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Electronic Supplementary Information

Yeast-laden hydrogel capsules for scalable trace lead removal from water

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Table of Contents

1. Supporting Discussion

1.1 Swelling ratios 2

1.2 Mesh size 3

1.3 Isotherm shape 4

1.4 PEGDA content in capsules relative to beads 4

1.5 Estimation of capsule wall thickness 4

1.6 Analysis of kinetics data 5

1.7 Predicting breakthrough in packed bed biofilter 6

1.8 Stress on capsules, maximum flow rate, and scalability 7

2. Supporting Figures

- Figure S1. *Microfluidics setup* 9
- Figure S2. *Yeast-laden hydrogel capsules* 10
- Figure S3. *Individual yeast cells within capsules and beads* 11
- Figure S4. *Image of capsule compression experiments* 12

3. Supporting Movie 13

4. References 14

1. Supporting Discussion

1.1 Swelling ratios

To measure hydrogel mesh size to prove effective and encapsulation, and to assess the effect of the presence of yeast cells on that mesh size, we prepared two hydrogel precursor solutions:

(1) Yeast-laden hydrogel: 4 ml PEGDA 700, 5.5 ml water, 0.5 g yeast powder, and 0.5 ml PI
(2) Yeast-free hydrogel: 4 ml PEGDA 700, 5.5 ml water, and 0.5 ml PI

The compositions of these solutions are selected so that the yeast-laden and yeast-free hydrogel have the same quantity of PEGDA and same average chain length between crosslinks, allowing for comparison on a uniform basis. The yeast loading in the yeast-laden hydrogel (5% (w/w)) is the same as in the yeast-laden beads and capsules studied in our work. The monomer solutions were poured into Petri dishes and exposed to UV light (365 nm) for 5 min to prepare two cylindrical hydrogel tablets, 3.5 cm in diameter. The original mass of these tablets was recorded, and they were immersed in excess deionized water for 24 hours, followed by a second immersion in fresh deionized water for another 24 hours to allow them to reach a swollen equilibrium. Dimensions and masses of the swollen state were recorded. The hydrogel tablets were subsequently dried in an oven, first at 70°C for 6 hours, then at 70°C under a vacuum for 6 hours, and finally at 105°C for 36 hours. The dimensions and masses of the dry state were also recorded. All dimensions and masses are shown in table 1 and are used to calculate the swelling ratio, which is a proxy for the mesh size of these hydrogels.

	Yeast-laden hydrogel			**Yeast-free hydrogel**		
	Original	*Dry*	*Swollen*	*Original*	*Dry*	*Swollen*
H (cm)	0.6	0.4	0.6	0.6	0.5	0.6
D (cm)	3.5	2.8	3.5	3.5	2.6	3.8
Mass (g)	4.80	2.26	5.51	5.06	2.19	5.52
V (cc)	5.77	2.46	5.77	5.77	2.65	6.80
Density (g/cc)	0.83	0.92	0.95	0.88	0.83	0.81

Table 1 | Dimensions and masses of cylindrical yeast-laden and yeast-free hydrogel tablets in various states of hydration.

Using these values, we compute two swelling ratios, which are shown in table 2.

$$Q = \frac{V_{swollen}}{V_{dry}} \tag{1.1.1}$$

$$q = \frac{m_{swollen}}{m_{dry}} \tag{1.1.2}$$

	Yeast-laden hydrogel	Yeast-free hydrogel
Q	2.34	2.57
q	2.44	2.52

Table 2 | Swelling ratios of yeast-laden and yeast-free hydrogels.

We observed that when yeast-laden and yeast-free hydrogels tablets were synthesized to have the same PEGDA content, cross-linking density, and degree of polymerization between junctions at synthesis, the yeast-free hydrogel had slightly higher swelling ratios (~5%), indicating a slight reduction in mesh size due to the presence of yeast.

