

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Microorganism inspired hydrogels: hierarchical super/macro-pore, rapid swelling rate and high adsorption

Qingsong Zhang^{1,2*}, Bingjie Chen¹, Lei Tao^{2*}, Mingyang Yan¹, Li Chen¹, Yen Wei^{2*}

RSC Advances Accepted Manuscript

* Q.S. Zhang

¹State Key Laboratory of Hollow Fiber Membrane Materials and Processes, School of Materials Science and Engineering, Tianjin Polytechnic University, Tianjin 300387, China; E-mail: zqs8011@163.com

²Department of Chemistry, Tsinghua University, Beijing 100084, China; E-mail: leitao@mail.tsinghua.edu.cn, weiyen@tsinghua.edu.cn

Abstract

Learning from the production of baking bread, rolls, cake, beer or Chinese steamed bread, a novel microorganism inspired macro/super-porous hydrogel composed of specific polymers and single-celled fungus yeast was prepared by production of carbon dioxide (CO₂) via fermentation method. The appearance, porous structure, swelling behavior and adsorption property of the resulting hydrogels were investigated by optical microscopes, scanning electron microscopes (SEM), UV/Vis spectroscopy and gravimetric methods. The resultant hydrogel presents yellowish brown color similar with ale yeast, and the integration of polymeric materials and Fungi has greatly improved the pore shape/size, swelling and adsorption property of hydrogels. Both super- and macro-pores with diameters ranging from 1 mm to 5 μ m exist in the hierarchical matrix of hydrogels. The super/macro-porous hydrogels can absorb water very rapidly and swell to equilibrium state in less than 60 min. With increasing yeast or sugar consumed, the adsorption capacity (Q_t) of hydrogels can be increased 1.39~1.87 times. After adsorbing cationic dye crystal violet (CV), pores of the hydrogel matrix were blocked and a dense layer was formed. By the same footstep, porous fiber, elastomer, ceramic and metals could be obtained which might have potential applications in the fields of cell culture, catalytic substrates, chemical separation and battery electrodes.

Keywords: hydrogel; porosity; yeast; fermentation; adsorption

I. Introduction

Natural porous materials, like rock, bamboo, wood, human skeleton and lotus root, are commonly found and acting vital roles in our surroundings. For example, soils are highly permeable and well sorted for the protection of plant life from drowning. The numerous porous materials have been prepared by worldwide researchers and used in the fields of scaffold materials for bone implants,¹ fuel cells,² noise elimination, ion exchangers,³ electromagnetic shielding,⁴ and gas separation⁵ due to great advantage in the aspect of controlled pore structure/size, bigger specific surface area, excellent permeability and damping property.

As a kind of porous materials, elastic soft hydrogels have three-dimensional networks that can contain huge amount of water (up to 90%), which allows them to be an attractive material for applications in chemical separation, tissue engineering, and drug delivery.⁶⁻¹⁰ Depending on the pore size, porous hydrogels are divided into microporous (10 to 100 nm), mesoporous (100-1000 nm), macroporous (1 to 100 μm) and superporous hydrogels (10 to 1000 μm , SPHs). Several techniques have been employed to introduce super/macro-porosity to hydrogels, such as phase separation,¹¹ freeze drying,¹² porogonic agent,¹³ emulsion template method,¹⁴⁻¹⁵ and so on. However, there are some disadvantages for above methods, like, a long period and inefficient process to solve, wash and soak in porogonic agent or emulsion template methods. Therefore, it is essential to create a novel technique to produce porous hydrogels with lower cost, high efficiency, and bigger pore or specific surface area.

To overcome this tough problem, a kind of novel microorganism inspired concept

based fermentation of yeast has been proposed. It is well known that we live in a world dominated by microbes, and baking bread, rolls, cake, beer or Chinese steamed bread are all porous food prepared by fermentation. Yeast could rise dough or create leavened dough and break down sugars by the way to create carbon dioxide (CO₂), H₂O or ethanol.¹⁶⁻¹⁷ After fermentation, the produced CO₂ gas could create various sized pores, and the obtained pores also can be fixed after removing dead single-celled fungus yeast when the nutrient materials are exhausted. Therefore, learning from fermentation process in microbiology,¹⁸ in the presence of nutrient material white sugar, single-celled fungus yeast to trigger the fermentation is involved in this article to generate pores in the hydrogels. Compared to other methods, preparation of porous hydrogels by yeast fermentation has significant benefits, such as simplified operation process, rapid pore formation, relatively inexpensive agents, bigger specific surface area and high adsorption capacity.

