

The Development of a Highly Efficient Photo-Initiator System and Its Application in the Photo-Immobilization of Activated Sludge

Xiangli Qiao,¹ Yanming Li²

¹School of Environmental Science and Engineering, Shanghai Jiaotong University, Shanghai 200240, China

²School of Mechanical Engineering, Shanghai Jiaotong University, Shanghai 200240, China

Correspondence to: X. Qiao (E-mail: qiao_xl@sjtu.edu.cn)

ABSTRACT: In this study, a highly efficient photo-initiator system was developed, which contained potassium persulfate, *N, N, N', N'*-tetramethylethylenediamine plus benzil dimethyl ketal. The photo-initiator system could successfully initiate poly (ethylene glycol) dimethyl-acrylate prepolymer to polymerize and crosslink under the irradiation of UV rays, in the presence of concentrated activated sludge, finally leading to the formation of immobilized activate sludge pellet beads. The presence of O₂ and thickness of the reaction solution did not influence the photo-immobilization process. Respiratory measurement result demonstrated that most activated sludge kept alive during the photo-immobilization. Mechanical strength of the immobilized cells could be improved by optimizing contents and ratio of the initiator system. The corresponding mechanism was also discussed. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 39838.

KEYWORDS: applications; biomaterials; gels; biocompatibility

Received 21 March 2013; accepted 8 August 2013

DOI: 10.1002/app.39838

INTRODUCTION

Hydrogel is three dimensional network polymer, which contains a large amount of water in swollen state. The water-containing polymer resembles a living tissue and exhibits excellent biocompatibility.¹ If cells, microorganism or bacteria were entrapped in the hydrogel, it is highly expected that most of their metabolic activities could be maintained or even improved in this water-rich micro-environment. Owing to being fixed in the network of hydrogel, microorganisms immobilized would not leak off hydrogel easily, in case the pollution of solution media.^{2,3} Because of high cell density, rapid biochemical reaction and easy separation of solid from liquid, immobilized cells have great potentials in the application of wastewater treatment, chemical synthesis and biochemical reaction, etc.^{4,5}

However, most hydrogels were synthesized by thermal polymerization, in which toxic monomer, chemical crosslinker and initiator were involved, accompanying high temperature.⁶ Furthermore, the polymerization always lasts for more than 4 or 5 h.⁷ If cells or bacteria were present in the reaction mixture, the toxic reagents and high temperature would seriously harm their activities, long polymerization time worsening the situation. In addition, oxygen was always wiped off the polymerization system, sharpening deactivation of the immobilized cells. On the other hand, low mechanical strength was another

drawback of the immobilized cells, which hampered their wide application.^{8,9} So how to maintain bioactivity of cells as much as possible during immobilization, and simultaneously improve carrier's mechanical strength still remained a challenge for scientists and engineers engaged in immobilization field.

Photo-polymerization is a widely accepted technology, which has experienced a rapid growth in the past few decades owing to its significant advantages, namely, simple, rapid, and mild.¹⁰ Unsaturated monomers or pre-polymers can be initiated to form three dimensional hydrogel within a fraction of a second upon illumination under physiological condition at ambient temperature, so it is highly expected that most activities of entrapped cells could be retained during the photo-immobilization. For example, protein and cells were reported to be successfully immobilized in polymer hydrogel by photo-polymerization,¹¹ and also yeast cells or fibroblast cells.¹²

But, it should be pointed out that transparency is an essential prerequisite condition for the smooth proceeding of photo-polymerization, for photon rays penetrate the whole system and photo-initiators or photo-sensitizers distributed evenly in it are excited to a higher state, finally leading to the production of large amount of free radicals initiating polymerization of unsaturated monomers.^{13,14} However, in fact, most immobilization systems were opaque, making penetration of light across the

systems impossible. For example, immobilization of activated sludge in the field of wastewater treatment. In such system, photo-rays irradiated were only absorbed by the upper or surface part, free radicals generated were quickly quenched by oxygen or impurities existed in the system, leading to the failure of immobilization. So the development of a highly efficient photo-initiator system making free radical photo-polymerization go well in a dark or grey system in presence of oxygen, is an urgent task for the wide application of photo-polymerization technology in immobilization field.

In the study, a novel complex photo-initiator system, which comprised potassium persulfate (KPS), *N, N, N', N'*-tetramethylethylenediamine (TMEDA) plus benzil dimethyl ketal (BDK), was developed. It was utilized to initiate the photo-polymerization of poly ethylene glycol prepolymer in the presence of concentrated activated sludge, to prepare the immobilized cells. High mechanical or highly-crosslinked immobilized PEG beads pellets could be obtained by adjusting and optimizing the reaction conditions, comparing with those obtained by thermal redox polymerization. The corresponding photo-immobilization mechanism involved was also discussed.

