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1 **Assimilation of hydrocarbons and lipids by means of biofilm formation**

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6
7 **ABSTRACT:** Hydrophobic organic compounds (HOCs) that are used as substrates by
8 bacteria, encompass a great variety of molecules, including contaminants such as
9 hydrocarbons and natural components of the organic matter such as lipids. It is now well
10 known that many bacterial strains use HOCs as carbon and energy sources for growth and
11 form biofilms at the HOCs-water interface that are referred to as oleolytic biofilms. The
12 formation of these biofilms appears to be a strategy to overcome the low accessibility of
13 nearly water-insoluble substrates and is therefore a critical process in the biodegradation of
14 hydrocarbons and lipids. Because oleolytic biofilms develop on a nutritive interface serving
15 as both substratum and substrate, they represent an original facet of biofilm biology.

16

17 **Introduction**

18 Early studies on hydrocarbon biodegradation led to the observation that hydrocarbon-
19 degrading bacteria had high affinity for oil droplets. Phase contrast and electron microscopy
20 examination of *Acinetobacter sp.* growing on *n*-hexadecane revealed hydrocarbon spheres
21 densely covered with bacterial cells and suggested close contact between the cells and oil
22 droplets (Kennedy *et al.*, 1975). Since then, similar observations have been reiterated with
23 various alkane-degrading strains, such as *Rhodococcus sp.* Q15 (Whyte *et al.*, 1999),
24 *Acinetobacter venetianus* RAG-1 (Baldi *et al.*, 1999), *Oleiphilus messinensis* (Golyshin *et al.*,
25 2002), *Pseudomonas* UP-2 (Zilber Kirschner *et al.*, 1980) and *Marinobacter*
26 *hydrocarbonoclasticus* SP17 (figure 1) (Klein *et al.*, 2008). Bacterial attachment to polycyclic
27 aromatic hydrocarbons (PAHs) has also been described for *Pseudomonas* spp. (Eriksson *et*

28 *al.*, 2002; Mulder *et al.*, 1998), *Sphingomonas sp.* CHY-1 (Willison, 2004) and
29 *Mycobacterium frederiksbergense* LB501T (Bastiaens *et al.*, 2000). In biphasic culture
30 medium containing a poorly water-soluble substrate and the aqueous phase, substrate-bound
31 cells often coexist with cells floating freely in the aqueous phase. Although the presence of
32 hydrocarbon-bound cells at the interface assumes interfacial growth, demonstration of actual
33 substrate degradation and growth of the attached cells has been provided in only a few cases
34 (Efroymsen and Alexander, 1991; Wick *et al.*, 2003; Zilber Kirschner *et al.*, 1980).

35 The sessile mode of life and the multilayered structure of cell populations growing at the
36 interface between hydrophobic organic compounds (HOCs) and water are reminiscent of
37 biofilms. During the last two decades, biofilms have been the subject of extensive
38 investigations. Most of our knowledge about the molecular biology of biofilms has been
39 derived from model strains such as *Pseudomonas aeruginosa* and *Escherichia coli*. This
40 research has revealed that biofilms are much more than the simple accretion of cells attached
41 to an interface. Biofilms are heterogeneous, highly organized structures possessing an
42 architecture that is essential to their functioning. Biofilm growth follows a stepwise pattern
43 involving differentiation and collective behavior of cells (Stewart and Franklin, 2008; Webb
44 *et al.*, 2003). Molecular studies of oleolytic biofilms growing at hydrophobic interfaces have
45 not yet gone far enough to conclude whether they share all the characteristics of extensively
46 studied model biofilms. However, properties characteristic of the biofilm lifestyle have been
47 identified. CSLM (Confocal Scanning Laser Microscopy) observation of a biofilm community
48 developing at polychlorinated biphenyl-water interface provided evidence of a stepwise
49 development pattern of the biofilm (Macedo *et al.*, 2005). Transcriptomic and proteomic
50 studies indicated that cells growing at the alkane-water interface experienced a profound
51 reshaping of their gene expression profile (Mounier *et al.*, 2014; Vaysse *et al.*, 2009; Vaysse
52 *et al.*, 2010). Extracellular polymeric substances (EPS), polysaccharides, DNA and proteins

53 have been detected during growth at the alkane-water interface, indicating production of an
54 extracellular matrix, which is a typical trait of biofilms (Ennouri *et al.*, 2017; Whyte *et al.*,
55 1999).