In the present work, series of microorganism inspired super/macro-porous hydrogels were facilely prepared by simultaneous reactions of gelation of polymerizable monomer like acrylamide (AM) and fermentation of yeast and sugar. The appearance, pore structure and swelling behavior of resulting hydrogels were investigated. Furthermore, the appearance of prepared hydrogels before and after adsorption of crystal violet (CV), adsorption dynamics under different yeast and sugar content, interior pore shape after adsorption of CV were studied by UV/Vis spectroscopy and scanning electron microscope (SEM), demonstrating the different pores with pore size ranging from 5 μm to 1 mm in the matrix of hydrogels,

which makes those hydrogels good candidate for the adsorption of dye. The concept of preparing porous materials by microorganism inspiration can also be translated to achieve other porous materials, such as fiber, elastomer, ceramic and metals for potential applications in cell culture, catalytic substrates, chemical separation and battery electrodes.

II. Experimental

II.1 Materials

Monomer acrylamide (AM) and cross-linker *N,N'*-methylene-bis-acrylamide (MBA) were of analytic grade and purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. The initiator ammonium peroxydisulfate (APS) and accelerator *N,N,N',N'*-tetramethylenediamine (TEMED) were of analytic grade and were used as received without further purification. Instant dry yeast was obtained from Angel Yeast Co., Ltd. The sugar was purchased from Beijing Zhongtang Sugar Industry Extend Co., Ltd. As a monovalent cationic dye, crystal violet (CV, analytical reagent grade) was used as received. Water was deionized by Millipore Direct-Q apparatus.

II.2 Porous hydrogel synthesis

At the same time of preparing 10 mL rehydration solution containing yeast and sugar, traditional pre-polymer solution was made by adding monomer AM and cross-linker MBA into 10 mL deionized water at room temperature under the continuous stirring. The weight ratio of MBA/AM and the concentration of AM were controlled to 5wt% and 10% (w/v), respectively. Thereafter rehydration solution was slowly poured into pre-polymer solution. Ten minutes later, 0.01g of ammonium persulfate (APS) and

160 mL of *N,N,N',N'*-tetramethylethylenediamine (TEMED) with 2% (v/v) were added to initiate and accelerate polymerization. The glass reaction vessel was sealed with retaining several gas-guide holes until fermentation polymerization was finished at 30°C for 4 h. The overall process of fermentation is to convert sugar to carbon dioxide gas (CO₂) and alcohol (CH₃CH₂OH) or H₂O. The resulting super/macro-porous hydrogel samples were simply named as HSYAB. In the present study, A means the sugar content, and B means the yeast content. For example, the hydrogel synthesized using 0.5 g sugar and 0.8 g yeast is expressed as HSY0508. When reaction was finished, all samples were cut into disc-shape pieces, and immersed in an excess of deionized water for 7 days to remove impurities by changing water.

II.3 Optical microscope

Firstly, 0.1 g of instant dry yeast was added into 10 mL aqueous solution under continuous stirring for 30 min. Then a drop of mixture solution was dripped onto a carry sheet glass, and was observed under Olympus IX71 phase-contrast microscope.

II.4 Interior morphology

The swollen or adsorbed hydrogels in deionized water were frozen to -60°C, and then fractured and freeze-dried on a FD-3 freeze-drier, Beijing Bilon Lab Equipment Co., Ltd. The morphology of the fractured specimens was observed on a JEOL JSM-5600LV SEM after sputter coating with gold under vacuum.

II.5 Swelling behavior

A gravimetric procedure was adopted to monitor the progress of the swelling process. In brief, swollen HSY with disc shape were taken out of the solution at regular time intervals, and then wiped with filter paper to remove excess water before weighing. The measurements were carried out until a constant weight was reached for each sample. The swelling ratio (SR) was calculated from following expression:

$$SR = \frac{M_t}{M_d} \quad (1)$$

where M_d is the weight of dried hydrogels, and M_t is the weight of water-swollen hydrogels after removing excess water from the surface at 20°C and specific times.

II.6 Adsorption kinetics of cationic dye crystal violet

For the adsorption kinetics of CV onto freeze-dried HSY hydrogels, they were immersed in 30 mL of 6 mg/L CV solution. The test temperature was 25°C. During the adsorption, the CV solution was withdrawn from the adsorption system at indicated times for the analysis of CV concentrations by using a UV/vis spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd) at $\lambda_{\max}=584$ nm. For each samples, the withdrawn solutions were feed back into the test system. The amounts of CV adsorbed on the HSY hydrogel at time t , q_t , were determined according to the following equation,

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (2)$$

where, V is the solution volume (mL), m is the weight of freeze-dried hydrogel (g), C_0 and C_t is the dye concentration at initial and indicated time (mg/mL), respectively.

