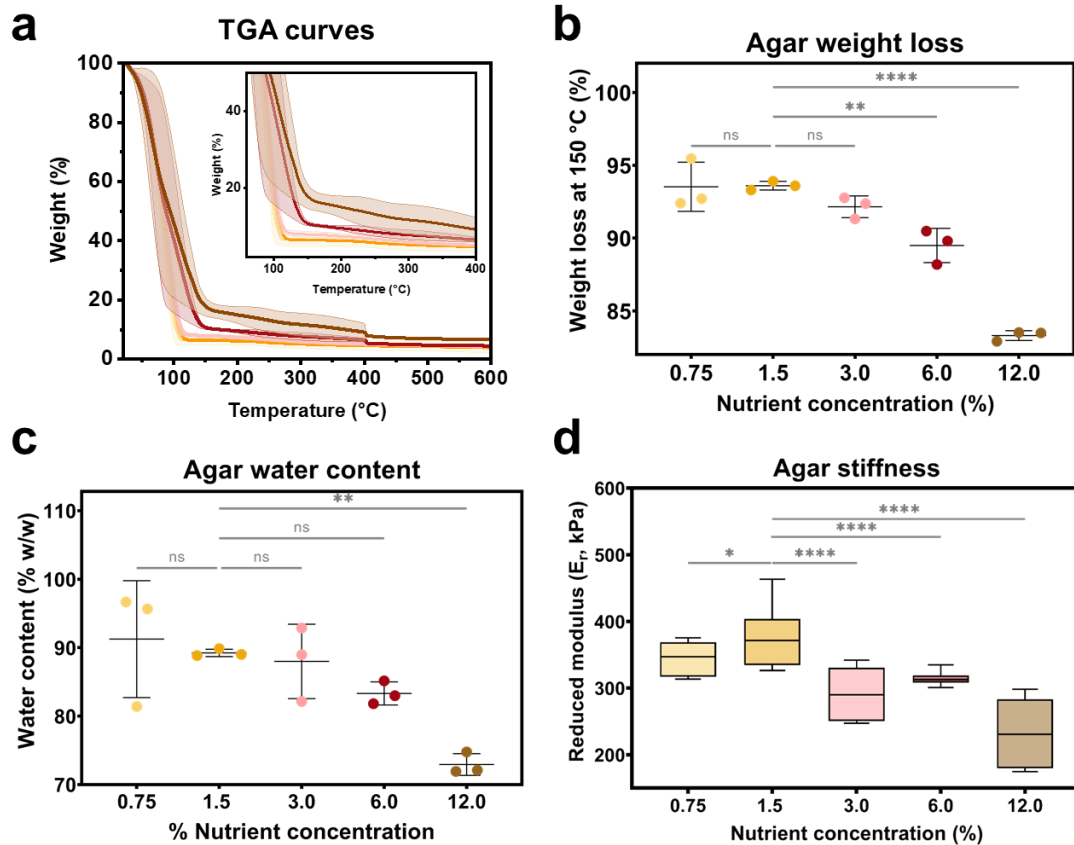


1. Salt-free agar characterization



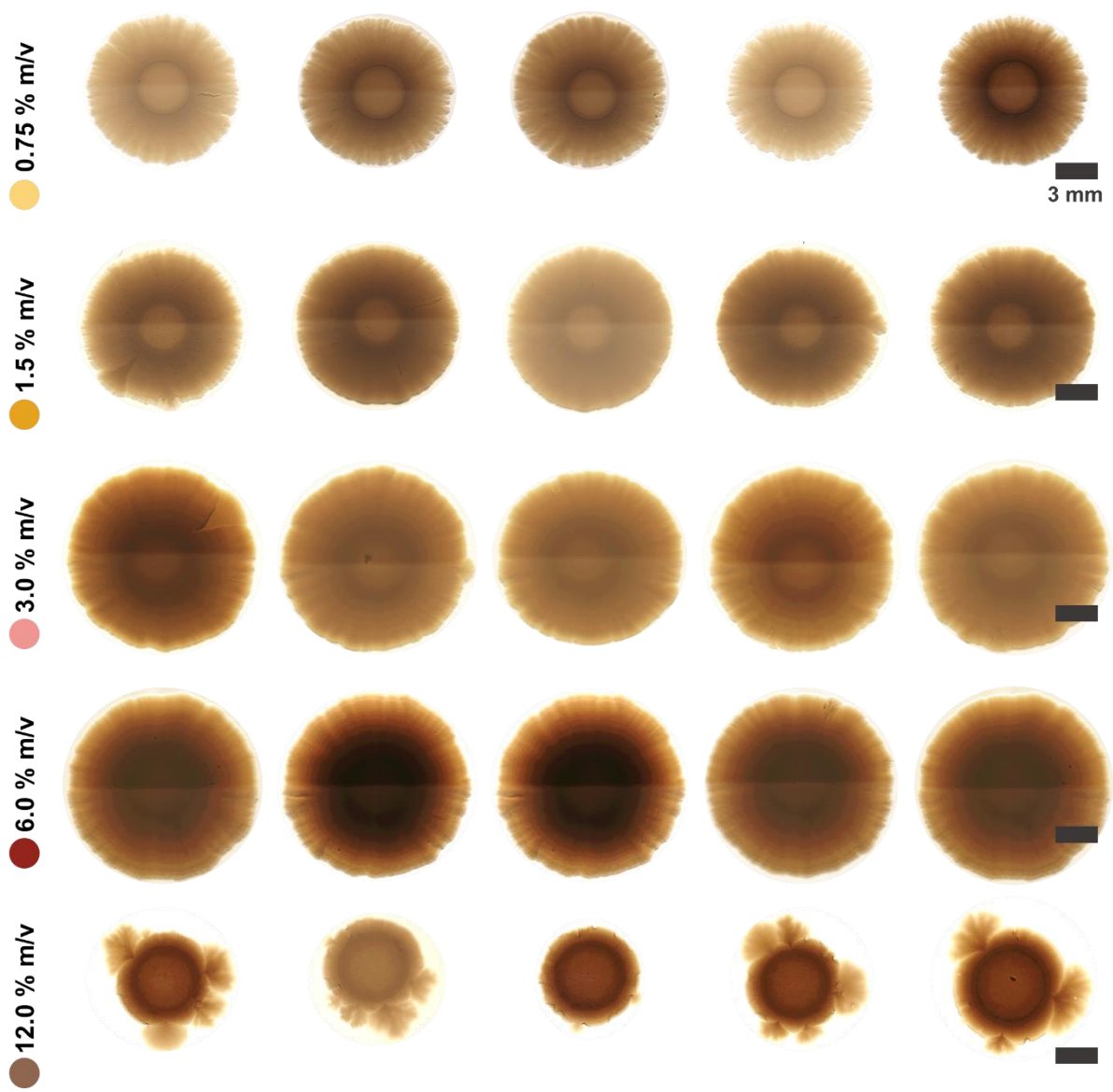
Supplementary Figure 1 Agar substrate properties. (a) Average Thermal Gravimetric Analysis (TGA) curves of each agar substrate between 90 and 600 °C. Inset is a zoom between 60 – 400 °C. N = 3. (b) Percentage of weight loss representing water loss at 150 °C for each agar substrate. (c) Water content of the agar substrates. N=3. The statistical analysis was done with One-way ANOVA ($p < 0.0001$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % w/v nutrient concentration condition was used as reference for the post-test multicomparisons. (d) Mechanical properties (stiffness) of the different agar substrates studied. N=14 microindentation curves of 2-3 agar substrates per condition. The number of individual measurements is $n = 10-23$ per condition. The statistical analysis was done with Man Whitney U test ($p < 0.00=1$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % nutrient concentration condition was used as reference for the post-test multicomparisons.