56 In this chapter, biofilms on HOCs refer to multilayered, matrix-embedded bacteria or bacterial
57 communities growing at the HOCs–water interface and using these compounds as a substrate.

58 In such biofilms, the energy and the carbon, which fuel bacterial growth, are provided by the
59 degradation of the substrate, which thus constitutes a nutritive interface serving as both
60 substrate and substratum.

61

62 **Multispecies biofilms on hydrophobic interface**

63 During enrichment procedures on hydrocarbons, microbiologists have very often observed
64 multispecies biofilms developing at the hydrocarbon–water interface. For example, Deppe *et*
65 *al.* (2005) have observed by phase contrast microscopy oil droplets covered by a biofilm
66 during isolation of a consortium enriched on crude oil from Arctic sea ice and seawater from
67 Spitzbergen. Unfortunately, such observations received little attention and in consequence
68 they are not always mentioned in the literature and are rarely fully documented, making it
69 difficult to measure the occurrence of these biofilms among bacterial species. Stach *et al.*
70 (2002) carried out a study devoted to the diversity of biofilm communities developing on
71 PAHs. Biofilms developing on naphthalene- and phenanthrene-coated flow cells were isolated
72 and their diversity compared with planktonic cultures enriched on the same hydrocarbons.
73 The biofilm system showed a three times higher diversity of cultivable bacteria as compared
74 to the enrichment culture. Molecular approaches revealed that the biofilm community
75 contained a greater diversity of active species and of PAH-degradation genes than the
76 planktonic enrichment community. The diversity of active species found in the biofilm closely
77 matched the diversity found in the PAH-contaminated soil used as inoculum. This study

78 demonstrates that biofilm cultures represent a means to obtain PAH-degrading communities
79 closely related to environmental situations suggesting that biofilm formation on hydrocarbons
80 is a likely lifestyle in natural ecosystems. The existence of biofilms at oil-water interfaces in
81 natural environments has been reported during the Deepwater Horizon oil spill where
82 bacterial flocs were observed in the oil plume. Synchrotron radiation-based Fourier-transform
83 infrared-spectra of these flocs indicated that their formation occurred on the surface of oil
84 droplets and revealed the presence of oil-degradation products, polysaccharides and proteins
85 (Baelum *et al.*, 2012; Hazen *et al.*, 2010).

86

87 **Cell adhesion to hydrophobic compounds**

88 In order to either develop a biofilm or to grow as single cell layers at the interface, bacteria
89 must first approach and then adhere to the interface. Bacteria can reach surfaces by passive
90 diffusion, random swimming, or taxis that is a directed motility in response to gradients of
91 chemical and physical stimuli. Chemotaxis has been observed in response to single ring
92 aromatic hydrocarbons, naphthalene, and hexadecane. Regrettably, no experiment designed to
93 determine whether this behavior led to biofilm formation at the interface between water and
94 hydrocarbons has been conducted (Lanfranconi *et al.*, 2003; Pandey and Jain, 2002). To date,
95 random mobility like swimming has never been shown to play a role in adhesion to HOCs.
96 Once cells have reached the interface, the initial adhesion step is a purely physicochemical
97 process described by the traditional and extended DLVO theories of colloidal stability, which
98 describes contact of cells to surfaces as the result of van der Waals interactions, Lewis acid-
99 base interactions, and electrostatic interactions (Hermansson, 1999). The intensity of these
100 interactions and hence the effectiveness of the binding depend on the cell surface properties
101 (hydrophobicity, charge, roughness, etc...) as well as interface properties. This means that
102 only bacteria exhibiting the proper surface properties will adhere on hydrophobic surfaces (for