The adsorption efficiency (A_E) was calculated by following equation,

$$A_E = \frac{q_t}{q_e} * 100\% \quad (3)$$

where, q_t is the adsorption amount of CV on the HSY hydrogel at a given time (mg/g), q_e is the maximum adsorption amount (mg/g), respectively. Here, q_e is the value of C_0V/m .

III. Results and discussion

It is known that yeasts are unicellular fungi that belong in the group of simple organisms called Fungi, which exist almost everywhere in natural environments, including the air. Due to a short shelf life of fresh yeast, the active dry yeast is best choice because it can be kept in small foil packets with very high cell activity for one to two years. The specific yeast used in active dry yeast is *Saccharomyces cerevisiae*, also known as ale yeast. In the case of size, the diameter of yeast cell in distilled water observed from optical microscope is about 3-4 μm .

The ale yeast needs to be activated by adding distilled water and sugar before polymerization to obtain a yellowish brown mixture solution. In this work, the rehydration of dried yeasts was firstly performed in warm water at 30°C, and then the white sugar was added to obtain a mixture solution of yeast and sugar. Hereafter, the ale yeast could wake from their dormant state and resume metabolism. With increasing fermentation time, the CO₂ bubbles become bigger on account of integration among bubbles (see Fig.S1 in Supporting Information).

[Fig. 1](#) is the network structure models for microorganism inspired super/macro-

porous hydrogel. As shown in Equation (4), the yeast cells gain energy from the conversion of the sugar into CO₂ and ethanol.



During the polymerization, the by-product CO₂ is expelled and bubbles through the reaction solution before dissipating into the air. The generated CO₂ gas bubbles rise consecutively from the glass mold, but is blocked by the cross-linking network structure of hydrogels, leading to the formation of super/macro-porous structures with various pore size. It is of interest to note that the pore wall between bigger pores also exist pores.

Traditional PAM hydrogels are transparent or opaque, which is strongly dependent on the crosslink density.¹⁹ Fig. 2 shows the appearance of pure PAM and HSY0508 hydrogel prepared by fermentation. It is seen that the pure swollen HSY00 hydrogel without any yeast shows transparency, while HSY0508 hydrogel exhibits brown. After freeze-drying, HSY00 hydrogel presents white with nothing pore in its surface in virtue of the formation of protection layer, but HSY0508 hydrogel exhibits porous structure with same color of yellowish brown. Accordingly, it is believed that the introduction of yeast could change the appearance and pores shape of HSY hydrogel.

Another question is that where the yeast cells are after fermentation and gelation. It is reasonable to suggest that the remaining dead yeast cells still exist in the matrix of HSY hydrogels. As seen in Fig. 3, large numbers of individual yeast cell clumped together in the interior of HSY0404 and HSY0604 hydrogels. It's known that yeasts

normally reproduce asexually-brewing yeasts through a process called “budding”, in which a “mother” cell grows a “daughter” cell that eventually separates to become fully independent yeast. Therefore, it is very difficult to completely remove yeast cells because there are numerous yeast cells distributing into the matrix of HSY hydrogels. The diameter of yeast cell in the matrix of HSY hydrogel is about 3 μm (see Fig.S2 in Supporting Information). And since yeast contains different levels of “sticky” carbohydrates on the surface of their cell walls, the areas with large numbers of yeast cells are flat, leading to invisibility of the pore shape.

To investigate the pore shape of HSY porous hydrogels, the disc HSY hydrogel samples were firstly freeze-dried at -60°C and observed by SEM. Fig. 4 shows the SEM micrographs of freeze-dried HSY porous hydrogels based on yeast, after being swollen up to water-equilibrium. In comparison with pure swollen PAM hydrogel (HSY00), HSY0504 and HSY0508 hydrogels had both bigger pores ranging from 10 μm to 1 mm and numerous closed-pores or interconnected capillary channels in their pore walls. The size of closed-pores in pore walls ranged from 5 to 35 μm . During the preparation, the pre-reaction solution contained certain amount of yeast and sugar which worked together to retain most of the bubbles, many of the CO_2 gas bubbles escaped from the pre-reaction solution because the viscosity of the solution was not high enough. Once the gelation was started, some CO_2 gas was stayed in the interior of HSY hydrogels. The accumulation of CO_2 gas leads to bigger pore, but there is still some CO_2 gas to be escaped. As a result, a compound hierarchical pore structure with super and macro pore was formed, which is the typical characteristics of yeast

inspired hydrogel. Furthermore, it is seen from the magnification graphs of Fig. 4 that no yeast cells were found from pure HSY00 hydrogel, but large numbers of yeast cells existed inside of the HSY0504 and HSY0508 hydrogels and clumped together, which was consistent with the result of Fig. 3. This kind of hierarchical and bigger pore structure may be especially useful in rapid absorbing solvent or separating dye.