We estimated the substrates (agar) free water content in every condition by carrying out thermal gravimetric analysis (TGA) (**Supplementary Figure 1a**). We tested the behavior of the substrates from 25 to 600 °C. The results show that the higher the nutrient concentration in the agar substrate, the lower content of free water is in the substrate (**Supplementary Figure 1a**). The loss of weakly linked water (Van der Waal forces) is indicated by a sharp loss of mass between 100 and 150 °C depending of the substrate. For substrates with low nutrient concentration, a lower temperature is needed to release weakly linked water compared to high nutrient concentration substrates. The total weight loss at 150 °C is correspond to the mass free water released (**Supplementary Figure 1b**).¹ Substrates with low nutrient concentration (0.75, 1.5 and 3.0 % w/v) lose ~90 % of their weight, while substrates with high nutrient concentration

1 (6.0 and 12.0 % w/v) lose 83 and 73 % of their weight respectively. We also analyzed the water
2 content of the salt-free LB-agar substrates by dehydration (**Supplementary Figure 1c**). Low
3 nutrient concentration substrates (0.75 and 1.5 % w/v) have 91 and 89 w/w % of water,
4 respectively. Higher nutrient concentration substrates (3.0, 6.0 and 12.0 % w/v) have 87, 83 and
5 73 % w/w of water.
6 Analyzing the mechanical properties of the agar substrates revealed that the higher their
7 nutrient content, the lower their stiffness (**Supplementary Figure 1d-e**).
8

1

2. Biofilm morphology



2

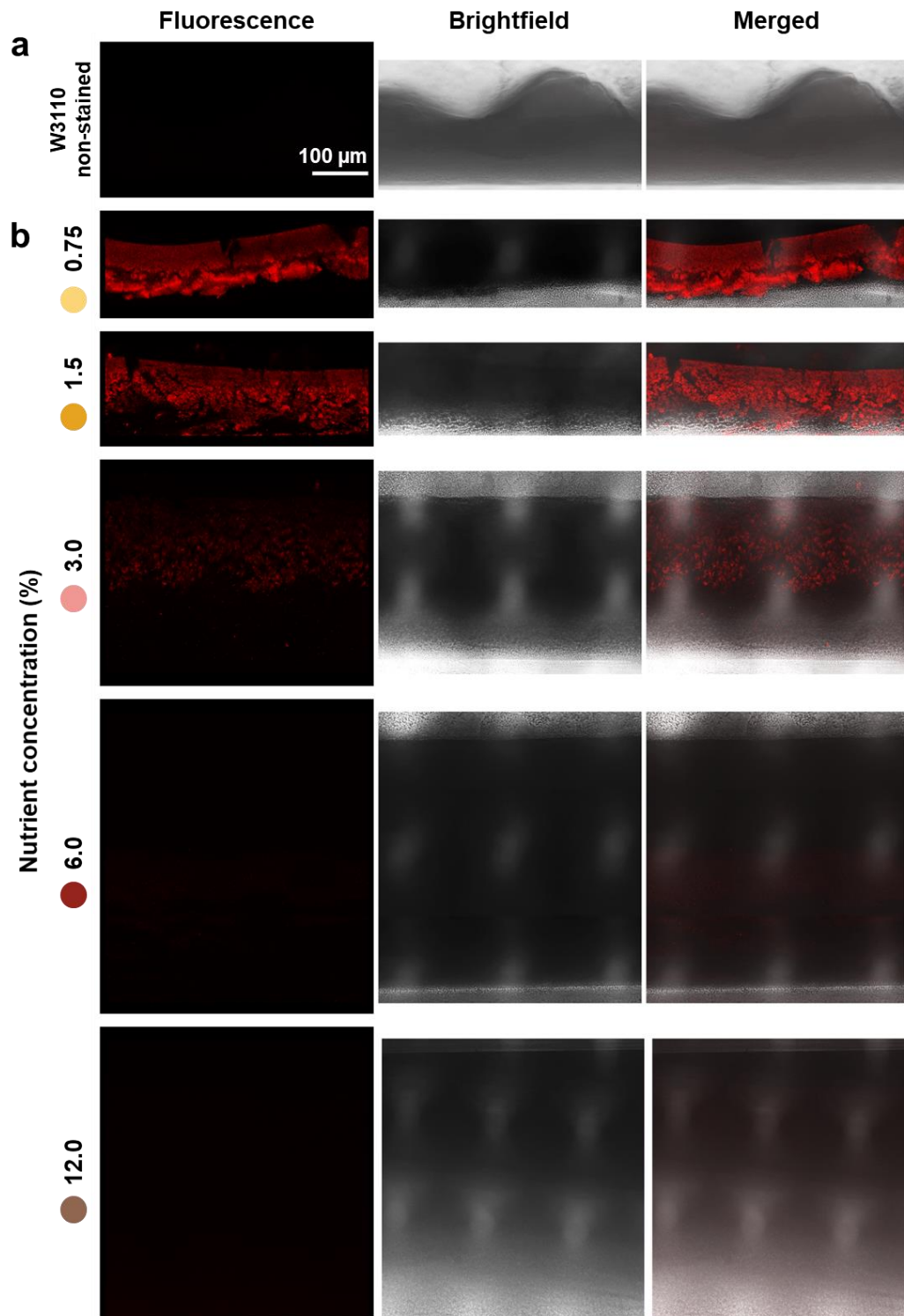
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Supplementary Figure 2 Images from biofilms grown at different substrate nutrient concentrations. The images are representative of the different morphologies. Scale bar = 3 mm.

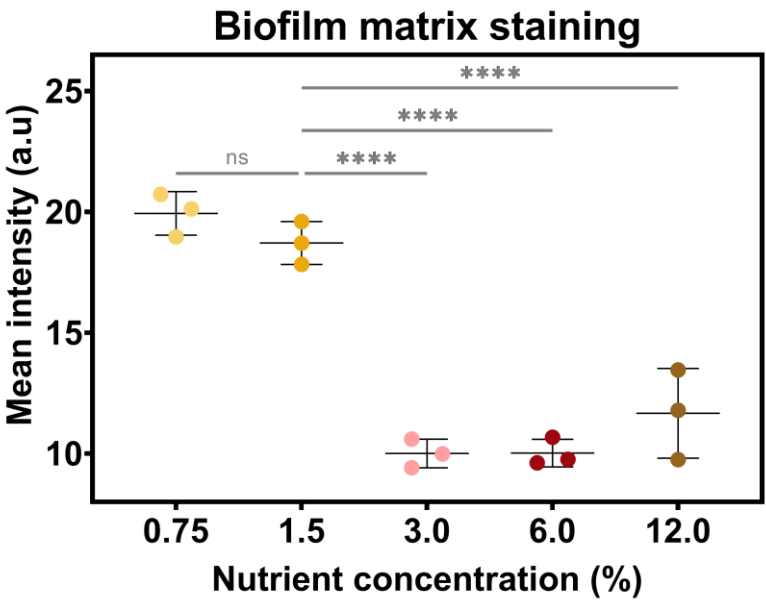
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1 3. Biofilm matrix staining with Direct Red 23



2
3 **Supplementary Figure 3 Cross-section of the biofilms obtained in different growth conditions.** Cross-section of *E.*
4 *coli* W3110 biofilms depicting fluorescence, brightfield and merged images. (a) The first row depicts the cross-section
5 of non-stained *E. coli* W3110 biofilm grown on salt-free LB agar containing 1.5 %w/v nutrients. (b) Cross-sections of
6 *E. coli* W3110 biofilms stained with Direct Red 23 grown on salt-free LB agar in increasing nutrient concentration. The
7 illumination pattern observed in the Brightfield and Merged column are due to the stitching process. The limit

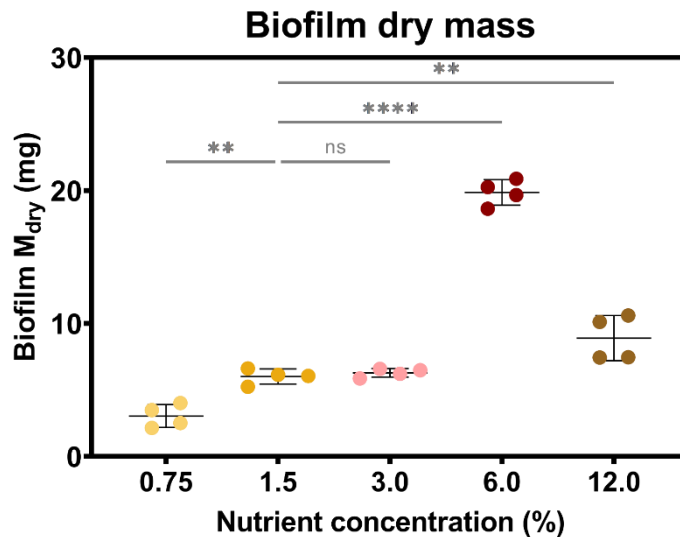
1 between agar substrate and biofilm of the 12 %w/v nutrient concentration condition is difficult to identify due to
2 smearing of the biofilm when preparing the cross-section.
3



