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Reactive Toughening of Polyvinyl alcohol Hydrogel and Its Wastewater Treatment Performance by Immobilizing with Microorganism

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\textbf{ABSTRACT:} In order to improve the hydraulic impact resistance of the polyvinyl alcohol (PVA) hydrogel as microorganism immobilization material and meet the requirements of long-time aeration of sewage treatment, the toughening PVA hydrogel beads were prepared by co-crosslinking with glycerol through the boric acid (H\textsubscript{3}BO\textsubscript{3}) - chemical crosslinking method. It was found that glycerol could increase consumption of H\textsubscript{3}BO\textsubscript{3} and decelerate the crosslinking reaction of PVA. The crosslinking structure of borate-PVA monodiol complex (abbreviation as BP) and PVA-borate-PVA didiol complex (abbreviation as BP\textsubscript{2}) formed, and the proportion of BP\textsubscript{2} increased by introduction of glycerol. Moreover, the pores of core layer and surface layer exhibited similar size and relatively uniform structure for PVA/glycerol hydrogel. With increasing glycerol content, the shear storage modulus (G') and the effective network density ($\nu_{e}$) increased first, decreased afterwards, and reached maximum in presence of 1.5 wt % glycerol, indicating the formation of dense network structure of the gel, resulting in an improvement of tensile property and the crushing strength of the gel beads. PVA/1.5 wt % glycerol immobilized with and without microorganism exhibited excellent hydrogel stability during long-term wastewater treatment process. The reactive toughening mechanism of glycerol on PVA hydrogel was explored. The value of oxygen uptake rate (OUR) and COD removal rate of the PVA hydrogel immobilized with activated sludge had no obvious difference with addition of glycerol, and a high microbial activity can be kept.
Keyword: polyvinyl alcohol (PVA) hydrogel; glycerol; porous structure; reactive toughening mechanism; waste water treatment

1. INTRODUCTION

Microbial cell immobilization techniques have been currently extended to wastewater treatments \(^1\), \(^2\). Compared with conventional free cell method, using activated sludge immobilization technology to such systems not only offers a high cell concentration in the reaction tank for increasing biodegradation efficiency, but also it is advantageous for the separation of liquid and solid in the settling tank \(^3\). It has been used to degrade many toxic substances, like phenol \(^4\), 4-chlorophenol \(^5\), 2,4-dichlorophenol \(^6\), quinoline \(^7\), phthalic acid esters \(^8\). And it’s also widely applied in wastewater treatment \(^9\)-\(^13\), like nitrogen removal, heavy metal removal, coke plant wastewater treatment, and so on.

Crosslinked poly(vinyl alcohol) (PVA) hydrogels with hydrophilic three-dimensional polymeric networks, are insoluble in water due to the presence of chemical or physical crosslinks, and exhibit good biocompatibility, non-toxicity, high elastic modulus and low cost, attracting great attention in microorganism immobilization for wastewater treatment \(^14\), \(^15\). However, in sewage aeration tank, the strong shear force produced by oxygen aeration and fluid turbulence made the hydrogel beads easy to be broken and damaged, which required the PVA hydrogel to possess high hydraulic impact resistance in order to meet the requirements of long-time running of sewage treatment \(^16\). Takei et al. \(^17\) prepared PVA hydrogel immobilized with microorganism through the sodium sulfate - chemical crosslinking method, resulting in the improvement of the mechanical strength of the hydrogel. Li et al. \(^18\) used acrylamide and N, N ‘ - methylene double acrylamide to modify PVA hydrogel by copolymerization method, and the product was not easy to break.
For PVA hydrogel beads prepared through the boric acid (H$_3$BO$_3$) - chemical crosslinking method $^{19-21}$, its surface crosslinked rapidly when contacting with the curing agent solution, forming a dense crosslinked shell, and hindering the curing agent solution to further disperse into core layer of the hydrogel, resulting in the low crosslinking degree and loose structure of the core layer. And thus the inhomogeneous crosslinking porous structure formed, leading to low mechanical strength of PVA hydrogel beads.

Glycerol is a simple polyol compound with low toxicity and a reactive molecule that undergoes all the usual reactions of alcohols $^{22, 23}$. The two terminal primary hydroxyl groups are more reactive than the internal secondary hydroxyl group $^{24}$. In particular, H$_3$BO$_3$ could react with glycerol and yield glyceroborate, which was highly presented in the patent literature $^{25, 26}$. In this work, the crosslinking reaction rate of the shell layer and core layer of the PVA hydrogel beads was controlled through the competition reaction of glycerol and PVA with H$_3$BO$_3$, and the homogeneous crosslinking network structure was tried to be achieved. As a result the mechanical strength of PVA hydrogel can be expected to be improved. The effect of glycerol on crosslinking reactive kinetics and crosslinking molecules structure of PVA was investigated. The network structure and toughening mechanism were explored. Furthermore, the PVA hydrogel was applied in the wastewater treatment by immobilizing with microorganism, and the hydraulic impact resistance and waste water treatment efficiency were investigated.