1.2 Mesh size

We use two approaches to estimate the mesh size of yeast-laden and yeast-free hydrogels, as described in references [1,2]. Reference [1] presents the classical Canal-Peppas theory and evaluates mesh size as:

$$\xi = \phi_s^{-\frac{1}{3}} l \left(2 C_\infty N_j\right)^{\frac{1}{2}} \tag{1.2.1}$$

Reference [2] corrects for the effects of junction functionality:

$$\xi = \phi_s^{-\frac{1}{3}} l \left(\left(1 - \frac{2}{f}\right) C_\infty \lambda N_j\right)^{\frac{1}{2}} \tag{1.2.2}$$

Here, the Flory characteristic ratio is C_∞, the junction functionality is f, the degree of polymerization between junctions is N_j, the repeating unit average bond length is l, the number of carbon-carbon bonds per repeating unit is λ, and the swollen polymer volume fraction is ϕ_s.

	Yeast-laden hydrogel	Yeast-free hydrogel	Difference (%)
$\boldsymbol{\phi_s}$ (from section 1.1)	0.427	0.389	
$\boldsymbol{l}$	0.15	0.15	
$\boldsymbol{f}$	4	4	
$\boldsymbol{C_\infty}$	4	4	
$\boldsymbol{\lambda}$	3	3	
$\boldsymbol{N_j}$	13	13	
Canal-Peppas [1] mesh size (nm)	2.03	2.09	2.87
Richbourg-Peppas [2] mesh size (nm)	1.76	1.81	2.76

Table 3 | Estimated mesh sizes of yeast-laden and yeast-free hydrogels.

As can be seen in table 3, the mesh size of yeast-free hydrogels is estimated to be very slightly (~2.8%) higher than the yeast-laden hydrogels.

1.3 Isotherm shape

The capacity of any absorbent or adsorbent is, in general, governed by the Langmuir isotherm or a similar isotherm[3]. These isotherms have two limiting regimes:

(1) At low supernatant concentrations c_{out} relative to the number of active sites, these isotherms reduce to a linear form for the concentration of absorbed micropollutant $c_{in} = Kc_{out}$, where K is the partition coefficient. In this case, the supernatant concentration limits micropollutant uptake by the absorbent or adsorbent.
(2) At high supernatant concentrations, the concentration of absorbed micropollutant is limited by the number of active sites available for micropollutant uptake, and $c_{in} \to a$, the number of active sites per unit volume.

The hydrogel materials have numerous active sites that weakly bind to lead ions. As such, we operate the hydrogels exclusively in the first limiting regime, and the isotherms have a linear shape. On the other hand, the yeast cells have a smaller number of strongly binding active sites, so we operate across all regimes, and the isotherms have a plateau-like shape (Fig. 3).

1.4 PEGDA content in capsules relative to beads

Confocal microscopy data shown in Fig. 2e is used to estimate the quantity of PEGDA hydrogel in the capsule walls, relative to solid beads. First, the image is split into multiple color channels to obtain the contribution of the capsule walls (Nile blue acrylamide) to fluorescence, independently of the yeast cells. The intensity of the wall fluorescence is then integrated over the entire area of the capsule cross-section, using source code in MATLAB. Selecting an appropriate cross-section within the capsule wall, we calculate the integrated fluorescence from fully polymerized PEGDA hydrogel. The ratio of the two integrated values yields the fraction (0.3239) of PEGDA hydrogel within the capsule, relative to a bead of the same volume. The beads thus contain ~3 times the PEGDA hydrogel as the capsules.

1.5 Estimation of capsule wall thickness

If R is the radius of the hydrogel beads/capsules, and l is the capsule thickness, the volume of hydrogel in the capsule and in the solid bead are given by:

$$V_{capsule} = \frac{4}{3}\pi(R^3 - (R-l)^3)$$

$$V_{bead} = \frac{4}{3}\pi R^3$$

These yield,

$$\frac{V_{capsule}}{V_{bead}} = 1 - \left(1 - \frac{l}{R}\right)^3$$

Or,

$$l = R\left(1 - \left[1 - \frac{V_{capsule}}{V_{bead}}\right]^{\frac{1}{3}}\right) \tag{1.5.1}$$

Using the fraction of PEGDA hydrogel in the capsule obtained in and a mean capsule diameter of 500 μm, we obtain a capsule wall thickness equal to 30.6 μm.