4
5 **Supplementary Figure 4** Intensity of the Direct Red fluorescent staining on biofilm cross-sections. The mean intensity
6 of the raw images of each cross-section correspond to the mean value of their histogram measured using the Fiji
7 software.²
8

4. Biofilm mass

We considered the *E. coli* W3110 biofilms wet mass (M_{wet}) as the sum of the masses of i) the water, and ii) the bacteria and matrix, also considered as the dry mass (M_{dry}). Within M_{dry} , we discriminate between i) curli fibers (as the main matrix component) and ii) bacteria and other secondary matrix components.

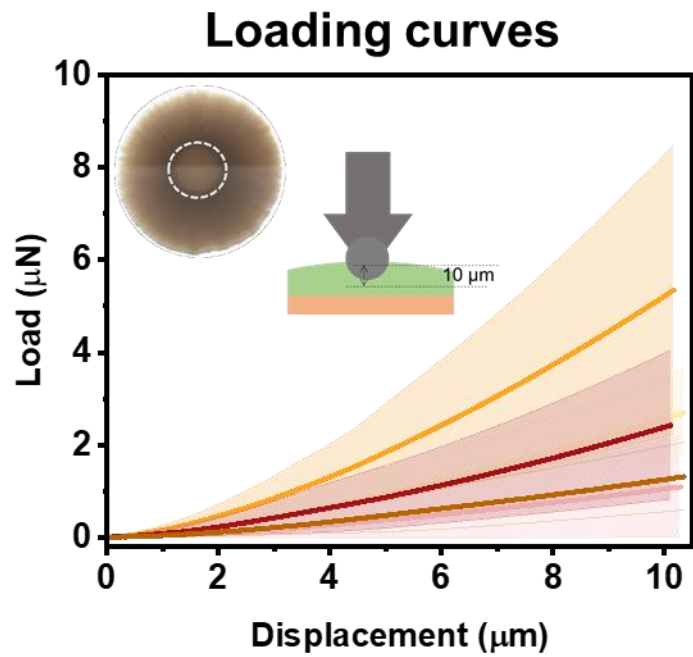


Supplementary Figure 5 Biofilm dry mass. Values correspond to the mass of a single biofilm calculated from four independent experiments. The statistical analysis was done with One-way ANOVA ($p < 0.0001$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % w/v nutrient concentration condition was used as reference for the post-test multicomparisons.

Supplementary Table 1 Composition of the *E. coli* W3110 biofilms grown on substrates of different nutrient concentration. The total mass corresponds to the sum of the water content and the dry mass. ^aThe bacteria mass was estimated by subtracting the curli mass given by the CsgA quantification after purification from the dry mass. The percentages of bacteria and CsgA are given with respect to the dry mass. N= 4 biofilms per experiment.

Substrate nutrient concentration (% w/v)	Biofilm size (mm)	Units	Total mass (M_{wet})	Water	Dry mass	CsgA ^a	Bacteria ^a
0.75	17 ± 1	mg	11 ± 3	8 ± 2	3.0 ± 0.9	0.01 ± 0.00	3.0 ± 0.9
		%	100	73	27	0.35	99.65
1.50	18 ± 2	mg	24 ± 2	18 ± 2	6.0 ± 0.6	0.02 ± 0.01	6.0 ± 0.6
		%	100	75	26	0.36	99.64
3.00	20 ± 2	mg	25 ± 1	18 ± 1	6.3 ± 0.3	0.03 ± 0.01	6.3 ± 0.3
		%	100	75	26	0.50	99.50
6.00	20 ± 2	mg	60 ± 5	40 ± 4	20 ± 1	0.05 ± 0.01	20 ± 1
		%	100	67	33	0.28	99.72
12.00	13 ± 1	mg	26 ± 5	18 ± 3	9 ± 2	0.01 ± 0.00	9 ± 2
		%	100	66	34	0.1	99.9

1 5. Microindentation loading curves



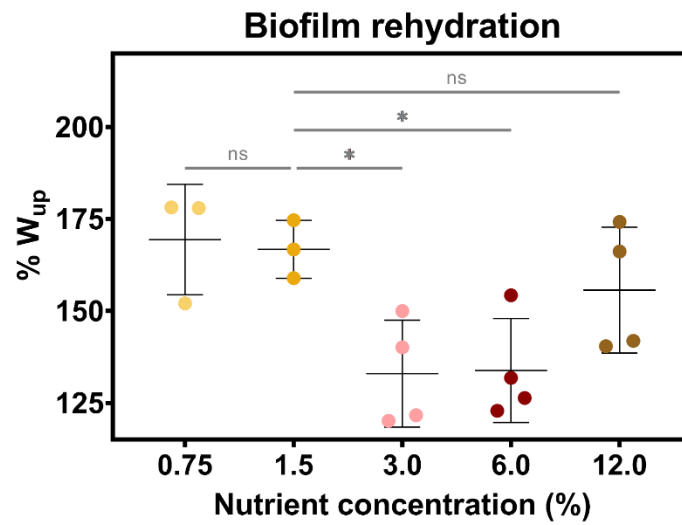
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3 **Supplementary Figure 6 Loading curves obtained from nanoindentation experiments on *E. coli* W3110 biofilms with**
4 **different nutrient concentrations.** Representative load–displacement curves when indenting the biofilm surface
5 (loading curve). N= 40. Color code: 0.75 % w/v nutrient concentration (yellow), 1.50 % w/v nutrient concentration
6 (orange), 3.00 % w/v nutrient concentration (pink), 6.00 % w/v nutrient concentration (red) and 12.0 % w/v nutrient
7 concentration (brown). In the inset, a scheme depicts the measurement: a spherical tip (50 μm) was indented in ~10
8 μm of the biofilm to avoid contribution of the underlying salt-free LB agar. For more details see the experimental
9 section.

10

1 6. Biofilm rehydration

2



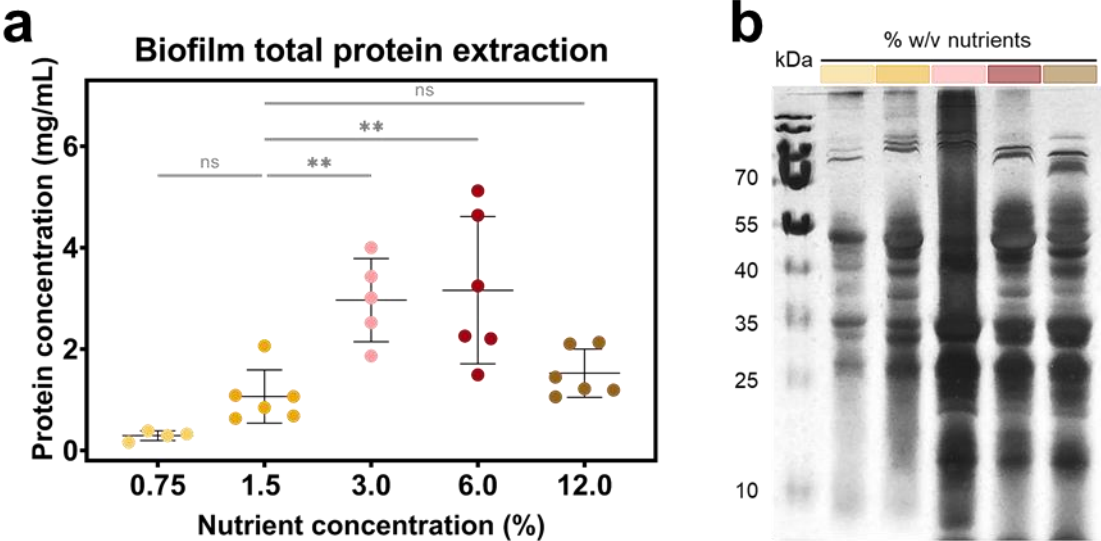
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4 **Supplementary Figure 7** Biofilm water uptake upon overnight rehydration of dry mass. The percentage of water
5 uptake of biofilms after rehydration (%W_{up}) was determined with respect to the biofilm initial wet mass: %W_{up} =
6 $(M_{\text{rewet}} - M_{\text{dry}}) / M_{\text{wet}} \times 100$ % w/w. M_{wet} , and M_{dry} stand for wet and dry masses, respectively, and M_{rewet} stand for the
7 biofilm weighed mass after rehydration.