2. EXPERIMENTAL

2.1 Materials

PVA with an average molecular weight ($M_n$) of 74,800 g/mol was supplied by Sichuan Vinylon Co. (China). Sodium alginate (Alg) was purchased from Kelong
Chemical Co. (Chengdu, China). Boric acid (H₃BO₃) and glycerol were purchased from Bodi Chemical Co. (Tianjin, China). Other chemical agents were all of analytical purity and used as received. The wastewater and activated sludge were collected from Chengdu Drainage Co. (Chengdu, China) on the day for preparation of PVA hydrogel beads.

### 2.2 Preparation of PVA hydrogel beads

PVA (10 g), Alg (1 g) and glycerol (0 - 2.5 g) were dissolved in distilled water (100 mL) by magnetic stirring at 95 °C. The solution was cooled to room temperature. The sludge (10 g) was added in the meantime for the sample immobilized with microorganisms. The mixture was then dropped into the saturated H₃BO₃ and CaCl₂ solution (3.0 wt %), keeping for 1h to form spherical beads. The formed beads were finally soaked and washed with distilled water, and stored in wastewater at room temperature. The diameter of hydrogel beads ranged from 4 mm - 6 mm.

### 2.3 Measurements

#### 2.3.1 ¹¹B NMR analysis

¹¹B NMR measurements of H₃BO₃, PVA/H₃BO₃ and PVA/glycerol/H₃BO₃ were performed with a Varian Inova-400 NMR spectrometer (Chesterfield of MI, USA). The samples were dissolved in D₂O, and the test was operated at room temperature and a frequency of 400 MHz.

#### 2.3.2 Mechanical property

The tensile property

The tensile property of the samples of PVA hydrogel was measured with a 4302 material testing machine from Instron Co. (USA) according to ISO 527-1993. Samples with a dumbbell shape and size 150×10×4 mm³ were prepared. The tensile speed and temperature were 20 mm/min and 23 °C, respectively.
The hydraulic impact property

PVA hydrogel beads were put into the distilled water, stirring at room temperature with stirring speed 2,000 r/min. The retention rate of gel beads without damage (the hydrogel beads could keep completely spherical shape and there was no obvious decrease for the diameter of beads ranging from 4 mm - 6 mm) as a function of time was calculated with the following equation 27:

\[
\text{Retention rate (\%) = \frac{(N_0 - N_t)}{N_0} \times 100}\% \quad \text{(2.1)}
\]

Where \(N_0\) was initial number of gel beads; \(N_t\) was the number of gel beads at time \(t\).

2.3.3 Rheological property

The viscoelasticity properties of PVA hydrogel were performed on Rheometer System Gemini 200 of Malvern Instrument Co. (UK) with parallel plates with diameter of 25 mm and a plate-to-plate distance of 1 - 2 mm. Both the strain and the frequency sweep experiments were performed at room temperature. In strain sweep measurements, the shear storage modulus (\(G'\)) and loss modulus (\(G''\)) were recorded at the strain of 0.005 - 100\% and the frequency of 1 Hz. In the frequency sweep experiments, \(G'\) and \(G''\) were measured in the linear viscoelastic regime, for frequencies ranging from 0.1 to 100 Hz, at a maximum strain, \(\gamma\), of 0.1\%. This \(\gamma\) value was determined by preliminary strain sweep experiments, in which \(G'\) and \(G''\) were measured as a function of strain at a fixed frequency value of 1 Hz to check if the deformation imposed on the gel structure during the rheological experiment was entirely reversible.

2.3.4 Scanning electron microscope analysis (SEM)

The fractured surface morphology of PVA hydrogel beads was observed with a JEOL JSM-5900LV scanning electron microscope (SEM) (Japan) at an acceleration voltage of 20 KV. The hydrogel beads were freezing dried and cryogenically fractured.
in liquid nitrogen. Then the samples were sputter-coated with gold for 2 - 3 min.

2.3.5 Oxygen uptake rate analysis (OUR)

100 mL distilled water was put into a flask, and aerated to make the dissolved oxygen saturated by air pump. Stop aeration and five grams of PVA hydrogel beads immobilized with sludge were added. The concentration of the dissolved oxygen (DO) variation with time was measured with AZ 8403 DO meter (China).