The hydrogels in general are mostly characterized by their swelling properties, and can be measured by weight, volume and dimension at different time intervals (to obtain swelling rate) or at equilibrium (to obtain swelling capacity). Here, swelling ratios are measured gravimetrically. Fig. 5 shows the curves of swelling ratio as a function of time for dried HSY hydrogels in deionized water at 20°C. At the beginning of swelling, HSY hydrogels can absorb water very rapidly and swell to equilibrium state in less than 60 min. But, conventional HSY00 hydrogels swell very slowly and the time of dried hydrogels reaching swelling equilibrium is about 180 min. For example, at $t=60$ min, the SR values of HSY00, HSY0504, HSY0508 hydrogels are 5.10, 8.37 and 10.91 g/g, respectively. This point confirmed high porosity causing by fermentation action of ale yeast. However, the SR value of HSY0510 hydrogel is only 7.10g/g, a sharply decrease in comparison with HSY0508. It can be ascribed to the block of pores resulting from the accumulation of ale yeast cells, which is in accordance with the results of Fig. 3 and Fig. 4.

Cationic dye crystal violet (CV) belongs to triphenylmethane group, and is harmful by inhalation, ingestion and skin contact, and has also been found to cause cancer and severe eye irritation to human beings.⁶⁻⁷ Bigger and numerous pores or

interconnected capillary channels may be favorable for CV absorption. To analyze the difference in the adsorption of CV on the HSY hydrogels with and without fermentation process, the appearance of HSY00 and HSY0504 at dried and absorbed state were observed, as shown in Fig.6. It is seen that HSY00 and HSY0504 hydrogels dried at room temperature present colorlessness and yellowish, respectively, which is similar with Fig.2. After taking out from CV solution, HSY0504 hydrogel shows amaranth, and the Chinese character below the HSY0504 hydrogel cannot be seen, which is completely opposite to that of HSY00 hydrogel. Also the remaining CV solution is so clear that all Chinese characters could be seen by naked eye. This phenomenon further indicates that dried HSY hydrogel fermented by yeast could swell and adsorb CV completely from the cationic CV solution on account of bigger and interconnected pore.

To analyze time dependence of cationic CV adsorption amount at different time, the time profile of CV adsorption onto HSY hydrogels with various yeast and sugar contents at 25°C was made, as shown in Fig.7. With increasing adsorption time from 0 to 570 min, the adsorption capacity (Q_t) increases from 0 to 0.71 mg/g. However, the value of Q_t has close relationship with the content of yeast and sugar. With the addition of yeast usage, the Q_t value of HSY00, HSY0504, HSY0508 and HSY0510 hydrogels at 570 min increases from 0.38 to 0.53, 0.68 and 0.71 mg/g. It means that, compared to pure HSY00 hydrogel, the adsorption capacity (Q_t) of HSY hydrogels with fermentation process increase 1.39~1.87 times at $t=570$ min. The more the yeast is used, the more CV molecules are adsorbed. Obviously,

super/macro-porous structures generated by CO₂ under the fermentation process of yeast are responsible for high adsorption amount of CV. Similar tendency can be found from the curves of Q_t of HSY hydrogels with various sugar contents *versus* time. In comparison with pure HSY00 hydrogel, the Q_t of HSY0604 hydrogel with fermentation process increases 1.77 times when t=570 min. The reason also arises from the bigger and interconnected pore structure because the sugar used in the experiment can be converted to CO₂ in the present or absence of oxygen.

On the other hand, a rapid increase of adsorption capacity (Q_t) occurs at 150~300 min. According to the results in Fig.5, HSY hydrogels reach the swelling equilibrium state at about 150 min. Then it can be assumed that the diffusion of water molecules into HSY hydrogel matrix plays a leading role at the primary stage of adsorption. Soon afterwards the CV molecules are quickly penetrated into the matrix of HSY hydrogels through capillary channels or electrostatic interactions.