8

7. Biofilm total protein extraction

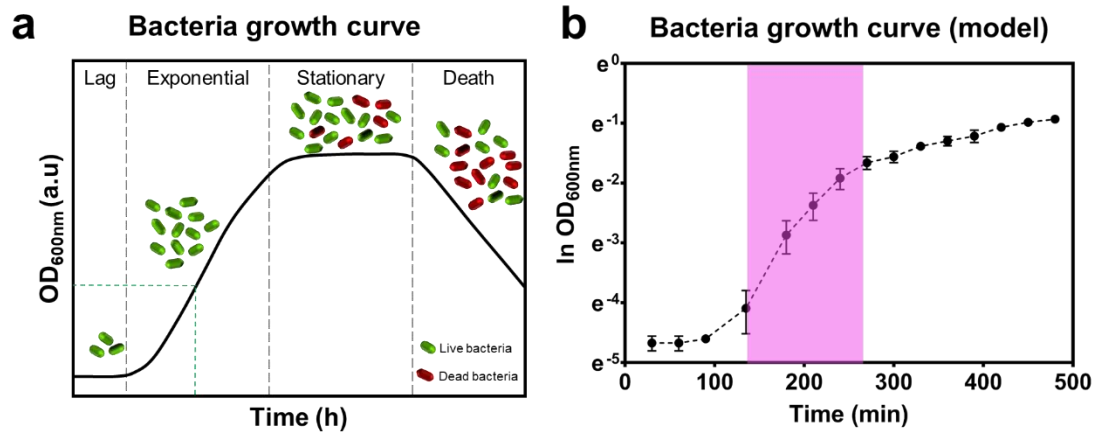
Biofilms grown on salt-free LB agar containing 3.0 and 6.0 % w/v nutrients had the highest total protein concentration (**Supplementary Figure 3**). The trend observed for the biofilm protein concentration is similar to the one observed for the size and wet mass of the biofilms, as well as for the dry mass of the biofilms. The SDS-PAGE electrophoresis gel run with aliquots of each total protein extraction showed that the pattern of proteins expressed by the bacteria is similar between conditions.



Supplementary Figure 8 (a) Biofilm total protein concentration (see experimental section for details). (b) SDS-PAGE electrophoresis gel with showing the protein pattern of the samples studied in (a).

8. Bacteria in liquid media

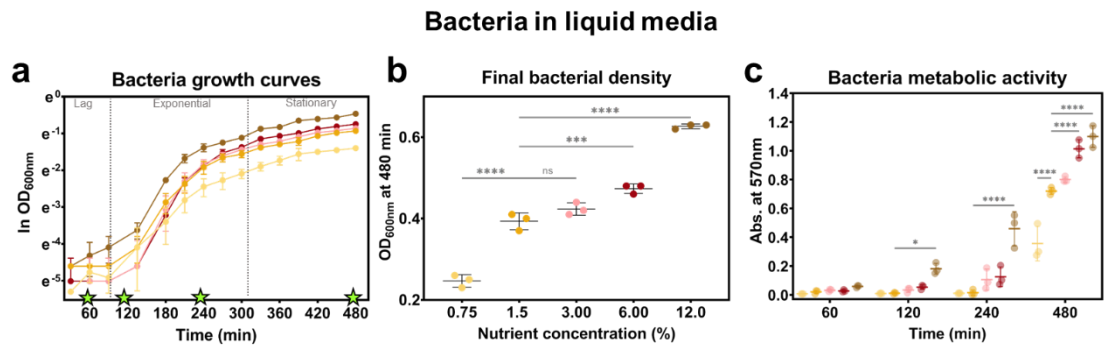
Growth curves allow studying the kinetics of bacterial growth in specific media. Typically, these curves present the four phases of life for bacterial culture: lag, exponential, stationary, and death (Supplementary Figure 9).³ In this work, the focus was set on the exponential phase, which is the phase where the bacteria have the most active metabolism.⁴



Supplementary Figure 9 Bacterial growth curve. (a) Bacteria grown in batch culture progress through four phases of growth: lag, exponential, stationary, and death. (b) Plotted growth curve $\ln(2) \text{OD}_{600\text{nm}}$ vs Time (min). Highlighted in pink is the linear section of the exponential phase.

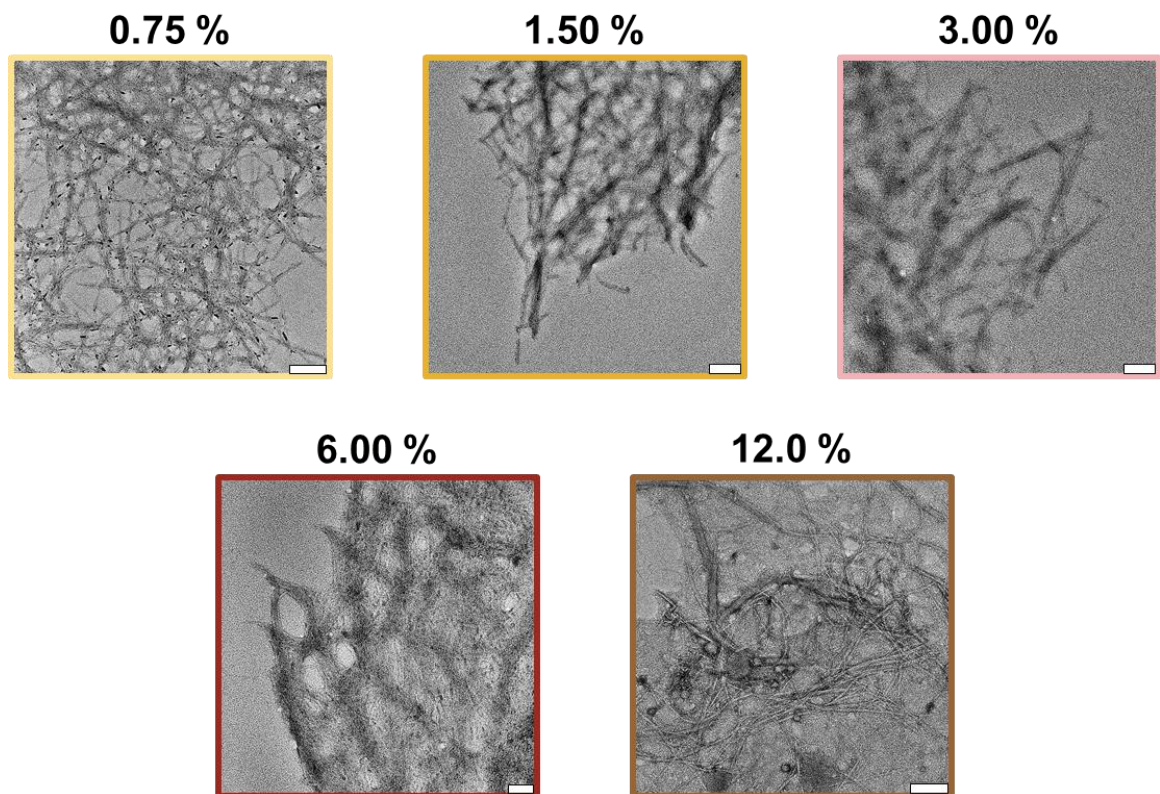
In order to study whether the differences of protein production are due to the growth and metabolism of the bacteria, bacterial growth was monitored for 8 hours in liquid salt-free LB media (**Supplementary Figure 9**).