EXPERIMENTAL

Materials

Polyethylene glycol dimethyl-acrylate pre-polymer (MW: 575), potassium persulfate, *N, N, N', N'*-tetramethylethylenediamine, benzil dimethyl ketal, benzophenone (BP), and 2,2-Diethoxyacetophenone (DEAP), etc. were all obtained from Aladdin Chemical Reagent Company and used without further purification. Chemical reagents utilized were all analytical grade.

Microorganisms

The sludge used for immobilization was obtained from aeration tank of a sewage treatment plant in Minhang District, Shanghai. The concentrated activated sludge used for immobilization was harvested by high-speed centrifugation (3000 rpm/min for 30min); its concentration was estimated to be around 51 g VSS l⁻¹, and its resulting microbial content was about 98%.

Immobilization of Concentrated Activated Sludge in PEG Hydrogel by UV Technology

The concentrated activated sludge obtained was used as the seed for immobilization. A typical immobilization was as follows: a certain amount of PEG pre-polymer was dissolved in 60 mL water, and concentrated activated sludge was added into it under the stirring of a magnetic stirrer. With the addition of photo-initiator into the sol and mixed them well, the reaction mixture was immediately poured into a reaction tank, whose top was covered with a quartz plate. Under the irradiation of UV rays from top for some time, a gelled polymer carrier was finally obtained, which was cut into 3 mm cubic pellets by a scalpel knife. The beads pellets were washed with sterile saline solution to remove excess bacteria.

The UV irradiation system was illustrated schematically in Figure 1.

Mechanical Test

The mechanical strength of the immobilized pellets was measured according to a method described by Reyes.¹⁵ One hundred

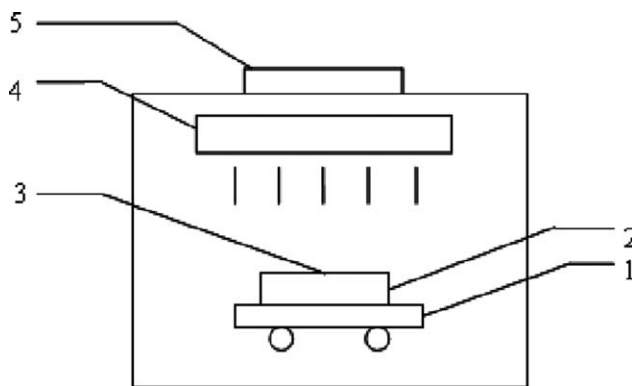


Figure 1. Schematic diagram of photoirradiation apparatus. (1) Mobile sample holder; (2) Reaction tank; (3) Top quartz plate; (4) UV lamp; (5) Ventilation; (4) UV lamp; (5) Ventilation.

of immobilized beads pellets were placed in a shaking flask containing 120 mL NaCl (0.85%) and five glass beads. They were incubated at 35°C under shaking at 300 rpm for 48 h. The flask contents were then filtered through a stainless steel sieve, and the number of the fractured beads was counted. The mechanical strength of the immobilized beads was expressed as fracture frequency $f(\%) = [N/N_t] \times 100$, where N is the number of fractured beads and N_t is the total number of gel beads. Six independent experiments were performed. Fracture frequency.

Water Content Measurement

The value of equilibrium water content, W_e , of the immobilized cells was determined according to the following equation:

$$W_e = \frac{W_s - W_d}{W} \times 100\%$$

where W_s and W_d are the weights of the fully swollen and completely dried immobilized beads, respectively.

Oxygen Uptake Rate (OUR)

The activity of the immobilized activated sludge was determined by the oxygen uptake rate of microbial cells, i.e., respiratory activity as described in document.¹⁶ Around 300 mL synthetic ammonia wastewater medium was placed in a 320-mL incubation vessel. After saturated with air, 30 mL of gel beads or the equal amount of free activated sludge were placed in the vessel. The oxygen electrode was then inserted into the medium solution and the vessel was sealed as a closed system through the entire operation. The measurement was taken under stirring. The time course of DO concentration change in the medium solution was measured at 25°C, which decreased linearly with time during the measurement. The current output of DO measured by the electrode linearly decreased with time at 25°C; therefore, OUR of the immobilized cell could be estimated from the slope of the DO decline curve when the oxygen concentration reached 2.75 ppm. Activity yield (Y_a) of the immobilized cells was defined as

$$Y_a = \frac{R_i}{R_m} \times 100$$

where R_i and R_m were the respiration activity of the immobilized cells and the concentrated activated sludge, respectively. All the measurements were taken in triplicate.