1.6 Analysis of kinetics data

Let c_t be the lead concentration in solution at time t, with initial value c_0. K is the partition coefficient, $k_c a$ is the mass transfer coefficient, and c_s is the concentration at time t in the solution equilibrium which is in equilibrium with the concentration inside the adsorbent. Let the average concentration inside the adsorbent be q_t. V is the volume of the supernatant and m is the volume of equivalent hydrogel. Then, we have the following equations based on equilibrium, mass transfer and pollutant mass balance respectively.

$$q_t = Kc_s \tag{1.6.1}$$

$$\frac{dc_t}{dt} = k_c a(c_t - c_s) \tag{1.6.2}$$

$$q_t = \frac{V}{m}(c_0 - c_t) \tag{1.6.3}$$

Combining these, we get

$$\frac{dc_t}{dt} = k_c a\left(\left(1 + \frac{V}{mK}\right)c_t - \frac{V}{mK}c_0\right)$$

Solving this differential equation for $c(t)$, with initial condition $c(0) = c_0$ yields

$$\frac{c_t}{c_0} = \frac{\frac{V}{mK} + e^{-\left(1+\frac{V}{mK}\right)k_c a t}}{\frac{V}{mK} + 1}$$

Let the dimensionless group $\alpha = V/mK$,

$$\frac{c_t}{c_0} = \frac{\alpha + e^{-(1+\alpha)k_c a t}}{1 + \alpha} \tag{1.6.4}$$

We define fitting parameters,

$$\phi_1 = \frac{\alpha}{1+\alpha} \tag{1.6.5}$$

$$\phi_2 = (1+\alpha)k_c a \tag{1.6.6}$$

yielding,

$$\frac{c_t}{c_0} = \phi_1 + (1-\phi_1)e^{-\phi_2 t} \tag{1.6.7}$$

The amount of lead adsorbed by the yeast is given by:

$$q_t = \frac{Vc_0}{m}\left(1 - \frac{c_t}{c_0}\right) = \frac{Vc_0}{m}(1-\phi_1)\left(1 + e^{-\phi_2 t}\right) \tag{1.6.8}$$

Equation 1.5.8 is fit to the data in Fig. 3f, and $\tau = 1/\phi_2$ are the time constants reported in the main text.

1.7 Predicting breakthrough in packed bed biofilters

With respect to a given micropollutant, breakthrough is complete, and the bed loses all ability to absorb that micropollutant when the entire bed has been saturated with that micropollutant. Bed saturation occurs when the absorbed micropollutant is in equilibrium with the inlet concentration of the micropollutant. Therefore, at the end of bed life, the total amount of micropollutant absorbed:

$$m_{hydrogel} c_{absorbed} = m_{hydrogel} K c_0$$

Where c_0 is the concentration at the inlet and K is the partition coefficient. To remove this amount of micropollutant from water in which its concentration is c_0, the volume of water that needs to have been cleaned is:

$$m_{water} = \frac{m_{hydrogel} c_{absorbed}}{c_0} = m_{hydrogel} K$$

This yields the volume of water cleaned at the end of bed life for any given micropollutant.

$$\frac{m_{water}}{m_{hydrogel}} = K \tag{1.7.1}$$

On the other hand, breakthrough begins and performance starts decreasing before this volume of water has been treated. If the residence time of the bed is τ_{bed} and the time it takes for the hydrogel microparticles to equilibrate with the given micropollutant is τ_{eq}, then breakthrough begins when that initial portion of the bed which has a residence time of $\tau_{bed} - \tau_{eq}$ has been saturated with the micropollutant. This implies:

$$m_{water} c_0 = \left(1 - \frac{\tau_{eq}}{\tau_{bed}}\right) m_{hydrogel} K c_0$$

$$\frac{m_{water}}{m_{hydrogel}} = \left(1 - \frac{\tau_{eq}}{\tau_{bed}}\right) K \tag{1.7.2}$$

The pre-factor in equation 1.7.2 depends on multiple parameters, such as the flow rate, the volume of the bed, the packing fraction (which is a function also of the compression of the hydrogels when the bed is pressurized by fluid flow), and the mass transfer coefficient of the micropollutant at the relevant flow conditions.