According to the bacterial growth curves (**Supplementary Figure 10a**), the differences in the optical density were observed at the endpoint of the experiment, which directly correlates to the number of bacteria in the suspension (**Supplementary Figure 10b**). The results suggest that the higher the nutrient concentration of the media, the higher the bacteria proliferation. Because the endpoint of our experiment was still at an early stationary phase (**Supplementary Figure 9a, Supplementary Figure 10a**), the optical density values acquired can be related to a majority of live bacteria. The bacteria metabolic activity was also studied in each phase of their growth curve (**Supplementary Figure 10c**). *E. coli* bacteria in liquid salt-free LB media containing 12.0 % w/v nutrients were more metabolically active from the beginning of the exponential phase compared to the other nutrient concentrations tested.



Supplementary Figure 10 Bacteria in liquid media. (a) 8-hour bacterial growth curves for each condition tested. (b) Final optical density values acquired from each condition at the endpoint of the experiment. The optical density refers to the number of bacteria produced in each solution. (c) Bacteria metabolic activity in liquid media measured using MTT at the times highlighted by green stars in panel a. All data presented here come from N = 3 independent biofilm cultures for each condition tested. The statistical analysis was done with One-way ANOVA ($p < 0.0001$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % w/v nutrient concentration condition was used as reference

1 9. TEM of the purified fibers



2

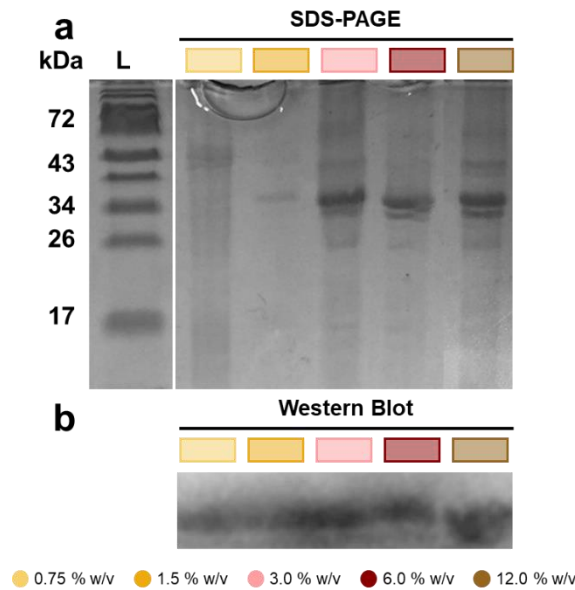
3 **Supplementary Figure 11** Transmission electronic microscopy images of the purified fibers. Scale bar = 100 nm.

4 Data come from 4-5 independent biofilm cultures for each condition tested.

10. Identification of purified curli amyloid fibers

a. Electrophoresis SDS-PAGE

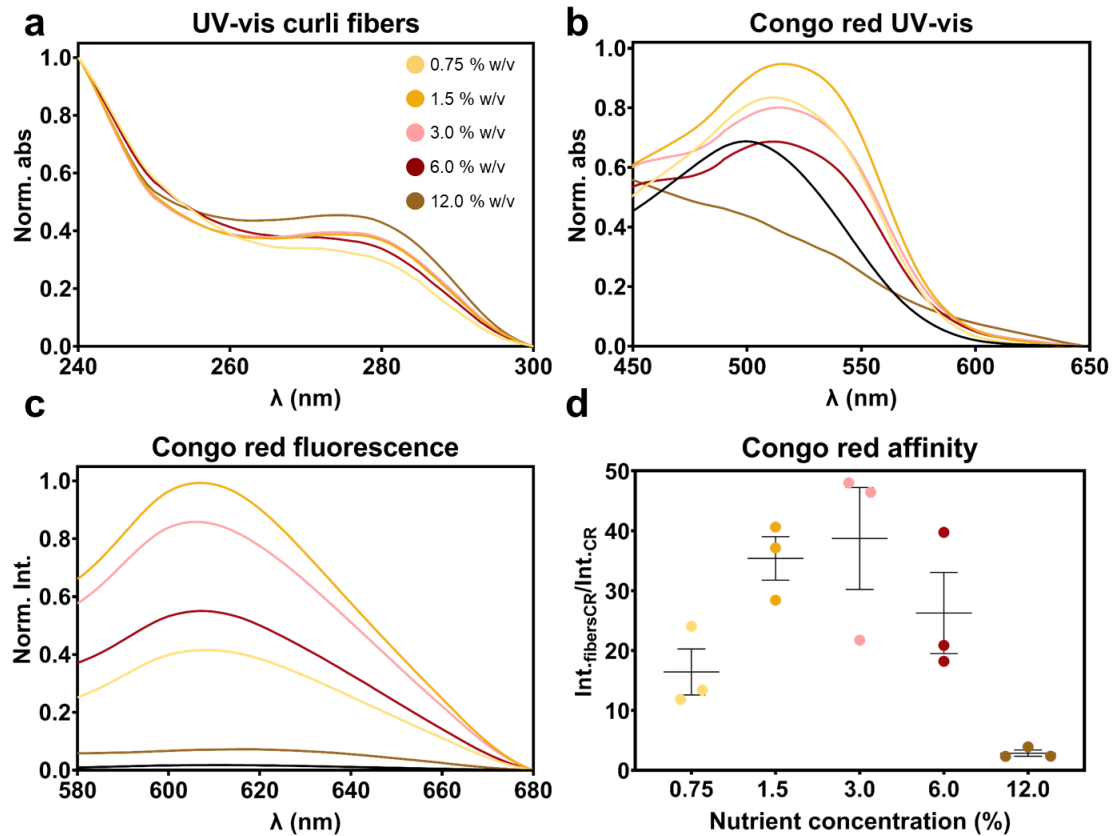
After the fiber purification process, the samples were treated with formic acid 100 % to depolymerize the fibers to their monomer CsgA. SDS-PAGE allowed us to identify bands at ~17 and ~34 kDa, molecular weight of the CsgA monomer and dimer, respectively (**Supplementary Figure 12a**). These bands were then further recognized by antibodies to CsgA in a Western Blot (**Supplementary Figure 12b**).⁵ The different intensities observed from the bands in the Western Blot matches the observations from the fiber yield quantification (**Figure 2d**).



Supplementary Figure 12 Identification of CsgA. (a) Electrophoresis SDS-PAGE gel. Molecular weight marker (L) was added to help identify the molecular weight of the bands in the different lanes showing CsgA dimer (34 kDa) after treating the extracted curli amyloid fibers from biofilms with formic acid. (b) Western Blots of samples in (a) stained with Anti-CsgA as primary antibody and Anti-Rabbit IgG as secondary antibody.

1

b. Binding to congo red



2

3 **Supplementary Figure 13 Identification of purified curli amyloid fibers with congo red (CR).** (a) UV-visible spectra of
 4 the purified curli amyloid fibers ($\lambda_{abs}=280$ nm). (b) UV-visible spectra of the binding of CR to the different samples of
 5 curli amyloid fibers. Free CR spectrum is indicated in black. (c) Fluorescence emission spectra of the curli fibers bound
 6 to CR, free CR spectrum is indicated in black ($\lambda_{exc}=540$ nm). (d) Values describing the increase in intensity of CR when
 7 bound to the purified fibers. Quantification of the increase was estimated by division of the area under the curve of
 8 each spectra of the probe with each fiber by the area under the curve of the emission spectra of the CR alone (values
 9 plotted indicating mean with SEM).