2.3.6 Chemical oxygen demand (COD) removal rate

COD value of the wastewater mainly depends on the composition and concentration of organic contaminants, and the absorbance of the organics has good correlation with COD $^{28,29}$. The wastewater solution with different concentrations was prepared, and the UV absorbance of the solution at 254 nm was measured with U3010 UV-visible spectrophotometer (Japan). The standard curve of UV absorbance-wastewater concentration was thus obtained.

In this work, 20 g PVA hydrogel beads immobilized with sludge (1.65 g) were added in a reactor containing 200 mL wastewater. After 6 hours’ aeration, the sample of 20 mL wastewater solution was taken out for UV measurement. The absorbance at 254 nm was measured, and the corresponding wastewater concentration can be obtained by the standard curve. The COD removal rate was then calculated with the following equation:

$$\text{COD removal rate} \, (\%) = \frac{(C_t - C_0)}{C_0} \times 100\% \quad (2.2)$$

Where $C_0$ was the original wastewater concentration before treatment, and $C_t$ was the wastewater concentration after treatment. The original COD of wastewater was 324 mg/L.

2.3.7 Statistical analysis

The quantitative results were obtained from triplicate samples and the data was
expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way analysis of variance, followed by post hoc Student's t-test. A value of p < 0.05 was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Effect of glycerol on crosslinking reaction kinetics of PVA

During the crosslinking reaction of PVA/glycerol system by using H$_3$BO$_3$ as the crosslinking agent, both the hydroxyl groups on the molecule of PVA and glycerol could react with H$_3$BO$_3$. Therefore, the effect of glycerol on the crosslinking reaction kinetics of PVA hydrogel was investigated. Fig.1 illustrated the molar concentration of H$_3$BO$_3$ versus crosslinking time of PVA hydrolgels with different content of glycerol. It can be seen that in the first 20 minutes of reaction, the molar concentration of H$_3$BO$_3$ decreased sharply, and reached equilibrium in about 60 minutes. With increasing content of glycerol, the molar equilibrium concentration of H$_3$BO$_3$ decreased first, reached minimum in presence of 1.5 wt % glycerol, and increased afterwards, indicating that glycerol could increase consumption of H$_3$BO$_3$ during the crosslinking reaction of PVA hydrogel. Under low content of glycerol, with increasing glycerol content in the PVA solution, the increment of glycerol volume fraction in the multi-phase system raised the free energy, resulting in higher interface diffusion coefficient of PVA system, which helped to the crosslinking reaction, thus the consumption of H$_3$BO$_3$ increased. However, under excessive addition of glycerol, the increment of glyceroborate volume fraction yielded by reaction of H$_3$BO$_3$ with glycerol reduced free energy, resulting in lower interface diffusion coefficient, which hindered the crosslinking reaction, and so the consumption of H$_3$BO$_3$ decreased$^{30-32}$. 
The crosslinking reaction kinetics of PVA composite hydrogel was analyzed by assuming that the crosslinking reaction process met the first order kinetic equation:

$$\frac{dB_t}{dt} = k (B_t - B_e)$$  \hspace{1cm} (3.1)

Where $t$ was crosslinking reaction time; $B_t$, molar concentration of $\text{H}_3\text{BO}_3$ at time $t$; $B_e$, molar concentration of $\text{H}_3\text{BO}_3$ when crosslinking reaction reached the equilibrium, and $\frac{dB_t}{dt}$, crosslinking reaction rate; $k$, the crosslinking reaction rate constant. The crosslinking reaction equation can be obtained by integral of the above equation:

$$B_t = B_0 - \frac{(B_0 - B_e)}{e^{kt}}$$  \hspace{1cm} (3.2)

Another form of the equation can be written as:

$$\text{lg}(B_t - B_e) = -kt/2.303 + \text{lg}(B_0 - B_e)$$  \hspace{1cm} (3.3)

By plotting the graph of $\text{lg}(B_t - B_e)$ versus time $t$, as shown in Fig.2, the crosslinking reaction rate constant $k$ can be obtained from the slope of the curve, which exhibited good linear relationship. The crosslinking reaction rate constant $k$ of PVA hydrogel beads with varying content of glycerol was listed in Tab.1. It can be seen that with increasing content of glycerol, the crosslinking reaction rate constant $k$
of PVA hydrogel decreased, indicating deceleration effect of glycerol on the crosslinking reaction.

