Instituto de Salud Carlos III FEDER for M.T.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest and that all data are available.

ORCID

María Tomás https://orcid.org/0000-0003-4501-0387
Thomas K. Wood https://orcid.org/0000-0002-6258-529X

REFERENCES

Barraud, N., Schleheck, D., Klebensberger, J., Webb, J.S., Hassett, D. J., Rice, S.A. et al. (2009) Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic diguanosine-5′-monophosphate levels and enhanced dispersal. *Journal of Bacteriology*, 191, 7333–7342.

Boyd, C.D. & O'Toole, G.A. (2012) Second messenger regulation of biofilm formation: breakthroughs in understanding c-di-GMP effector systems. *Annual Review of Cell and Developmental Biology*, 28, 439–462.

Cámara, M., Green, W., MacPhee, C.E., Rakowska, P.D., Raval, R., Richardson, M.C. et al. (2022) Economic significance of biofilms: a multidisciplinary and cross-sectoral challenge. *npj Biofilms Microbiomes*, 8, 42.

Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.-L. & Brüssow, H. (2004) Phage-host interaction: an ecological perspective. *Journal of Bacteriology*, 186, 3677–3686.

Corinaldesi, C., Dell'Anno, A. & Danovaro, R. (2007) Early diagenesis and trophic role of extracellular DNA in different benthic ecosystems. *Limnology and Oceanography*, 52, 1710–1717.

Davies, D.G. & Marques, C.N.H. (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *Journal of Bacteriology*, 191, 1393–1403.

Dell'Anno, A. & Danovaro, R. (2005) Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science*, 309, 2179.

Dunstan, R.A., Bamert, R.S., Belousoff, M.J., Short, F.L., Barlow, C. K., Pickard, D.J. et al. (2021) Mechanistic insights into the capsule-targeting depolymerase from a *Klebsiella pneumoniae* bacteriophage. *Microbiology Spectrum*, 9, e01023-01021.

Dürwald, A., Zühlke, M.-K., Schlüter, R., Gebbe, R., Bartosik, D., Unfried, F. et al. (2021) Reaching out in anticipation: bacterial membrane extensions represent a permanent investment in polysaccharide sensing and utilization. *Environmental Microbiology*, 23, 3149–3163.

Flemming, H.-C. & Wingender, J. (2010) The biofilm matrix. *Nature Reviews Microbiology*, 8, 623–633.

Flemming, H.-C. & Wuertz, S. (2019) Bacteria and archaea on earth and their abundance in biofilms. *Nature Reviews Microbiology*, 17, 247–260.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A. & Kjelleberg, S. (2016) Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology*, 14, 563–575.

Hall-Stoodley, L., Costerton, J.W. & Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nature Reviews Microbiology*, 2, 95–108.

Henrici, A.T. (1933) Studies of freshwater bacteria. I. A direct microscopic technique. *Journal of Bacteriology*, 25, 277–287.

Hong, S.H., Hegde, M., Kim, J., Wang, X., Jayaraman, A. & Wood, T. K. (2012) Synthetic quorum-sensing circuit to control consortial biofilm formation and dispersal in a microfluidic device. *Nature Communications*, 3, 613.

Jefferson, K.K. (2004) What drives bacteria to produce a biofilm? *FEMS Microbiology Letters*, 236, 163–173.

Kaplan, J.B., Ragunath, C., Ramasubbu, N. & Fine, D.H. (2003) Detachment of *Actinobacillus actinomycetemcomitans* biofilm cells by an endogenous beta-hexosaminidase activity. *Journal of Bacteriology*, 185, 4693–4698.

Katharios-Lanwermeyer, S., O'Toole, G.A. & Galperin, M.Y. (2022) Biofilm maintenance as an active process: evidence that biofilms work hard to stay put. *Journal of Bacteriology*, 204, e00587-00521.

Kimkes, T.E.P. & Heinemann, M. (2019) How bacteria recognise and respond to surface contact. *FEMS Microbiology Reviews*, 44, 106–122.

López, D., Vlamakis, H., Losick, R. & Kolter, R. (2009) Cannibalism enhances biofilm development in *Bacillus subtilis*. *Molecular Microbiology*, 74, 609–618.

Ma, L., Conover, M., Lu, H., Parsek, M.R., Bayles, K., & Wozniak, D.J. (2009) Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. *PLoS Pathogens*, 5, e1000354.

Ma, Q., Zhang, G. & Wood, T.K. (2011a) *Escherichia coli* BdcA controls biofilm dispersal in *Pseudomonas aeruginosa* and *Rhizobium meliloti*. *BMC Research News*, 4, 447.

Ma, Q., Yang, Z., Pu, M., Peti, W. & Wood, T.K. (2011b) Engineering a novel c-di-GMP-binding protein for biofilm dispersal. *Environmental Microbiology*, 13, 631–642.

Newell, P.D., Boyd, C.D., Sondermann, H. & O'Toole, G.A. (2011) A c-di-GMP effector system controls cell adhesion by inside-out signaling and surface protein cleavage. *PLoS Biology*, 9, e1000587.

O'Toole, G., Kaplan, H.B. & Kolter, R. (2000) Biofilm formation as microbial development. *Annual Review of Microbiology*, 54, 49–79.

Petrova, O.E. & Sauer, K. (2016) Escaping the biofilm in more than one way: desorption, detachment or dispersion. *Current Opinion in Microbiology*, 30, 67–78.