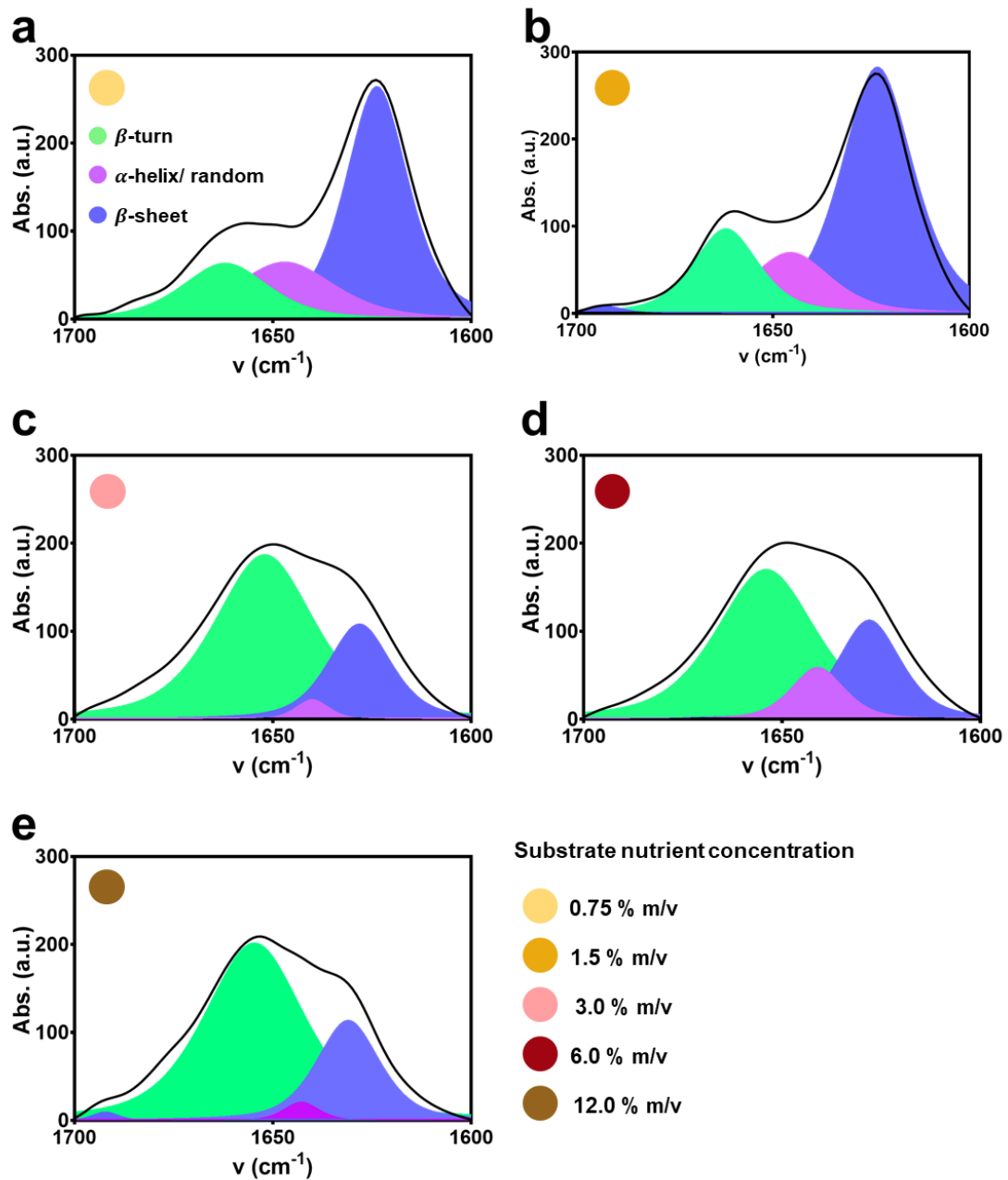
10 Congo red (CR) is a common dye to identify the presence of amyloid fibers. Although it is not
 11 clear how CR bounds to the amyloid fibers, two binding modes have been proposed.⁶ The CR
 12 could interact along the fiber axis with the β -sheet by electrostatic or hydrophobic interactions,
 13 or the CR could intercalate in parallel between two β -strands.⁶

14 The purified curli fibers from the different biofilms were first identified with this dye
 15 (**Supplementary Figure 13**). The presence of fibers was assessed by protein absorbance
 16 (Abs_{280nm}) after the purification process (**Supplementary Figure 13a**). With the exception of the
 17 fibers grown in biofilms with the highest nutrient availability, all samples presented a red-shift
 18 in the CR absorbance spectrum signifying the binding between CR and fibers (**Supplementary**
 19 **Figure 13b**).⁷

20 To further study the behavior of the fibers grown on high nutrient availability (12.0 % w/v
 21 nutrient concentration) fluorescence studies were performed (**Supplementary Figure 13c-d**).

1 When bound to the fibers, the congo red emission spectra exhibit an increase in its intensity
2 after an excitation at $\lambda_{exc}= 540$ nm (**Supplementary Figure 13c**). The intensity values of the
3 different spectra varied depending on the structure of the fibers bound to CR. When normalizing
4 the intensity value against the free CR signal, fibers produced on substrates containing 1.5 and
5 3.0 % w/v of nutrient showed an increase of c.a. 30 - 40 times of the CR intensity, while fibers
6 produced on substrates containing 0.75 and 6.0 % w/v of nutrient showed an increase of c.a. 10
7 times of the CR intensity (**Supplementary Figure 13d**). The differences in these values among
8 the different samples suggest changes in the structure of the purified fibers. The trend observed
9 in this experiment, follows the trend observed for the binding of ThioT with the fibers (**Figure**
10 **3a-b**).

1 11. FTIR-spectra of purified curli fibers



2
3 **Supplementary Figure 14 Amide I' spectra of curli fibers purified from *E. coli* biofilms.** Area-normalized FTIR
4 absorbance spectra in the Amide I' region of the different samples. The spectra were curve-fitted using the number
5 of spectral components identified by second derivative on the Fourier self-deconvoluted spectra. N = 3.

1 **Supplementary Table 2 Secondary structure analysis of the purified curli fibers assessed by ATR-FTIR.** The values
2 correspond to Figure 3d. N = 3.

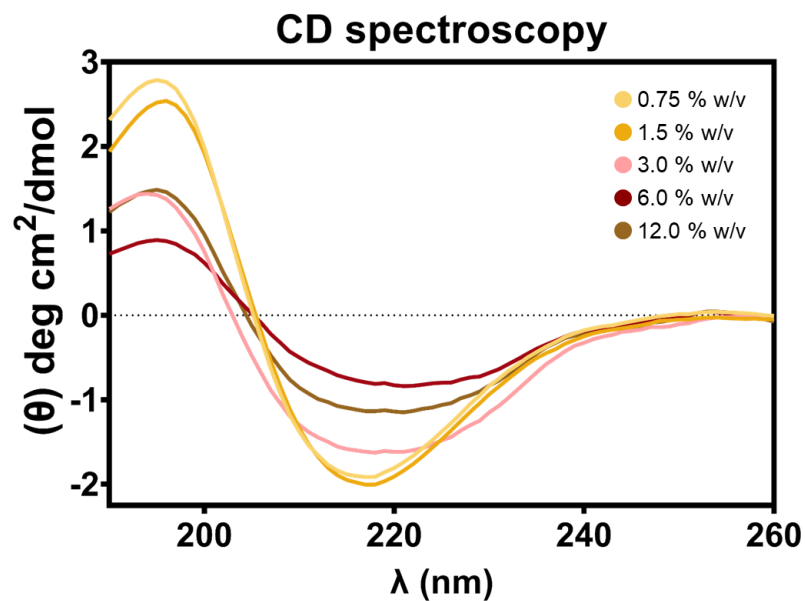
Substrate nutrient concentration (% w/v)	Secondary structure content (%)		
	β - sheet	α - helix/ random	Turns
0.75	58.03 $\pm$ 0.41	22.44 $\pm$ 0.12	19.82 $\pm$ 0.10
1.50	57.33 $\pm$ 1.04	29.63 $\pm$ 3.84	16.08 $\pm$ 1.63
3.00	30.08 $\pm$ 5.98	4.87 $\pm$ 3.00	65.01 $\pm$ 5.74
6.00	25.23 $\pm$ 3.66	14.84 $\pm$ 10.41	59.82 $\pm$ 6.92
12.00	26.72 $\pm$ 1.88	3.41 $\pm$ 0.78	69.86 $\pm$ 2.61