![Graph showing crosslinking reaction kinetics of PVA hydrogel with various content of glycerol.](image)

**Fig.2 Crosslinking reaction kinetics of PVA hydrogel with various content of glycerol.**

Data points represent mean ± SD (n = 3), P = 0.03

**Tab.1 Crosslinking reaction rate constant PVA with H$_3$BO$_3$**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reaction rate constant k (h$^{-1}$)</th>
<th>Equilibrium H$_3$BO$_3$ molar concentration (mmol L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>1.40</td>
<td>39.26</td>
</tr>
<tr>
<td>PVA/0.5 wt % glycerol</td>
<td>1.30</td>
<td>39.02</td>
</tr>
<tr>
<td>PVA/1.0 wt % glycerol</td>
<td>1.21</td>
<td>35.82</td>
</tr>
<tr>
<td>PVA/1.5 wt % glycerol</td>
<td>1.16</td>
<td>35.31</td>
</tr>
<tr>
<td>PVA/2.0 wt % glycerol</td>
<td>1.07</td>
<td>37.86</td>
</tr>
<tr>
<td>PVA/2.5 wt % glycerol</td>
<td>1.06</td>
<td>37.68</td>
</tr>
</tbody>
</table>

**3.2 Effect of glycerol on crosslinking molecular structure of PVA hydrogel**

H$_3$BO$_3$ in water dissociated into borate ion (H$_4$BO$_4^-$) and hydrogen ion (H$^+$), while H$_4$BO$_4^-$ can react with Cis-ortho-di-hydroxyl groups on the molecular chain of polyol to yield single and bi-crosslinking products$^{33,34}$. PVA with linear hydrocarbon chains containing a lot of 1,3-type hydroxyl groups reacted with H$_3$BO$_3$, resulting in the formation of crosslinking structure of borate-PVA monodiol complex.
(abbreviation as BP) and PVA-borate-PVA didiol complex (abbreviation as BP_2), as shown in Fig.1s. Meanwhile, only crosslinking caused by formation of BP_2 made PVA generate a three dimensional crosslinking network structure, resulting in the formation of hydrogel. Glycerol with three hydroxyl groups on its molecule reacts with H_3BO_3 to form an organic ligand. Compared with PVA, glycerol as a small molecule compound has higher reactive activity when reacting with H_3BO_3. For PVA/glycerol system, H_3BO_3 first reacted with glycerol, and yielded glyceroborate (abbreviation as BG_2), and then the ester exchange reaction occurred between BG_2 and PVA, resulting in the formation of crosslinking structure of BP, BP_2 and borate-PVA-glycerol (abbreviation as BPG), as shown in Fig.2s.

The $^{11}$B NMR spectrums of H_3BO_3, PVA/H_3BO_3 and PVA/glycerol/H_3BO_3 were shown in Fig.3. It can be seen that for pure H_3BO_3, the peak at 4.38 ppm was attributed to the chemical shift of H_3BO_3/H_4BO_4$. For PVA/H_3BO_3 system, three peaks can be observed. The chemical shift of H_3BO_3/H_4BO_4 appeared at 5.59 ppm, which was higher than that of pure H_3BO_3, and the peak became broader. It was perhaps mainly due to the consumption of H_3BO_3 by PVA and relatively low molar concentration of H_3BO_3/H_4BO_4$. The chemical shifts of BP_2 and BP exhibited at 1.71 ppm and 2.37 ppm, respectively. For PVA/glycerol/H_3BO_3 system, the peaks at 6.01 ppm, 1.69 ppm and 2.42 ppm belonged to the chemical shifts of H_3BO_3/H_4BO_4$, BP_2 and BP respectively. A new peak appearing at 4.21 ppm was attributed to the chemical shift of BG_2. The chemical shifts of BP_2 and BP in PVA/H_3BO_3 and PVA/glycerol/H_3BO_3 had partial overlap. The Peakfit software was used to separate them, and the proportion of integration area of each peak in the total peak area was calculated, and listed in Tab.2. It can be seen that compared with PVA/H_3BO_3 system, the proportion of BP_2 in the two crosslinking structures was obviously higher for
PVA/glycerol/H₃BO₃ system, indicating that glycerol could improve the crosslinking degree of PVA hydrogel.

![Fig.3](image)

**Fig.3** $^1$H NMR spectrum of H₃BO₃ (a), PVA/H₃BO₃ (b) and PVA/glycerol/H₃BO₃ (c) in D₂O solution

**Tab.2** $^1$H NMR results of H₃BO₃, PVA/H₃BO₃ and PVA/glycerol/H₃BO₃

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chemical shift (ppm)</th>
<th>Type of B</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃BO₃</td>
<td>4.38</td>
<td>H₃BO₃/H₄BO₄⁻</td>
<td>100</td>
</tr>
<tr>
<td>PVA/H₃BO₃</td>
<td>5.59</td>
<td>H₃BO₃/H₄BO₄⁻</td>
<td>80.97</td>
</tr>
<tr>
<td></td>
<td>2.37</td>
<td>BP</td>
<td>12.01</td>
</tr>
<tr>
<td></td>
<td>1.71</td>
<td>BP₂</td>
<td>7.02</td>
</tr>
<tr>
<td>PVA/glycerol/H₃BO₃</td>
<td>6.01</td>
<td>H₃BO₃/H₄BO₄⁻</td>
<td>28.70</td>
</tr>
<tr>
<td></td>
<td>4.21</td>
<td>BG₂</td>
<td>64.51</td>
</tr>
<tr>
<td></td>
<td>2.42</td>
<td>BP</td>
<td>3.66</td>
</tr>
<tr>
<td></td>
<td>1.69</td>
<td>BP₂</td>
<td>3.13</td>
</tr>
</tbody>
</table>