In reality, the isotherms for yeast-laden hydrogel capsules are not linear, but Langmuir-like (supplementary information 1.3), so K refers to the local partition coefficient, or the slope of the isotherm at $c_e = c_0 = 100$ ppb. The value of K is estimated to be 2500. As described in methods, $\tau_{eq} = 3.83$ min, and $\tau_{bed} = 8.45$ min. Therefore, we get, for the end of bed life,

$$\frac{m_{water}}{m_{hydrogel}} = 1367$$

This value is a good estimate of the point at which the breakthrough curve crosses the USEPA threshold ($m_{water}/m_{hydrogel}$ = 1200) in Fig. 4e.

1.8 Stress on capsules, maximum flow rate, and scalability

Considering the bioreactor's operational parameters and capsule dimensions ($Q = 1.667 \times 10^{-9} m^3/s$; $u = 2.831 \times 10^{-5}\, m/s$; $R_{bioreactor} = 4.34\, mm$; $L_{bioreactor} = 4.2\, cm$; $\Delta P = 29.726\, kPa$; $D_{capsule} = 500\, \mu m$), we estimated a developed drag force (F_{drag}) of ~0.8 μN and a maximum stress (σ) of ~4.00 Pa on each yeast-laden hydrogel capsule, though the following equations:

$$A_{bioreactor} = \pi R_{bioreactor}^2$$

$$V_{bioreactor} = A_{bioreactor} L_{bioreactor}$$

$$A_{capsule} = \pi \left(\frac{D_{capsule}}{2}\right)^2$$

$$V_{capsule} = \frac{4}{3}\pi \left(\frac{D_{capsule}}{2}\right)^3$$

$$\sigma = \frac{\left[\frac{\Delta P \times V_{capsule}}{V_{bioreactor}}\right]}{A_{capsule}} \tag{1.8.1}$$

$$F_{drag} = \sigma A_{capsule} \tag{1.8.2}$$

For the projections, we approximated the theoretical maximum flow rate Q (Q_{max}) that could occur corresponding to the maximum load that the capsules can withstand without bursting ($P_{max} = 0.88\, N$) (which was acquired by the mechanical testing), assuming that the operating flow rate

($Q = 1.667 \times 10^{-9} m^3/s$) corresponds to a drag force of ~0.8 μN, as calculated above. Hence, this Q_{max} was calculated to be equal to 1.86 L/sec or ~160 m^3/day. To put this into perspective, the standard flow rate for a US household kitchen faucet is 0.18 L/sec [4], while small communities ($\leq$ 10,000) have an average daily wastewater flow $\leq$ 3,800 m^3/day [5], so a flow of 160 m^3/day would correspond to a community with up to 400 people.