Furthermore, as mentioned above, the maximum adsorption capacity (Q_t) of HSY hydrogels are all less than 0.80 mg/g. But the adsorption efficiency (A_E) is relatively high. The values of A_E for HSY0510 and HSY0604 hydrogels reach 85% and 90%, respectively. Then it can be inferred that the HSY hydrogels prepared by fermentation possess excellent adsorption efficiency of CV molecules.

To further confirm the adsorption property of CV onto HSY hydrogels, the SEM graphs of HSY00, HSY0508 and HSY0510 hydrogels after adsorption were shown in Fig.8. In comparison with compound pore structure with super and macro-pore of HSY0508 and HSY0510 hydrogels, HSY00 hydrogel presents

well-distributed pore shape. But it's difficult to find any aggregation of CV and change of pore shape for HSY00 hydrogel. In the case of the HSY0508 and HSY0510 hydrogel, numerous pores with micrometer size are full of pore walls of HSY hydrogel matrix, from which it is also hard to find aggregation of CV. However, it can be found from graphs of HSY0508 that smaller pores among bigger pores were blocked by CV molecules. The whole bigger pore seems like a dense film. The adsorbed HSY0510 presents similar property. The inside walls were completely covered, remaining several closed-pores. It is also of interest to note from graphs of HSY0510 that lots of yeast cells are widely distributed in the interior of HSY0510 hydrogels. The white dots are dead yeast cells, which is in good agreement with the results in Fig.3. Above results indicate that the hydrogels prepared by fermentation of ale yeast really are possessed of high adsorption capacity, which is in good accordance with the results in Fig.7.

IV. Conclusions

In summary, the compound super/macro-porous hydrogels were successfully prepared by fermentation of ale yeast and present yellowish brown. The generated CO₂ gas during fermentation and gelation process serves as porogenic agent, leading to the formation of hierarchical porous structure. Both bigger and smaller pores ranging from 5µm to 1mm exist in the matrix of hydrogels. The resulting hydrogel can reach equilibrium state within 60 min. Compared to pure hydrogel, the adsorption

capacity (Q_t) of hydrogels with fermentation process increased 1.39~1.87 times at $t=570$ min. The adsorption efficiency (A_E) of hydrogels prepared by fermentation of yeast exceeds 85%. The pore size, swelling ratios and adsorption capacity can be regulated according to the feed ratio of yeast and sugar.

Acknowledgements

This work has been supported by National Nature Science Foundation of China (21104058, 31200719 & 21174103), and the grant from the Applied Basic Research and Advanced Technology Programs of Science and Technology Commission Foundation of Tianjin (12JCQNJC01400 & 12JCQNJC08600).

Notes and references

- 1 J. Li, H. Yang, H. Wang and J. Ruan, *Mater. Sci. Eng. C*, 2014, **34**, 110-114.
- 2 P. Xu and J. H. Dickeerson, *J. Power Sources*, 2013, **237**, 64-73.
- 3 H. Aghayan, A.R. Khanchi and A.R. Mahjoub, *Appl. Surf. Sci.*, 2013, **274**, 7-14.
- 4 X. Yin, Y. Xue, L. Zhang and L. Cheng, *Ceram. Int.*, 2012, **38**, 2421-2427.
- 5 W. Zhang, M. Gaggl, G. J.G. Gluth and F. Behrendt. *J. Environ. Sci.*, 2014, **26**, 140-146.
- 6 K.L. Spiller, S.J. Laurencin, D. Charlton, S.A. Maher and A.M. Lowman, *Acta Biomater.*, 2008, **4**, 17-25.
- 7 Y. Qiu and K. Park, *Adv. Drug Deliv. Rev.*, 2001, **53**, 321-339.
- 8 I. Tomatsu, K. Peng and A. Kros, *Adv. Drug Deliv. Rev.*, 2011, **63**, 1257-1266.
- 9 K. Deligkaris, T.S. Tadele, W. Olthuis and A. van den Berg, *Sensor. Actuat. B: Chem.*, 2010, **147**, 765-774.
- 10 C.S. Shen, Y. Shen, Y.Z. Wen, H.Y. Wang and W.P. Liu, *Water Res.*, 2011, **45**, 5200-5210.
- 11 A. Matalanis, U. Lesmes, E.A. Decker and D. J. McClements, *Food Hydrocolloid.*, 2010, **24**, 689-701.