The observation of the internal morphology of PVA hydrogel and PVA/glycerol hydrogel was carried out. Fig.4 showed SEM images of the cross section, core layer and surface layer of cross section of PVA hydrogel and PVA/glycerol hydrogel. It can be seen that, for PVA hydrogel, the morphology of core layer and surface layer had obvious differences: the pores were dense and small in the surface layer, whereas the
pores were loose and large in the core layer. For PVA/glycerol hydrogel, the pores of core layer and surface layer of exhibited similar size and relatively uniform structure.

The pore size distribution for the PVA hydrogel and PVA/glycerol hydrogel was summarized in Fig.5. It can be clearly observed that for PVA hydrogel, the diameter of pore on the surface layer ranged from 0 - 12 µm, and focused on 2 µm - 4 µm, while the pore size distribution of core layer ranged from 0 - 21 µm, and focused on 0 - 6 µm. However, for PVA/glycerol hydrogel, the pore on the surface layer had larger and uniform size, and the diameter of pore ranged from 0 - 9 µm, and focused on 3 µm - 6 µm. Meanwhile, the pore size distribution of core layer ranged from 0 - 10 µm, and focused on 4 µm - 6 µm. This indicated that introduction of glycerol could control pore size of surface layer and core layer of PVA hydrogel beads, and make the porous structure more uniform.
Based on the above analysis results, the crosslinking reaction mechanism of PVA system can be deduced:

For PVA in absence of glycerol, the crosslinking reaction of PVA with H$_3$BO$_3$ started on the surface layer of the liquid drops of PVA solution and the formed dense crosslinking networks may hinder H$_4$BO$_4^-$ to penetrate into core layer of PVA liquid drops, resulting in a relatively low crosslinking degree and inhomogeneous network structure.

For PVA in presence of glycerol, the crosslinking process included the following two steps:

Step I: glycerol and PVA on the surface layer of the liquid drops reacted with H$_4$BO$_4^-$ to yield glyceroborate and PVA-borate crosslinking networks, respectively. Besides, the reactive rate of the former is higher than that of the latter. H$_4$BO$_4^-$ and
glyceroborate can percolate into the core of the drops.

Step II: ester exchange reaction of glyceroborate and PVA in the core layer of the drops promoted the formation of crosslinking networks in the core layer. This path of reaction made the crosslinking reaction rate constant of PVA/H$_3$BO$_3$ decrease, resulting in a relatively uniform porous structure of the PVA hydrogel beads.

3.3 Effect of glycerol on crosslinking network structure of PVA hydrogel

Fig.6 showed the strain dependence at the frequency of 1 Hz of the shear storage modulus (G'), and loss modulus (G'') for PVA hydrogel with different content of glycerol. At very low strain amplitudes, the loss modulus of the hydrogel was lower than the storage modulus, which was consistent with the existence of a network structure. In addition, it can be noted that, for small strain amplitudes, G' was independent of the strain amplitude, which indicated that the deformation imposed on the network structure was entirely reversible. At higher strain amplitudes, G' was a decreasing function of the strain amplitude and the deformation was no longer reversible.

![Fig.6 Strain dependence of G' and G'' for PVA hydrogel with various content of glycerol](image-url)
The frequency dependence of $G'$ was plotted in Fig. 7 for PVA hydrogel with different content of glycerol. It can be seen that $G'$ does not depend on the test frequency in the range between 0.1 and 100 Hz for all samples, indicating that the elastic behavior of these samples predominated over their viscous behavior, and a perfect network formed. With increasing content of glycerol, $G'$ increased first, reached maximum in presence of 1.5 wt % glycerol and decreased afterwards.