2. Supporting Figures

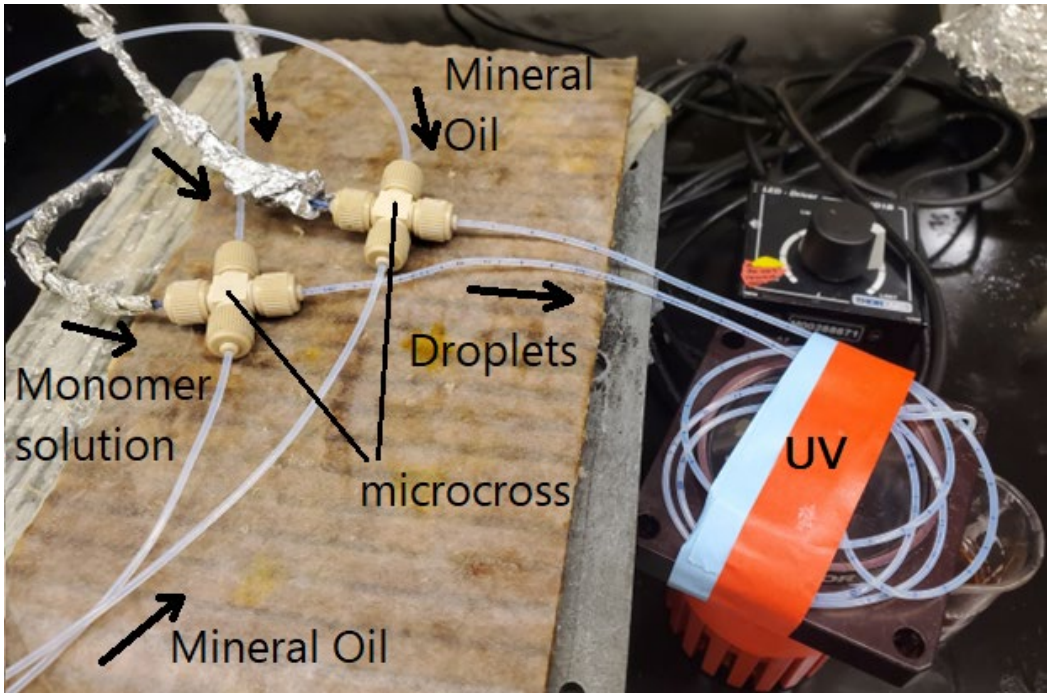

Fig. S1 | Microfluidics setup. Using multiple microcrosses with the same UV lamp enables rapid scale up of hydrogel synthesis.

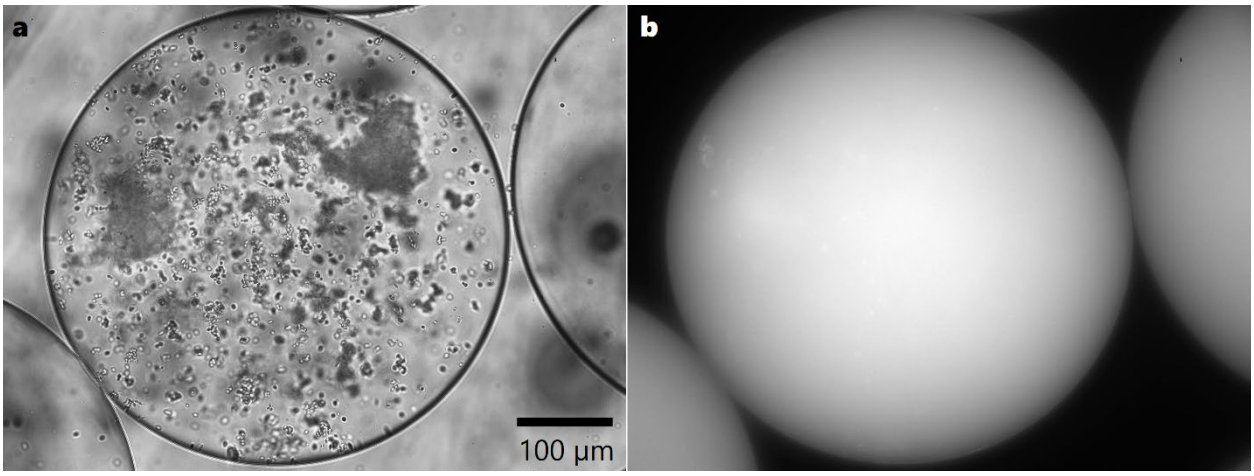

Fig. S2 | Yeast-laden hydrogel capsules visualized using (a) optical microscopy and (b) fluorescence microscopy. These capsules contain 1% (w/w) yeast cells, which can be seen through the capsule walls. The yeast cells are stained with Calcfluor, resulting in fluorescence as seen in (b). No yeast cells or fluorescence is seen outside the capsule walls.