Table I. Effect of Different Photo-Initiators on the Preparation of Immobilization Activated Sludge Under N₂ Protection

Photo-initiator (0.08M)	Irradiation time (min)/ Outer appearance			
	5 min	10 min	20 min	30 min
BP	Liquid mixture	Liquid mixture	Thick Liquid	Viscous liquid
DEOAP	Liquid matrix	Brittle matrix	Soft matrix	elastic matrix
BDK	Elastic matrix	Hard matrix +	Hard matrix +*	Hard matrix+*

(C_{PEG prepolymer} : 12%; C_{concentrated activated sludge} : 8%; temperature: 25°C; mixture depth: 2 mm) "+" indicate that many tiny holes existed across the hard beads; "*" indicate that most cells were killed during irradiation.

RESULTS AND DISCUSSION

Selection of Efficient Photo-Initiator

UV polymerization in the presence of a suitable photoinitiator is one of the most efficient methods for the preparation of highly crosslinked polymers or hydrogels from multifunctional monomers, as suitable initiator can efficiently generate reactive species under UV irradiation, e.g. free radicals, which initiated polymerization and crosslinking of multi-functional monomers and oligomers.

Among radical-type photo-initiators currently utilized in UV-curable system, BP, DEOAP, and DBK were found to give better performances.^{17,18} So in the study, we selected the three initiators to test their efficiency in initiating the photo-immobilization of concentrated activated sludge from PEG prepolymer, reaction solution depth being set at 2mm. To prolong lifetime of free radicals, the experiments were conducted under N₂ protection and the result was demonstrated in Table I.

From data in Table I, we can see that comparing with BP and DEAP, BDK exhibited better photo-initiating performance. Under the same reaction condition, BDK effectively initiated PEG pre-polymer to polymerize and crosslink in the presence of activated sludge, leading to the formation of elastic immobilized activated sludge beads pellets under UV irradiation for 5 min. With an increase in irradiation time, an increase in mechanical strength of the immobilization matrix was observed, and also the change of their outer appearance. Color of immobilized cells was transparent grey at 5 min UV irradiation [Figure 2(a)], while turned opaque white at 30min, with many pinholes penetrating the beads pellets [Figure 2(b)]. However, when the irradiation time lasted for more than 10 min, the obtained pellets would release much cell debris when they were washed with water, indicating that most bacteria activities were seriously damaged.

Comparing with BDK, DEAP exhibited a relative low initiating efficiency: the prepared immobilized matrix varied from liquid at 5 min irradiation time to elastic solid at 30 min.

Of the three photo-initiators tested, BP had the lowest initiating efficiency in the immobilization of activated sludge. The reaction mixture remained liquid, even the irradiation time was prolonged to 30 min.

It was due to the great difference in polarity between BP and water molecule, making the movement of BP molecule and the corresponding free radicals produced greatly limited. It is impossible for the excited BP molecules and free radicals gener-

ated in the upper place of the system to dissipate easily to the surrounding, finally leading to the failure of photo-immobilization.

For DBK and DEAP, the production of free radicals occurred by a fragmentation reaction process named Norrish I.¹⁹ Upon irradiation, cleavage of C—C bond of DEOAP and DBK took place, producing two radicals, a benzoyl radical and a $\cdot\text{C}_6\text{H}_5$ radical. $\cdot\text{C}_6\text{H}_5$ radical was unstable, further generating $\cdot\text{CH}_2$ radicals [(1) in Figure 3]. In our system, DBK was dissolved in N-vinyl-2-pyrrolidone in advance. As N-vinyl-2-pyrrolidone could mixed thoroughly with water, DBK was evenly distributed in the whole system, thus free radicals generated in the upper part of the system rapidly diffused to the surrounding position. Because Benzoyl and $\cdot\text{CH}_2$ radical were two highly reactive species, they rapidly attacked double bonds of PEG prepolymer to polymerize [see path (3) in Figure 4], crosslink [path (4) in Figure 4], or cyclize [path (5) in Figure 4], leading to the formation of hydrogels. It is reasonable to expect that with an increase in irradiation time, higher mechanical strength hydrogels could be obtained, as long irradiation time meant more protons were absorbed by the system and more free radicals were produced. The free radicals generated attacked the double bonds of PEG prepolymer, leading to the formation of more compact and crosslinked hydrogels.

For DEAP, a benzoyl radical and a $\cdot\text{C}_6\text{H}_5$ radical were produced under UV irradiation [(2) in Figure 3]. The former was the major initiating species, while the other fragment, $\cdot\text{C}_6\text{H}_5$, was relatively less reactive and not prone to free radical polymerization, but tended to participate in termination by coupling reaction. Less free radical meant low crosslinking density, thus lower mechanical immobilized beads were obtained with DEAP, comparing with those obtained with DBK.