Equilibrium water content (EWC), volume fraction of polymer in the hydrogel at equilibrium swelling, $\Phi_1$, and volume fraction of the crosslinking polymer in the relaxed state, $\Phi_2$, can be calculated as follows $^{37,38}$:

$$ECW(\%) = \frac{W_e - W_d}{W_e} \times 100$$  \hspace{1cm} (3.4)

$$\Phi_1 = \frac{W_d / \rho_p}{W_d / \rho_p + (W_e - W_d) / \rho_s}$$  \hspace{1cm} (3.5)
\[
\phi_e = \frac{W_d / \rho_p}{W_d / \rho_p + (W_r - W_d) / \rho_s}
\]  

(3.6)

Where \(W_d\) was the weight of the dry gel, \(W_r\) was the weight of the relaxed gel and \(W_e\) was the weight of gel in the equilibrium swollen state, \(\rho_p\) and \(\rho_s\) were the densities of polymer matrix and water, respectively. For a blend of PVA and Alg as the matrix, the density of the polymer matrix (\(\rho_p\)) was the same for all the hydrogels and calculated as follows:

\[
\rho_p = \frac{W_{PVA} + W_{Alg}}{W_{PVA} / \rho_{PVA} + W_{Alg} / \rho_{Alg}}
\]

(3.7)

Where \(W_{PVA}\) and \(W_{Alg}\) were the weight fractions of PVA and Alg, respectively, and \(\rho_{PVA}\) and \(\rho_{Alg}\) were the densities of PVA and Alg, respectively.

The effective network density (\(\nu_e\)) of the PVA hydrogel was determined from the following equation based on the rubber elasticity theory:

\[
G = RT \nu_e \phi_1^{1/3} \phi_2^{2/3}
\]

(3.8)

Where \(R\) was the gas constant, \(T\) was the temperature. The average molecular mass between crosslinks, \(M_c\), was calculated as follows:

\[
M_c = \frac{\rho_p}{\nu_e}
\]

(3.9)

The value of \(G'\), \(\nu_e\) and \(M_c\) of PVA hydrogel with different content of glycerol was calculated and listed in Tab.3. It can be seen that, with increasing glycerol content, \(G'\) and \(\nu_e\) increased first, decreased afterwards, and reached maximum in presence of 1.5 wt % glycerol, at this point \(M_c\) reached minimum indicating that a relatively low content of glycerol could promote the formation of relatively uniform and dense network structure in PVA hydrogel, which was coincident with the formation of BP\(_2\) crosslinking structure. Excessive addition of glycerol led to the retarding of PVA crosslinking reaction and decrease of crosslinking density of the whole system.
Tab. 3 Network parameters of PVA hydrogel with various content of glycerol

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\rho_p$ (g/cm$^3$)</th>
<th>$\Phi_1$</th>
<th>$\Phi_2$</th>
<th>$G'$ (KPa)</th>
<th>$\nu_e$ (mol/m$^3$)</th>
<th>$M_c$ (kg/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>1.219</td>
<td>0.1325</td>
<td>1</td>
<td>7.13</td>
<td>5.55</td>
<td>2.20×10$^2$</td>
</tr>
<tr>
<td>PVA/0.5 wt % glycerol</td>
<td>1.219</td>
<td>0.1222</td>
<td>1</td>
<td>10.3</td>
<td>8.23</td>
<td>1.48×10$^2$</td>
</tr>
<tr>
<td>PVA/1.0 wt % glycerol</td>
<td>1.219</td>
<td>0.1308</td>
<td>1</td>
<td>24.9</td>
<td>19.46</td>
<td>0.63×10$^2$</td>
</tr>
<tr>
<td>PVA/1.5 wt % glycerol</td>
<td>1.219</td>
<td>0.1213</td>
<td>1</td>
<td>31.7</td>
<td>25.41</td>
<td>0.48×10$^2$</td>
</tr>
<tr>
<td>PVA/2.0 wt % glycerol</td>
<td>1.219</td>
<td>0.1252</td>
<td>1</td>
<td>13.4</td>
<td>10.63</td>
<td>1.15×10$^2$</td>
</tr>
<tr>
<td>PVA/2.5 wt % glycerol</td>
<td>1.219</td>
<td>0.1262</td>
<td>1</td>
<td>11.3</td>
<td>8.94</td>
<td>1.36×10$^2$</td>
</tr>
</tbody>
</table>

Fig. 8 showed SEM images of cross section of PVA hydrogel beads with and without glycerol. It can be seen that PVA gel beads in absence of glycerol presented closed pores. By addition of 1.5 wt % glycerol, many large open pores formed, so as to provide channels for mass transfer. By addition of 2.5 wt % glycerol, a lot of small dense pores appeared on the wall of large pores.

3.4 Effect of glycerol on mechanical properties of PVA hydrogel

The tensile mechanical property of PVA hydrogel with varying content of glycerol was depicted in Fig. 9. It can be seen that with increasing content of glycerol, the tensile strength and the elongation at break of PVA hydrogel increased
dramatically, and reached maximum at 1.5 wt % glycerol, and then decreased, which was coincident with the $\nu_e$ tendency.