- 12 N. Kato and S.H. Gehrke, *Colloid. Surface. B.* 2004, **38**, 191-198.
- 13 P. S. Gils, D. Ray and P.K. Sahoo, *Int. J. Biol. Macromol.*, 2009, **45**, 364-371.
- 14 A. Barbeeta, G. Rizzitelli, R. Bedini, R. Pecci and M. Dentini, *Soft Matter*, 2010, **6**, 1785-1792.
- 15 S.Y. An, Mi.P. Ngoc Bui, Y. J. Nam, K. N. Han, C. A. Li, J. Choo, E. K. Lee, S. Katoh, Y. Kumada and G. H. Seong, *J. colloid. Interf. Sci.*, 2009, **331**, 98-103.
- 16 R. Gugerli, V. Breguet, U. Stockar and I.W. Marison, *Food Hydrocolloid.*, 2004, **18**, 703-715.
- 17 R.P. Jones and P.F. Greenfield, *Enzyme Microb. Tech.*, 1982, **4**, 210-223.
- 18 M.J.R. Nout, *Encyclopedia of Food Safety*, 2014, **3**, 168-177.
- 19 E.A. Kuru, N.Orakdogan and O.Okay. *Eur. Polym. J.*, 2007, **43**, 2913-2921.

Caption of Figures

Fig. 1 The network structure schematic diagram of microorganism inspired super/macro-porous hydrogel.

Fig. 2 The appearance of pure PAM and hydrogel prepared by fermentation.

Fig. 3 The clumped yeast cells of HSY0404 and HSY0604 hydrogels.

Fig. 4 SEM images of HSY00, HSY0504 and HSY 0508 hydrogels.

Fig. 5 The curves of swelling ratios of HSY hydrogel *versus* time at 20°C.

Fig. 6 The appearance of HSY00 and HSY0504 hydrogels at dried and adsorbed state, and CV solution after adsorption.

Fig. 7 Time profiles of CV absorption onto HSY hydrogels at 25°C.

Fig. 8 SEM images of HSY00, HSY0508 and HSY0510 hydrogels after adsorption of CV molecules.

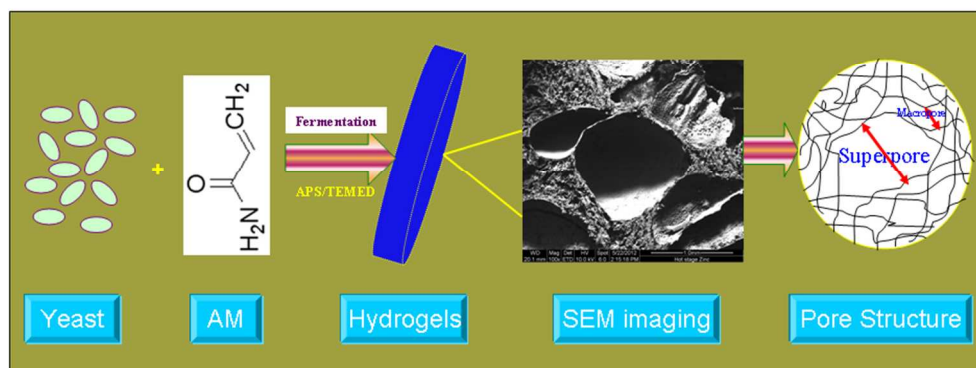


Fig. 1 The network structure schematic diagram of microorganism inspired super/macro-porous hydrogel.
254x190mm (96 x 96 DPI)

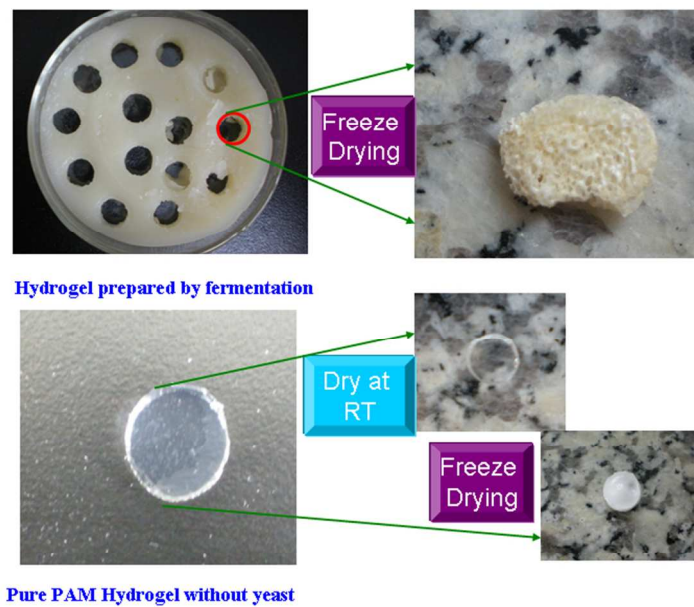


Fig. 2 The appearance of pure PAM and hydrogel prepared by fermentation.
254x190mm (96 x 96 DPI)