Pinchuk, G.E., Ammons, C., Culley, D.E., Li, S.-M.W., McLean, J. S., Romine, M.F. et al. (2008) Utilization of DNA as a sole source of phosphorus, carbon, and energy by *Shewanella* spp.: ecological and physiological implications for dissimilatory metal reduction. *Applied and Environmental Microbiology*, 74, 1198–1208.

Poosarla, V.G., Wood, T.L., Zhu, L., Miller, D.S., Yin, B. & Wood, T.K. (2017) Dispersal and inhibitory roles of mannose, 2-deoxy-d-glucose and N-acetylgalactosaminidase on the biofilm of Desulfovibrio vulgaris. *Environmental Microbiology Reports*, 9, 779–787.

Pu, M. & Wood, T.K. (2010) Tyrosine phosphatase TpbA controls rugose colony formation in *Pseudomonas aeruginosa* by dephosphorylating diguanylate cyclase TpbB. *Biochemical and Biophysical Research Communications*, 402, 351–355.

Rahmani-Badi, A., Sepehr, S., Fallahi, H. & Heidari-Keshel, S. (2015) Dissection of the cis-2-decenoic acid signaling network in *Pseudomonas aeruginosa* using microarray technique. *Frontiers in Microbiology*, 6, 383.

Rumbaugh, K.P. & Sauer, K. (2020) Biofilm dispersion. *Nature Reviews Microbiology*, 18, 571–586.

Sanchez-Torres, V., Maeda, T. & Wood, T.K. (2010) Global regulator H-NS and lipoprotein NlpI influence production of extracellular DNA in *Escherichia coli*. *Biochemical and Biophysical Research Communications*, 401, 197–202.

Sauer, K., Cullen, M.C., Rickard, A.H., Zeef, L.A.H., Davies, D.G. & Gilbert, P. (2004) Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *Journal of Bacteriology*, 186, 7312–7326.

Schmidt, T.M. (2012) Bacteria battling for survival. In: Kolter, R. & Maloy, S. (Eds.) *Microbes and evolution*. Washington, D.C.: American Society of Microbiology, pp. 59–64.

Song, S. & Wood, T.K. (2021) 'Viable but non-culturable cells' are dead. *Environmental Microbiology*, 23, 2335–2338.

Ueda, A. & Wood, T.K. (2009) Connecting quorum sensing, c-di-GMP, Pel polysaccharide, and biofilm formation in *Pseudomonas aeruginosa* through tyrosine phosphatase TpbA (PA3885). *PLOS Pathogens*, 5, e1000483.

Visnapuu, A., Van der Gucht, M., Wagemans, J. & Lavigne, R. (2022) Deconstructing the phage–bacterial biofilm interaction as a basis to establish new antibiofilm strategies. *Viruses*, 14, 1057.

Wasmund, K., Pelikan, C., Schintlmeister, A., Wagner, M., Watzka, M., Richter, A. et al. (2021) Genomic insights into diverse bacterial taxa that degrade extracellular DNA in marine sediments. *Nature Microbiology*, 6, 885–898.

Wood, T.K., Hong, S.H. & Ma, Q. (2011) Engineering biofilm formation and dispersal. *Trends in Biotechnology*, 29, 87–94.

Wood, T.L., Gong, T., Zhu, L., Miller, J., Miller, D.S., Yin, B. et al. (2018) Rhamnolipids from *Pseudomonas aeruginosa* disperse the biofilms of sulfate-reducing bacteria. *npj Biofilms Microbiomes*, 4, 22.

Yu, S., Su, T., Wu, H., Liu, S., Wang, D., Zhao, T. et al. (2015) PslG, a self-produced glycosyl hydrolase, triggers biofilm disassembly by disrupting exopolysaccharide matrix. *Cell Research*, 25, 1352–1367.

Zezzi do Valle Gomes, M. & Nitschke, M. (2012) Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. *Food Control*, 25, 441–447.

Zhang, X.-S., García Contreras, R. & Wood, T.K. (2007) YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity. *Journal of Bacteriology*, 189, 3051–3062.

Zhao, T., Zhang, Y., Wu, H., Wang, D., Chen, Y., Zhu, M.-J. et al. (2018) Extracellular aminopeptidase modulates biofilm development of *Pseudomonas aeruginosa* by affecting matrix exopolysaccharide and bacterial cell death. *Environmental Microbiology Reports*, 10, 583–593.

Zhu, L., Poosarla, V.G., Song, S., Wood, T.L., Miller, D.S., Yin, B. et al. (2018) Glycoside hydrolase DisH from *Desulfovibrio vulgaris* degrades the *N*-acetylgalactosamine component of diverse biofilms. *Environmental Microbiology*, 20, 2026–2037.

Zrelli, K., Galy, O., Latour-Lambert, P., Kirwan, L., Ghigo, J.M., Beloin, C. et al. (2013) Bacterial biofilm mechanical properties persist upon antibiotic treatment and survive cell death. *New Journal of Physics*, 15, 125026.

How to cite this article: Muthami, J.M., Fernández-García, L., Tomás, M. & Wood, T.K. (2022) What is the fate of the biofilm matrix? *Environmental Microbiology*, 1–5. Available from: https://doi.org/10.1111/1462-2920.16097