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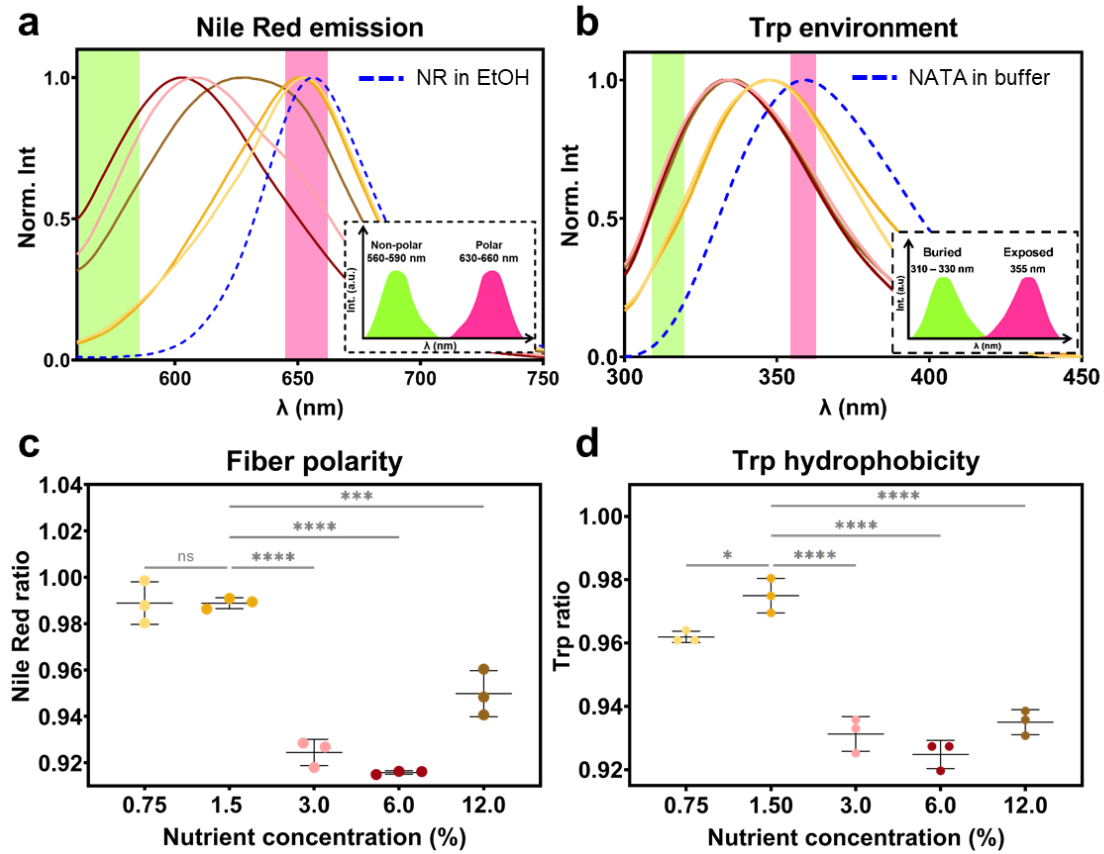
12. Fiber structure by circular dichroism (CD) spectrometry

The purified curli fibers showed differences in their β -sheet structure as described in their CD spectra (**Supplementary Figure 14**). All fibers tested in this experiment were normalized by CsgA monomer concentration, meaning there is the same protein concentration in each sample tested. Because of this, lower signal intensity means lower β -sheet content. Hence, fibers from biofilms grown on agar substrates containing lower nutrient concentration (0.75 and 1.5 % w/v), present high β -sheet content than those from biofilms grown on agar substrates containing higher nutrient concentration (3.0 % w/v or higher). These results are complementary to those of acquired with the ATR-FTIR experiments.



Supplementary Figure 15 CD spectrometry of the purified curli fibers. All fibers were normalized by CsgA monomer concentration. Data come from N = 3 independent biofilm cultures for each condition tested.

1 13. Fiber polarity



Supplementary Figure 16 Fiber polarity. (a) Fluorescence spectra of fibers stained with Nile Red (NR). The spectra of NR in buffer is represented as references for the emission of NR in a hydrophobic and hydrophilic environment, respectively. A sketch of the position of peaks for the NR in non-polar and polar environments is represented in the inset.⁸ The shadowed areas in the main plot indicate these exposure extremes. N=3-4 independent biofilm cultures for each condition tested. (b) Intrinsic fluorescence of the fibers through Trp emission. The spectrum of soluble Trp (NATA) in buffer is represented as reference for the maximum exposure possible of the Trp to the surface ($\lambda_{exc} = 280$ nm). A sketch of the position of peaks for buried and exposed Trp is represented in the inset.⁹ The shadowed areas in the main plot indicate these exposure extremes. N=3-4 independent biofilm cultures for each condition tested. (c) Ratio between the position emission maximum of each condition, taking NR in buffer as a reference. (d) Ratio between the position emission maximum of each condition, taking NATA in buffer as a reference. Data come from N = 3 independent biofilm cultures for each condition tested. The statistical analysis was done with One-way ANOVA ($p < 0.0001$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % w/v nutrient concentration condition was used as reference for the post-test multicompairs.

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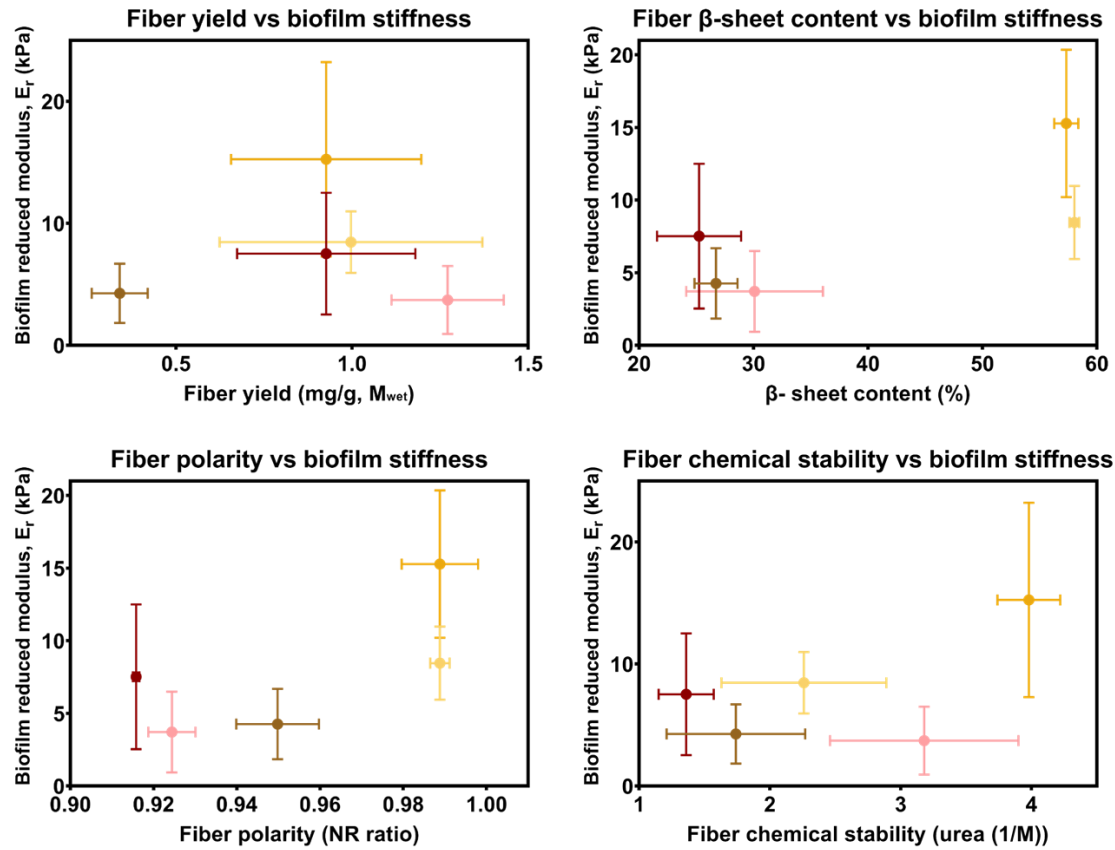


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1 15. Structure/function relationship between purified curli fibers and biofilm
2 mechanical properties



3
4 Supplementary Figure 18 Biofilm mechanical properties as a function of different properties of the purified curli
5 fibers.