103 a detailed review of bacterial adhesion to hydrocarbon, see Abbasnezhad *et al.*, 2011). In
104 contrast to adhesion to biotic surfaces or insoluble polysaccharides like chitin or cellulose, no
105 specific adhesins or receptors recognizing hydrocarbons or lipids have been identified so far.
106 In many cases, adhered cells exhibit surface properties that are different from their soluble
107 substrate-grown counterparts. These alterations of the cell surface are thought to strengthen
108 adhesion after the initial interaction with the interface. For example, anthracene-grown cells
109 of *Mycobacterium sp.* LB501T are more hydrophobic and more negatively charged than
110 glucose-grown cells (Wick *et al.*, 2002). Changes in cell surface can be achieved by
111 modification, production, or removal of their surface molecules. In Gram-positive bacteria,
112 the presence and the chain length of mycolic acids are correlated with hydrophobicity and
113 adherence (Bendinger *et al.*, 1993). Lipopolysaccharides are important determinants of cell
114 surface properties in Gram-negative bacteria. Their chain length variation or their removal
115 from the cell surface has been shown to be important for interacting with hydrocarbons (Al-
116 Tahhan *et al.*, 2000). Capsular polysaccharides are another class of surface molecules playing
117 a role in adhesion to hydrophobic compounds (Baldi *et al.*, 1999).

118 Adhesion to hydrophobic surfaces is also mediated by extracellular appendages such as
119 fimbriae and pili. The importance of fimbriae in adherence to *n*-hexadecane was demonstrated
120 by the isolation of a non-adherent mutant of *Acinetobacter calcoaceticus* RAG-1, which was
121 devoid of fimbriae and defective for growth on hydrocarbons. The reappearance of fimbriae
122 in adherent revertants was a strong argument in favor of the involvement of fimbriae in
123 adhesion to hexadecane (Rosenberg *et al.*, 1982). The strains *Acinetobacter haemolyticus* AR-
124 46 and *Acinetobacter sp.* Tol 5 produce pili at their cell surfaces when grown on *n*-
125 hexadecane or triglycerides, respectively. Although the function of these organelles has not
126 been elucidated it was presumed that they play a role during the adhesion to the hydrophobic
127 substrate (Bihari *et al.*, 2007; Katsutoshi *et al.*, 2011). Changes in surface properties of cells

128 grown on hydrophobic substrates and inhibition of adhesion in the presence of soluble
129 substrates indicate that the process of adhesion is regulated and that cells are able to respond
130 to contact with hydrophobic interfaces.

131

132 **Regulation and determinism of biofilm formation at the HOC-water interfaces**

133 In biofilms growing on an inert substratum, all nutrients are supplied through the aqueous
134 phase. In biofilms on HOCs, the situation is very different. These biofilms develop in a
135 biphasic medium where the electron donor is provided by the non-aqueous phase and the
136 electron acceptor (*e.g.*, oxygen) is available from the aqueous phase. It results in a geometry
137 in which one side of the biofilm is in close association with the electron source, while the
138 other contacts the source of the electron acceptor. Thus, cells within the biofilm experience
139 two opposite gradients of acceptor and donor of electron generated by their simultaneous
140 diffusion and consumption. These microscale chemical gradients presumably contribute to the
141 physiological heterogeneity in the biofilm and exert a control on its development. The
142 experiment carried out by Joannis-Cassan *et al.* (2005) demonstrated that biofilm growth on
143 hydrocarbons can be limited either by carbon or by oxygen depletion. The authors studied
144 biofilm growth in a liquid–liquid system consisting of an emulsion obtained by stirring
145 dodecane in mineral medium. Biofilm growth occurred at the surface of a dodecane droplet.
146 During growth, the droplet diameter was reduced from 200 μm to 160 μm . Biofilm growth
147 ceased when it reached a maximum thickness of about 80 μm . A series of experiments
148 demonstrated that inhibition of growth was caused by the diffusion limitation of both
149 dodecane and oxygen within the biofilm but not by others factors such as nutrient exhaustion
150 or product inhibition (Joannis-Cassan *et al.*, 2005).

151 Although adhesion to hydrocarbons does not seem to depend on the recognition of molecular
152 structures, many HOC-degrading bacteria show a preference or specificity to the surface of