![Graph showing mechanical properties of PVA hydrogel with various content of glycerol.](image)

**Fig. 9** Mechanical properties of PVA hydrogel with various content of glycerol.

Data points represent mean ± SD (n = 3), P = 0.04

Tab. 4 showed the effect of glycerol content on the hydraulic impact resistance of PVA hydrogel beads. It can be seen that addition of glycerol improved the crushing strength of the gel beads. With increasing content of glycerol, the retention rate of gel beads increased first, and reached the maximum at 1.5 wt % glycerol, and then decreased. These results indicated that proper content of glycerol could toughen and strengthen PVA hydrogel.

<table>
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<tr>
<th>Sample</th>
<th>Time (h)</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>8.5</th>
<th>9</th>
<th>10</th>
<th>10.5</th>
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<tr>
<td>PVA</td>
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<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>PVA/0.5 wt % glycerol</td>
<td></td>
<td>64</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PVA/1.0 wt % glycerol</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>60</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PVA/1.5 wt % glycerol</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>96</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>PVA/2.0 wt % glycerol</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>68</td>
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<td>0</td>
</tr>
<tr>
<td>PVA/2.5 wt % glycerol</td>
<td></td>
<td>100</td>
<td>100</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>PVA/3.0 wt % glycerol</td>
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<td>100</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Furthermore, the long-term hydraulic stability of PVA/1.5 wt % glycerol...
hydrogel beads and PVA/1.5 wt % glycerol hydrogel beads immobilized with microorganism in the process of wastewater treatment was investigated. Fig.3 showed photos of the hydrogel samples before and after long-term aeration. It can be seen that after 11 - 22 months aeration test, the surface of the hydrogel beads was not damaged, and the shape and size of the beads were kept well, indicating that PVA/glycerol hydrogel had excellent long-term hydraulic stability.

Based on the above discussion of crosslinking reaction kinetics, the crosslinking molecular structure, porous crosslinking network structure and mechanical properties of PVA/glycerol hydrogel, the reactive toughening mechanism of such hydrogel was deduced: introduction of glycerol changed the crosslinking reaction pathway of PVA by slowing down the crosslinking reaction of PVA with H$_3$BO$_3$ on the surface layer of the liquid drop of PVA solution and promoting the crosslinking reaction in its core layer, resulting in the formation of more regular and uniform porous structure of the hydrogel. In the meantime, the proportion of BP$_2$ crosslinking structure and the effective network density increased, resulting in the improvement of mechanical strength and modulus, and the hydraulic impact resistance of PVA hydrogel beads.

**3.5 Effect of glycerol on the microbial activity of PVA hydrogel immobilized with microorganism**

The PVA hydrogel immobilized with activated sludge was applied in wastewater treatment. OUR was an important parameter to characterize the microbial activity of activated sludge in the wastewater treatment process, which can be calculated with the following equation $^{39}$:

$$OUR = \frac{DO_2 - DO_1}{t_2 - t_1}$$

(3.10)

Where OUR was the oxygen concentration consumed per minute; DO$_1$ was the dissolved oxygen concentration at time $t_1$; DO$_2$ was the dissolved oxygen
concentration at time \( t_2 \); \( t \) was the measuring time.

The effect of glycerol on DO variation of PVA immobilized beads with time in the saturated aerated distilled water was depicted in Fig.10. The value of OUR was calculated and fluctuated between 0.103 - 0.113.

![Fig.10 ρ\[DO\]-t curve of PVA hydrogel beads with various content of glycerol.](image)

Data points represent mean ± SD (n = 3), \( P = 0.04 \)

The effect of glycerol content on the COD removal rate of PVA hydrogel was investigated, as shown in Fig.11. The COD removal rate of PVA hydrogel with different addition of glycerol fluctuated and reached up to 80 - 85\%, implying that the formed large pores of PVA hydrogel beads were beneficial to supply channels for mass transfer of microorganism, and the high microbial activity of the gel beads can be kept well. All the PVA hydrogel beads with porous structure had good permeability, providing channels for mass transfer, while the microbial reaction mainly controlled the waste water treatment efficiency. Therefore, the microbial activity and COD removal rate had no significant change by addition of glycerol.
For the COD removal of PVA/glycerol hydrogel, the dosage was 20 g PVA gel per 200 mL, which meant 100 g/L (glycerol concentration between 0.5 - 2.5%). Thus, the possible carbon release in 6 hours' aeration was estimated as \((10 \text{ to } 15)/100 \times 6 \times 100 \times ((0.5\% \text{ to } 2.5\%)/40\%) = 0.5 - 6 \text{ mg/L}^{40, 41}\). The data were significantly lower than that under normal wastewater treatment process and then the carbon release process can be ignored.