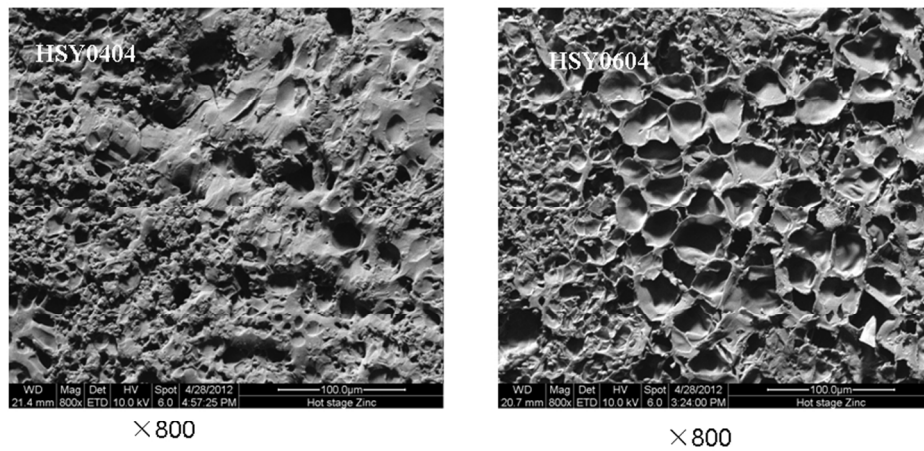


Fig. 3 The clumped yeast cells of HSY0404 and HSY0604 hydrogels.
254x190mm (96 x 96 DPI)

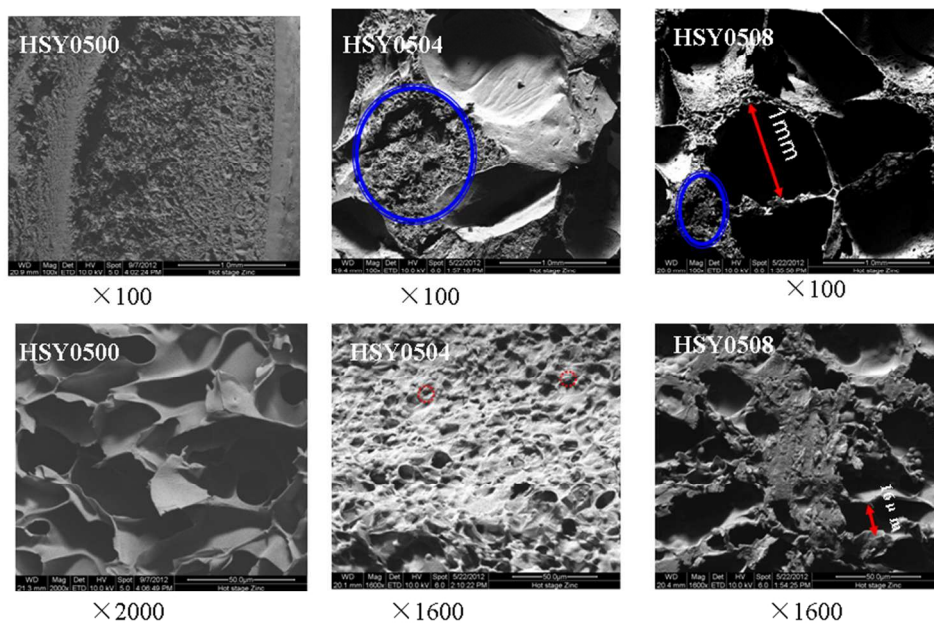


Fig. 4 SEM images of HSY00, HSY0504 and HSY 0508 hydrogels.
254x190mm (96 x 96 DPI)