153 metabolizable hydrocarbons or lipids for biofilm formation (Johnsen and Karlson, 2004;
154 Klein *et al.*, 2008; Rodrigues *et al.*, 2005). The substrate/substratum specificity of biofilm on
155 hydrocarbons is certainly one remarkable feature that distinguishes them from other biofilms.
156 Biofilm formation tends to occur preferentially on less-soluble substrates and seems to be
157 regulated in function of substrate availability. Screening for biofilm formation capacity by
158 isolated PAHs-degrading strains showed that the majority of the tested strains formed biofilm
159 in microtiter wells coated with PAH crystals. For strains capable of growing on different
160 PAHs, it was observed that the percentage of adhering cells decreased with the solubility of
161 the PAHs, indicating that aqueous solubility of the substrate exerts a regulation on biofilm
162 development (Johnsen and Karlson, 2004). *Pseudomonas putida* ATCC 17514 exhibits
163 different growth patterns depending on the PAH properties on which it is feeding. CSLM
164 observation of a gfp-labeled derivative of this strain showed that growth on phenanthrene
165 occurred by forming a biofilm at the crystal surface, while on fluorene, which is more soluble
166 than phenanthrene, *P. putida* grew randomly between the crystals feeding on dissolved PAH
167 (Rodrigues *et al.*, 2005). Insoluble substrate preference for biofilm formation has also been
168 observed in *M. hydrocarbonoclasticus* SP17. This bacterium forms biofilms on a variety of
169 HOCs, including *n*-alkanes, wax esters, and triglycerides, but is unable to form biofilm (in
170 presence of acetate as substrate) on non-metabolizable alkanes (branched alkanes and *n*-
171 alkanes with more than 28 carbon atoms) and forms only weak biofilms on polystyrene with
172 10 times less biomass than on paraffin (Ennouri *et al.*, 2017; Klein *et al.*, 2008). The
173 preference for insoluble substrates suggests that bacteria forming biofilms on HOCs are able
174 to detect and recognize nutritive interfaces. It is reasonable to anticipate that control of
175 biofilm formation by substrate/substratum is exerted through a signal transduction pathway
176 and genetic regulatory mechanisms. Indeed, induction of genes at an interface was
177 demonstrated for the *pra* gene encoding the PA protein, an alkane inducible extracellular

178 protein exhibiting an emulsifying activity involved in hexadecane assimilation, and the *rhlR*
179 gene coding for the transcriptional activator of rhamnolipids biosynthesis. Studies with liquid
180 cultures on hexadecane of *P. aeruginosa* harboring a *pra::gfp* or *rhlR::gfp* fusion revealed
181 specific transcriptional activity at the *n*-hexadecane–water interface (Holden *et al.*, 2002).

182

183 **Biofilm formation as an adaptive response to optimize acquisition of insoluble HOCs**

184 The first intuitive indications that biofilms could favor access to poorly soluble HOCs came
185 from the observations that biofilm formation occurs in function of substrate solubility since it
186 was shown that the more insoluble the substrate is, the more growth occurs at the water–HOC
187 interface. Moreover, it was observed that several strains growing at the interface between
188 nearly insoluble hydrocarbons and water do not release emulsifier or surface-active
189 compounds into the bulk medium (Bouchez *et al.*, 1997; Bouchez-Naïtali *et al.*, 1999;
190 Bouchez-Naïtali *et al.*, 2001; Klein *et al.*, 2008; Wick *et al.*, 2002). In these cases, cells do not
191 access the substrate by surfactant-mediated transfer, during which cells contact emulsified,
192 solubilized or pseudo solubilized hydrocarbons. Access to the insoluble substrate rather
193 occurs by direct contact of the cells or extracellular structures with the hydrocarbon–water
194 interface. Rosenberg demonstrated the importance of adhesion to hydrocarbons in the growth
195 of *Acinetobacter calcoaceticus* RAG-1 on hexadecane in absence of any emulsifier
196 (Rosenberg and Rosenberg, 1981). Thus, biofilm formation and adhesion to hydrocarbons
197 would promote growth on hydrocarbons by facilitating interfacial access. The strongest
198 evidence of an increase in access to HOCs by adhesion or biofilm formation arose from
199 kinetic studies showing that growth at the interface occurs faster than the mass transfer rate of
200 HOCs in the absence of bacteria would suggest (Bouchez-Naïtali *et al.*, 2001; Calvillo and
201 Alexander, 1996; Harms and Zehnder, 1995; Wick *et al.*, 2002).