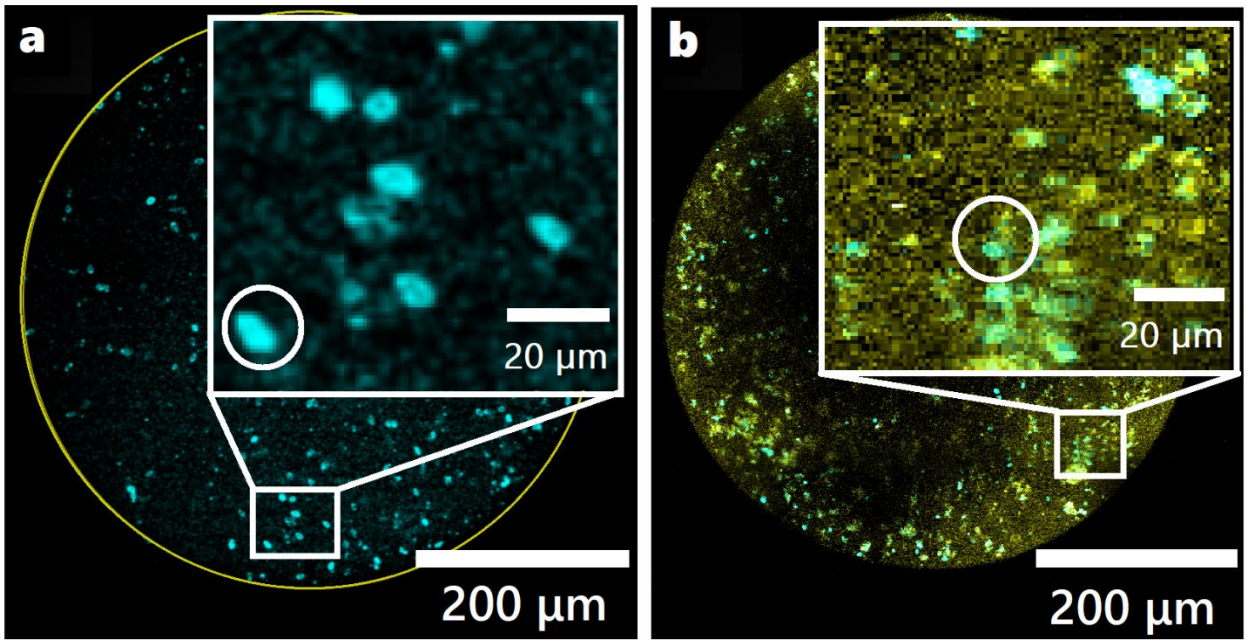

Fig. S3 | Individual yeast cells within beads and capsules shown in Fig. 2d,e. The yeast cells (cyan) are seen to be intact after polymerization.

Fig. S4 | Image of mechanical characterization setup. Yeast-laden hydrogel capsules are lightly held between the load cell and the base of the instrument and water is poured into the gap to keep the capsules hydrated. Experiments are then performed as described in methods.

3. Supporting Movie

Movie S1 | Movie showing capsule compression experiments for mechanical characterization of yeast-laden hydrogel capsules.

4. References

[1] T. Canal and N. A. Peppas, ‘Correlation of mesh size and equilibrium degree of swelling of polymeric networks,’ *J. Biomed. Mater. Res.*, **23(10)**, 1103-1237 (1989)

[2] N. R. Richbourg and N. A. Peppas, ‘The swollen polymer network hypothesis: Quantitative models of hydrogel swelling, stiffness, and solute transport,’ *Prog. Polym. Sci.*, **105**, 101243 (2020)

[3] I. Langmuir, ‘THE CONSTITUTION AND FUNDAMENTAL PROPERTIES OF SOLIDS AND LIQUIDS. PART I. SOLIDS.’ *J. Am. Chem. Soc.*, **38(11)**, 2221-2295 (1916)

[4] Aquasana - What is a Typical Home Water Flow Rate? URL: https://www.aquasana.com/info/what-is-a-typical-home-water-flow-rate-pd.html Accessed: 2023-10-17

[5] USEPA - About Small Wastewater Systems URL: https://www.epa.gov/small-and-rural-wastewater-systems/about-small-wastewater-systems Accessed: 2023-10-17