4. CONCLUSIONS

The toughening PVA hydrogel beads were prepared by introduction of glycerol through \(\text{H}_3\text{BO}_3\)-chemical crosslinking method. It was found that addition of glycerol could increase consumption of the \(\text{H}_3\text{BO}_3\) and decelerate the crosslinking reaction PVA hydrogel. The \(^{11}\text{B}\) NMR analysis results showed that compared with PVA/\(\text{H}_3\text{BO}_3\) system, the proportion of BP\(_2\) in the two crosslinking structures was obviously higher for PVA/glycerol/\(\text{H}_3\text{BO}_3\) system. Besides, the introduction of glycerol could control pore size of surface layer and core layer of PVA hydrogel beads, and make the porous structure more uniform. The crosslinking reaction mechanism of PVA system with addition of glycerol was deduced. With increasing glycerol content,
G' and ν increased first, decreased afterwards, and reached maximum in presence of 1.5 wt % glycerol, at this point Mc reached minimum indicating the formation of a relatively uniform and dense network structure of PVA hydrogel, resulting in an improvement of tensile property and the hydraulic impact resistance of PVA hydrogel beads. Compared with pure PVA gel beads, many large open pores formed for PVA/glycerol hydrogel beads, so as to provide channels for mass transfer. Moreover, PVA/1.5 wt % glycerol immobilized with and without microorganism exhibited excellent hydrogel stability during long-term wastewater treatment process. The reactive toughening mechanism of glycerol on PVA hydrogel was also explored. More importantly, the value of OUR and COD removal rate of the PVA hydrogel fluctuated with addition of glycerol implying that the high microbial activity of the gel beads can be kept well.

REFERENCES

1. S. Shi, Y. Qu, Q. Ma, X. Zhang, J. Zhou and F. Ma, Bioresource technology, 2015, 190, 159-166.


**Figure Captions**

Fig. 1 The molar concentration of H$_3$BO$_3$ versus crosslinking time of PVA hydrogel with various content of glycerol. Data points represent mean ± SD (n = 3), P = 0.004

Fig. 2 Crosslinking reaction kinetics of PVA hydrogel with various content of glycerol. Data points represent mean ± SD (n = 3), P = 0.003

Fig. 3 $^{11}$B NMR spectrum of H$_3$BO$_3$ (a), PVA/H$_3$BO$_3$ (b) and PVA/glycerol/H$_3$BO$_3$ (c) in D$_2$O solution

Fig. 4 SEM image of the cross section of PVA hydrogel and PVA/glycerol hydrogel

Fig. 5 Pore size distribution of PVA hydrogel and PVA/glycerol hydrogel. (a) Surface layer of PVA hydrogel (b) Core layer of the PVA hydrogel (c) Surface layer of PVA/glycerol hydrogel (d) Core layer of the PVA/glycerol hydrogel

Fig. 6 Strain dependence of G’ and G” for PVA hydrogel with various content of glycerol

Fig. 7 Frequency dependence of G’ for PVA hydrogel with various content of glycerol

Fig. 8 SEM image of the cross section of PVA hydrogel beads various content of
glycerol (Magnification: 2000 x)

Fig. 9 Mechanical properties of PVA hydrogel with various content of glycerol. Data points represent mean ± SD (n = 3), P = 0.004.

Fig. 10 ρ[DO]-t curve of PVA hydrogel beads with various content of glycerol. Data points represent mean ± SD (n = 3), P = 0.004.

Fig. 11 Effect of glycerol content on COD removal rate of PVA hydrogel beads. Column with error bars represent mean ± SD (n = 3), P = 0.002.

Fig. 1s Schematic representation of reaction formula of PVA with H$_3$BO$_3$.

Fig. 2s Schematic representation of reaction formula of PVA/glycerol with H$_3$BO$_3$.

Fig. 3s The image of the PVA/1.5 wt % glycerol hydrogel beads before aeration (a), PVA/1.5 wt % glycerol hydrogel beads after aeration for 22 months (b), PVA/1.5 wt % glycerol hydrogel beads immobilized with microorganism before aeration (c) and PVA/1.5 wt % glycerol hydrogel beads immobilized with microorganism after aeration for 11 months (d).

Table Captions

Tab. 1 Crosslinking reaction rate constant of PVA with H$_3$BO$_3$.

Tab. 2 $^{11}$B NMR results of H$_3$BO$_3$, PVA/H$_3$BO$_3$ and PVA/glycerol/H$_3$BO$_3$.

Tab. 3 Network parameters of PVA hydrogel with various content of glycerol.

Tab. 4 Crushing strength of PVA hydrogel beads with various content of glycerol.