202 Mechanisms employed in biofilms for accessing HOCs are still poorly understood. On the one
203 hand, it is not difficult to imagine that biofilms offer a way to optimize the effect of known
204 mechanisms of acquisition of poorly soluble hydrophobic substrates. Surfactant production
205 within a biofilm would limit its dilution in the bulk phase, facilitating the formation of
206 micelles by keeping the concentration of the surfactant close to the critical micelle
207 concentration (CMC). Biofilms also offer the advantage of holding the cell population in the
208 vicinity of the HOC–water interface thus stimulating the mass transfer of HOCs by shortening
209 the diffusive pathway (Wick *et al.*, 2002). On the other hand, biofilm lifestyle might offer
210 possibilities of biofilm-specific mechanisms for HOCs accession. Biofilms are typically
211 characterized by dense cell clusters embedded in extracellular polymeric substances. The
212 formation of such structures involves profound changes in cell physiology and behavior
213 requiring regulation of the expression of hundreds of genes. Such changes in cellular
214 physiology has been indeed revealed by transcriptomic and proteomic studies on biofilms of
215 *M. hydrocarbonoclasticus* growing at HOCs-water interfaces (Mounier *et al.*, 2014; Vaysse *et*
216 *al.*, 2009; Vaysse *et al.*, 2010). The transition from the planktonic to the biofilm mode on
217 HOCs entails change in the expression level of more than one thousand genes . Although
218 most of these genes are of unknown function some of them are involved in cellular processes
219 like lipid, alkanes and central metabolisms, chemotaxis, motility, transport and protein
220 secretion. In view of such a reshaping of cellular functions and structural organization, the
221 existence of biofilm-specific mechanisms for HOCs accession is conceivable.

222 For instance, biofilms can improve the assimilation of HOCs through their extracellular
223 matrix. Many functions currently attributed to the biofilm matrix, *e.g.* adhesion to surfaces
224 and retention of enzymes and metabolites, can have implications in the assimilation of HOCs
225 (Flemming and Wingender, 2010). The retention properties of the matrix could maintain exo-
226 products in the vicinity of cells and prevent their loss in the bulk medium. The action of

227 biosurfactants, which has been demonstrated to improve assimilation of hydrocarbons in some
228 cases (Perfumo *et al.*, 2010), could be greatly increased within a matrix by enabling their
229 accumulation up to the CMC and thus allow micellar transport of hydrocarbons (Guha and
230 Jaffé, 1996). In addition, EPS of the biofilm matrix may act as sorbents or emulsifiers that
231 could stimulate the mass transfer rate of HOCs (Harms *et al.*, 2010). Various strains of
232 *Acinetobacter sp.* produce extracellular complexes of polysaccharides or lipo-polysaccharides
233 and proteins called bio-emulsans that have the capacity to emulsify and increase the solubility
234 of hydrocarbons (Barkay *et al.*, 1999; for reviews see Ron and Rosenberg, 2002; Ron and
235 Rosenberg, 2010). Although the emulsifying activities were not localized within a biofilm
236 matrix, these results indicate that biopolymers could increase mass transfer rates of
237 hydrocarbons and hence stimulate their biodegradation. Direct interactions between EPS and
238 hydrocarbons have been evidenced in *A. venetianus* VE-C cells growing on diesel fuel where
239 nano-droplets incorporated in an extracellular matrix containing glyco-conjugates were
240 observed (Baldi *et al.*, 1999; Baldi *et al.*, 2003). Similarly, oil droplets were completely
241 covered with cells and EPS in a culture of *Rhodococcus sp.* strain Q15 on diesel fuel (Whyte
242 *et al.*, 1999). In both cases, EPS mediated the adhesion of cells to hydrocarbons and are
243 thought to participate in the uptake of hydrocarbons, although the mechanisms involved
244 remain unknown. Biofilm EPS can also serve as an adsorbent to store HOCs and allow their
245 subsequent utilization by the biofilm community (Wolfaardt *et al.*, 1995).

246 Biofilm matrices comprise extracellular proteins with various functions such as hydrolytic
247 enzymes or adhesion (Flemming and Wingender, 2010). The involvement of extracellular
248 proteins in alkane utilization was first evidenced in *A. calcoaceticus* ADP1 as it was shown
249 that a Type 2 Secretion System (T2SS) mutant showed reduced growth on alkanes (Parche *et*
250 *al.*, 1997). The OmpA-like AlnA protein of *A. radioresistens* KA53 and the PA protein from
251 *P. aeruginosa* PG201 and S7B1 are two extracellular proteins that have been shown to play a

252 role in alkane utilization in planktonic culture (Hardegger *et al.*, 1994; Kenichi *et al.*, 1977).
253 These two proteins exhibit emulsifying properties that have been claimed to be the basis of
254 their function in alkane assimilation although no clear cause-to-effect relationship has been
255 established. During biofilm development on alkanes or triglycerides *M.*
256 *hydrocarbonoclasticus* SP17 uses cytoplasmic proteins released by cell lysis and proteins
257 secreted through the T2SS to form a proteinaceous matrix (Ennouri *et al.*, 2017). It was
258 hypothesized that the surface activity of proteins could be exploited in oleolytic biofilm
259 matrices to form a conditioning film at the HOC-water interface that could promote cell
260 adhesion and colonization. Matrix proteins could also participate in the mass transfer of
261 HOCs to the biofilm cells by forming micelles or acting as mobile sorbents.