6

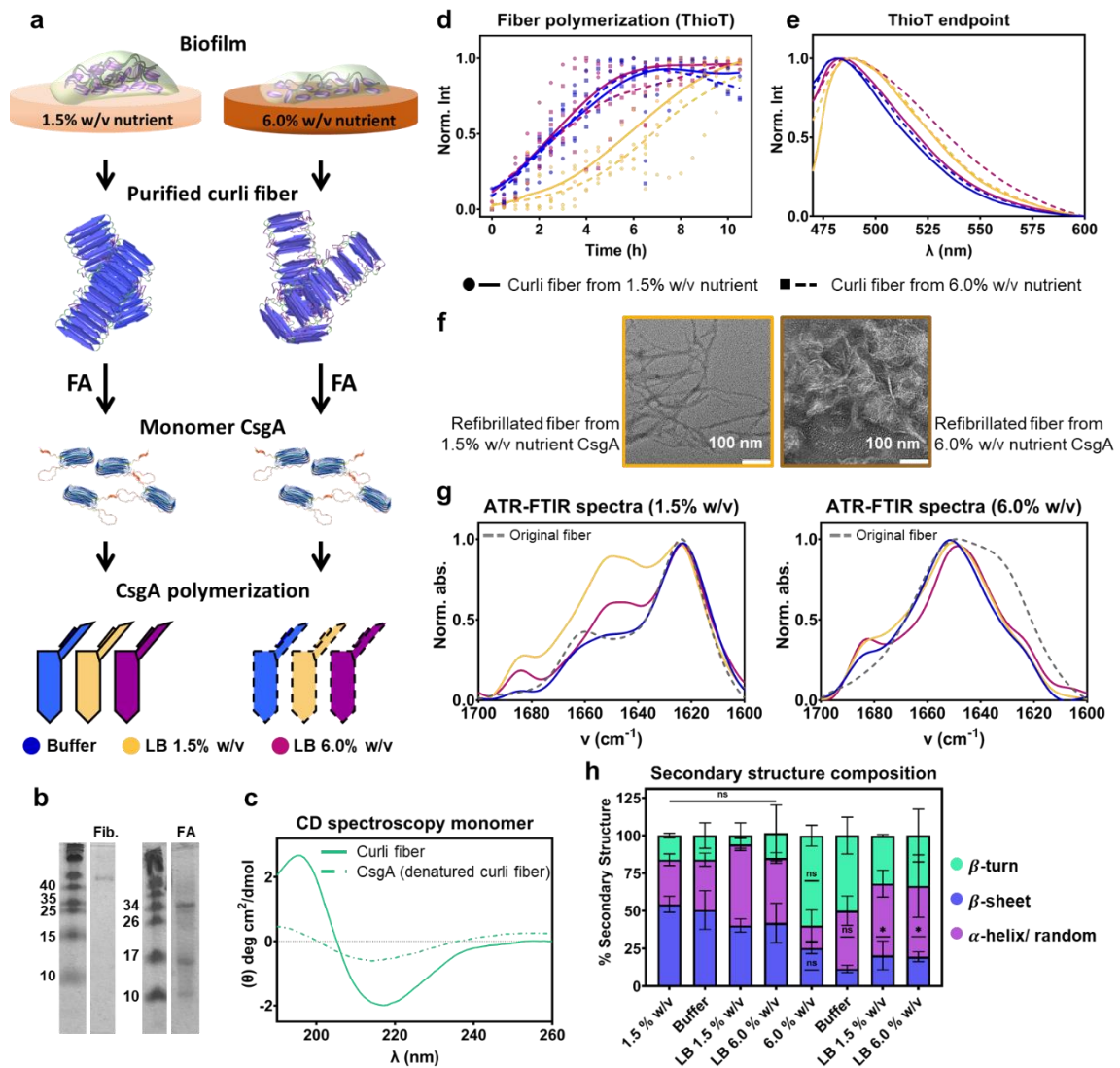
1 16. Proof of concept: from purified curli fibers to CsgA to fibers again

2 We purified the curli fibers from biofilms grown in two different conditions of the biofilm: LB
3 agar substrates containing 1.5% w/v and 6.0% w/v nutrients. We then reduced these fibers to
4 their monomer state (CsgA), and fibrillated them again in various buffers (**Supplementary Figure**
5 **19**). To favor clarity, the curli fibers purified from biofilms grown on LB agar substrates
6 containing 1.5% w/v nutrients are “Fiber A” and the curli fibers purified from biofilms grown on
7 LB agar substrates containing 6.0% w/v nutrients are “Fiber B”.

8 CsgA monomers or polymers were identified by a band of the SDS-PAGE around 15 kDa and two
9 bands around 30 kDa⁵, as well as by CD spectroscopy with a peak close to 200 nm⁵
10 (**Supplementary Figure 19 b and c**). The increase of ThioT emission indicates that the previously
11 denatured fibers can repolymerize in each of the media tested (**Supplementary Figure 19d**).
12 Fibers grown in LB 1.5% w/v show a slower polymerization process for the monomers of fiber A
13 and fiber B compared to the other conditions tested. Fibers grown in buffer or LB 6.0% w/v have
14 similar polymerization curves.

15 At the end of the polymerization experiment, each sample was centrifuged to separate the
16 resulting fibers from the unpolymerized CsgA (supernatant) and restained with ThioT for fiber
17 identification (**Supplementary Figure 19e**). Because of the low yield in each sample, the
18 concentration of fibers measured by ThioT emission was not normalized to any concentration.
19 In all cases, the ThioT emission spectra observed displayed the shape expected in the presence
20 of amyloid fibers in the sample. Moreover, electron microscopy (TEM) confirmed the existence
21 of fibers (**Supplementary Figure 19f**).

22 The ATR-FTIR spectra of the newly polymerized fibers in each medium showed spectra with
23 peaks in similar positions to those of their respective original fibers (before denaturation with
24 FA) (**Supplementary Figure 19g**). Upon detailed analysis of these spectra, band assignment and
25 secondary structure fitting suggest the structure of the newly polymerized fibers in each media
26 follow the same trend as the structure of their respective original fibers (Fiber A and Fiber B,
27 respectively), amidst the statistical differences observed (**Supplementary Figure 19h**). These
28 exploratory results suggest that depending on the environmental cues bacteria are under, they
29 might express the CsgA monomer with a different secondary structure, thus defining different
30 structural conformations of the mature curli fibers.



Supplementary Figure 19 Curli fiber denaturation and characterization of refibrillated fibers. (a) Schematic workflow. (b) Coomassie stain SDS-PAGE purified curli fibers (Fib.) and of samples after fiber denaturation with formic acid (FA). Molecular size markers (in kDa) are indicated in the corresponding ladder. (c) CD spectra of the purified curli fibers and of the CsgA monomer after denaturation. (d) CsgA polymerization followed by ThioT emission. (e) ThioT fluorescence emission of the dye bound to the fibers after CsgA polymerization in (d). (f) TEM images of the polymerized fibers. Scale bar = 100 nm. (g) Amide I' spectra of polymerized fibers. Area-normalized FTIR absorbance spectra in the Amide I' region of the different samples. The spectra were curve-fitted using the number of spectral components identified by second derivative on the Fourier self-deconvoluted spectra. (h) Distribution of the three types of secondary structure in the polymerized fibers. The data was obtained from the Amide I' region of each spectra. The statistical analysis was done with One-way ANOVA ($p < 0.0001$, **** | $p < 0.001$, *** | $p < 0.01$, ** | $p < 0.05$, * | ns = non-significant), where the 1.5 % w/v nutrient concentration condition was used as reference for the post-test multicomparisons. All data came from two independent denaturation experiments, except fiber polymerization that was done four times for each condition (duplicate of each denaturation experiment).

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