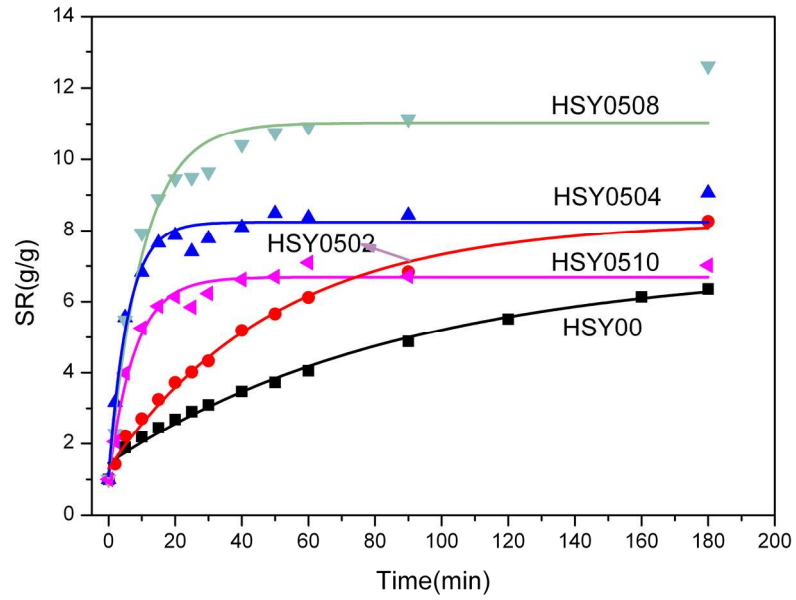


Fig. 5 The curves of swelling ratios of HSY hydrogel versus time at 20°C.
187x130mm (300 x 300 DPI)



Fig. 6 The appearance of HSY00 and HSY0504 hydrogels at dried and adsorbed state, and CV solution after adsorption.
254x190mm (96 x 96 DPI)

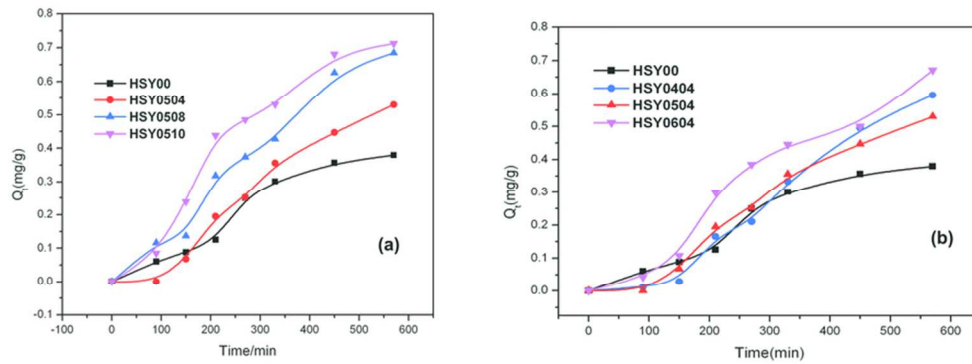


Fig.7 Time profiles of CV absorption onto HSY hydrogels at 25°C.
77x29mm (300 x 300 DPI)

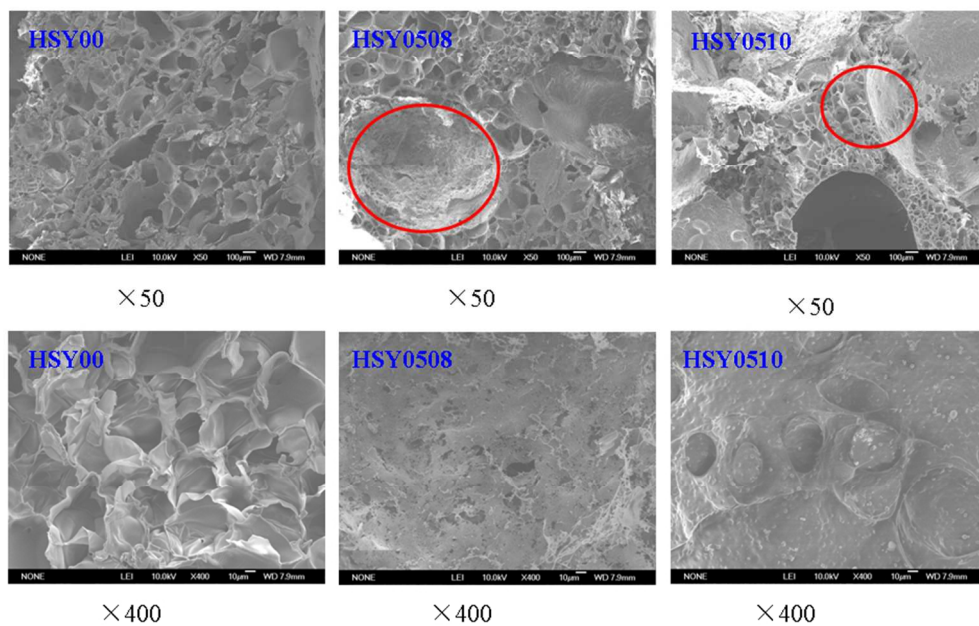


Fig. 8 SEM images of HSY00, HSY0508 and HSY0510 hydrogels after adsorption of CV molecules. 254x190mm (96 x 96 DPI)