262

263 **Research Needs**

264 The most exciting aspect of biofilms on HOCs is certainly to identify the features that
265 distinguish them from other biofilms, that is to say, their substrate/substratum specificity and
266 their capacity to overcome the low accessibility of a hydrophobic substrate. These two
267 properties make biofilm formation a very efficient adaptive strategy to assimilate HOCs,
268 which can provide a serious advantage in environments where carbon sources are scarce. The
269 processes by which biofilms stimulate interfacial accession to nearly insoluble substrates
270 remain to be elucidated. Substrate specificity of biofilm formation for HOCs surfaces
271 presumably involves surface sensing and signal transduction pathways, which have not been
272 revealed yet. Biofilm development during the assimilation of HOCs most likely requires
273 coordination of fundamental processes such as architectural biofilm organization, physico-
274 chemical interactions between biofilm and substrate, and the control of gene expression.
275 Investigation of these processes will require multidisciplinary approaches aimed at (1)
276 identifying the genes/proteins involved in biofilm formation, (2) deciphering the architecture

277 of biofilms, and (3) characterizing at the physico-chemical level the interactions between
278 biofilm components (cells and extracellular matrix) with hydrophobic substrates. So far,
279 investigations on biofilms on HOCs have been conducted on different strains growing on
280 various substrates in diverse experimental setups. It was therefore not possible to correlate
281 these results in order to draw a picture of the functioning of these biofilms. The study of
282 additional?? model bacteria, chosen for their ability to form readily and reproducibly biofilms
283 on HOCs, their genetic amenability, and the availability of their genome sequence would
284 ensure the complementarity of the data obtained from multidisciplinary approaches. In
285 addition to studies examining model single species biofilms on HOCs, investigations of the
286 activities and biodiversity of multispecies biofilms isolated from samples collected from
287 various environments are critical to gain full understanding of the ecological significance of
288 these biofilms.

289 Due to their wide distribution in the environment, their recalcitrance, and their deleterious
290 effect on human health, hydrocarbons have been the main molecules used in studies of
291 biofilm formation on HOCs. However, other classes of HOCs should also be taken into
292 consideration. In the natural environment, lipids represent a very abundant class of HOCs. For
293 example in sea water they represent up to about 15 % of the organic carbon and its
294 biodegradation is relevant to the global carbon cycle (Lee *et al.*, 2004). Consistent with this,
295 some bacterial strains isolated for their hydrocarbon-degrading capacities also form biofilms
296 on a larger panel of HOCs. This suggests that HOCs-degrading bacteria may have the ability
297 to form oleolytic biofilms that can cope with several types of hydrophobic organic carbon
298 they may encounter in the environment by adapting their physiology according to the
299 chemical nature of the HOC.

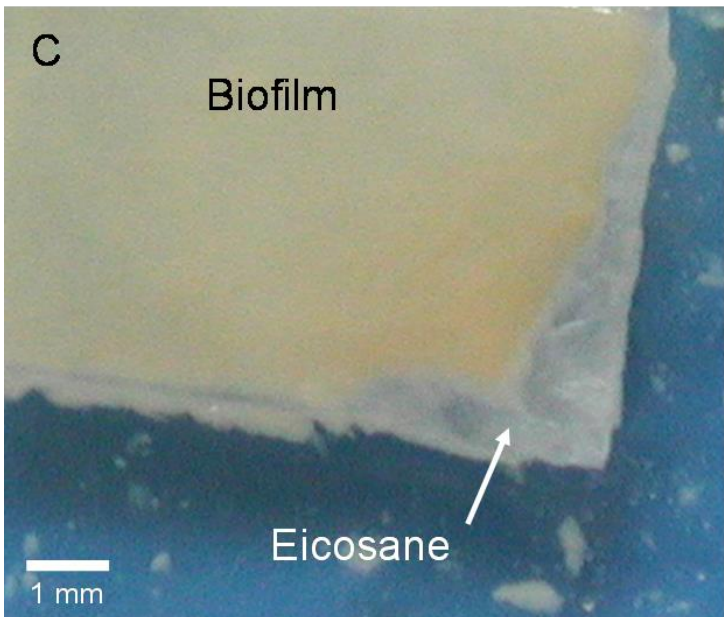
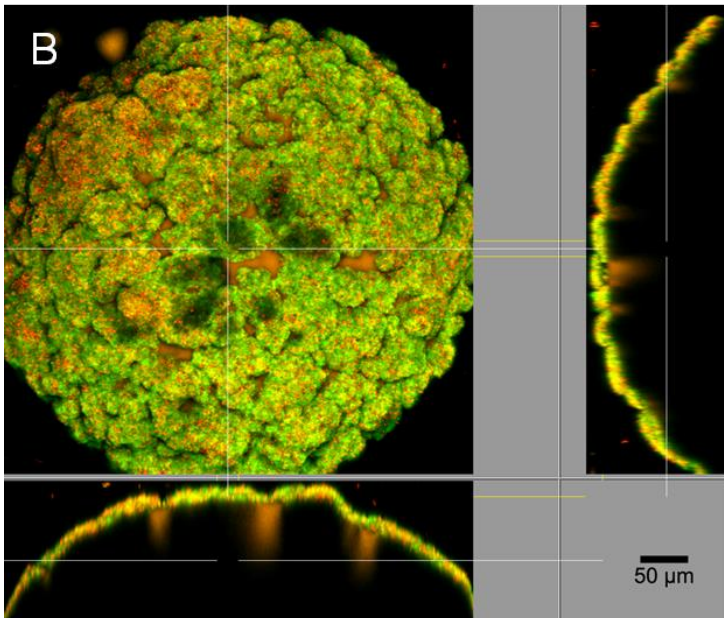
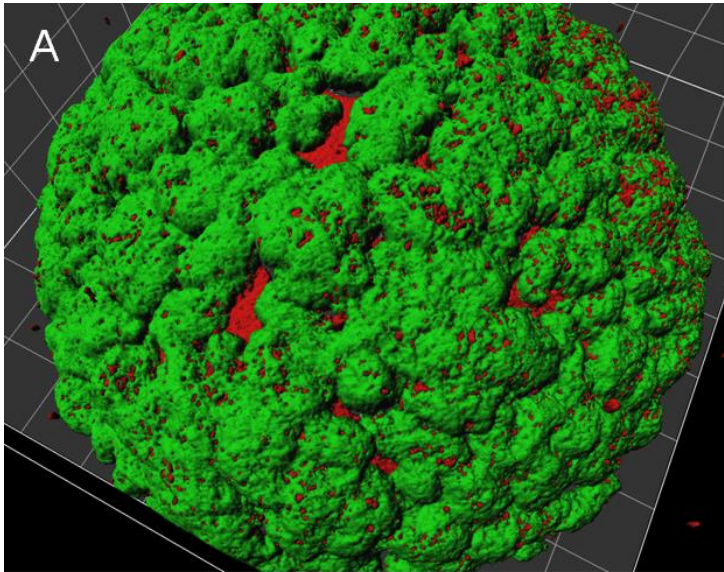
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302 **Figure 1**

303 *M. hydrocarbonoclasticus* SP17 biofilms growing on alkanes.

304 A and B, confocal scanning laser microscopy images of a biofilm covered *n*-hexadecane
305 droplet. Hydrophobic regions including bacteria as well as hydrocarbon were stained with red
306 Nile (red signal), glycoconjugates were stained with PSA lectin (green signal). In A, the data
307 are presented as an isosurface projection where the two signals have been split. In B, the
308 dataset is presented as an XYZ projection. The two signals were not separated, colocalized
309 signals of the green and red channel appear in yellow indicating the colocalization of Nile red
310 and lectin stain. Images courtesy by Pierre-Jo Vaysse and Thomas R. Neu (Helmholtz Centre
311 for Environmental Research - UFZ, Magdeburg, Germany). C, picture of *M.*
312 *hydrocarbonoclasticus* SP17 biofilm growing at the surface of solid eicosane.



314

315

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