Figure 2. Experimental photos of the immobilized cells prepared for 5 min (a) and 30 min (b) UV irradiation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

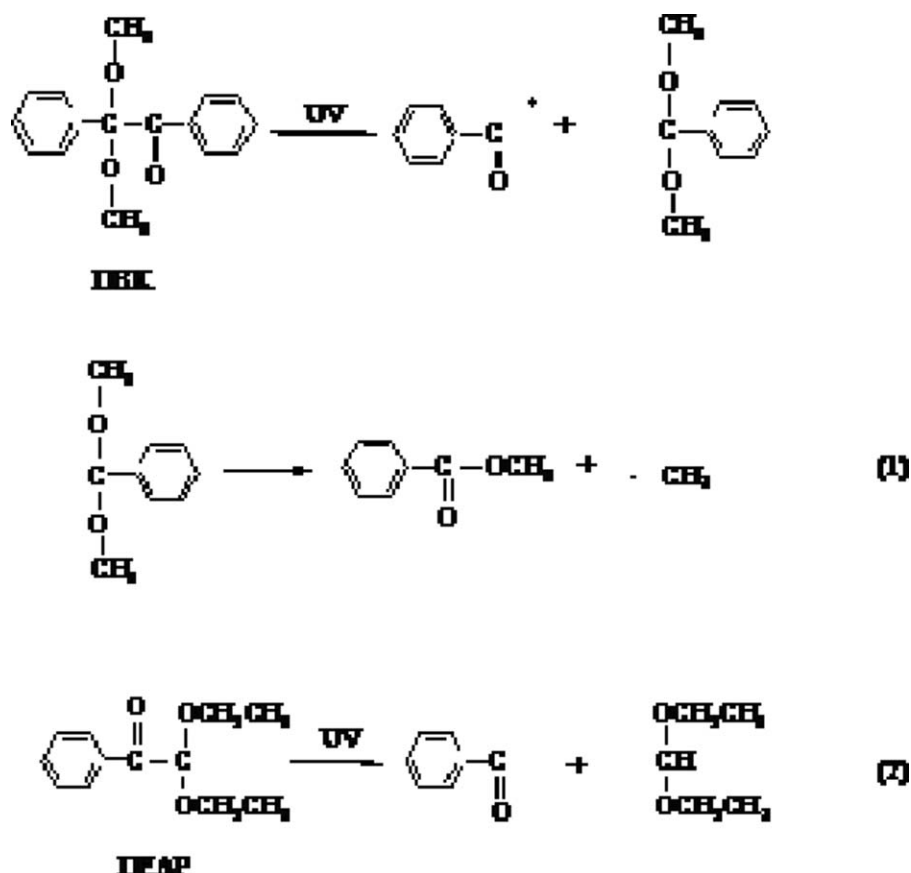


Figure 3. Photo-initiating mechanism of the initiator DBK and DEAP.

In addition, it also can be seen from Table I that although mechanical strength of the beads pellets improved with irradiation time, activities of immobilized bacteria were greatly damaged. To maintain bacteria activity during immobilization, irradiation time should be short as much as possible. In our study, 5 min irradiation time was selected.

Influence of O₂ and Solution Depth on Photopolymerization

However, light attenuate rapidly across solution, so photopolymerization can not go on in large scale. In our study, not only the monomer and solvent absorbed and screened UV light, but also the concentrated activated sludge did, so depth of the

reaction solution would be largely limited. Besides, oxygen was another important factor inhibiting photo-polymerization. So, in the next step, we conducted the photo-immobilization in air or in N₂ atmosphere, and also investigated the influence of solution height on cell immobilization (listed in Table II).

Data in Table II demonstrated that with an increasing in mixture depth from 2 mm to 15 mm, the obtained immobilized matrix varied from elastic hydrogel to liquid. This indicated that although DBK could effectively initiate the polymerization and crosslinking of PEG prepolymer in the presence of activated sludge, while with an increase in solution depth, the degree of polymerization and crosslinking of PEG prepolymer sharply decreased, making the whole system remain liquid, even the irradiation time was prolonged to 15 min (data not shown). This may be ascribed to the following reason: light intensity decreased exponentially with depth, as described by Beer's law. The initiator was consumed at a rate proportional to the local light intensity, thus the concentration of free radicals near the light source is much higher than that far away. Because of unfavorable surface-to-volume ratio, free radical concentration of the whole system decreased, making the total monomer conversion decreased abruptly across the opaque system.

On the other hand, the presence of oxygen quenched the free radicals produced, leading to the failure of the immobilization. As oxygen readily react with carbon-based polymerizing radicals at the diffusion-controlled limit to form peroxy radicals that

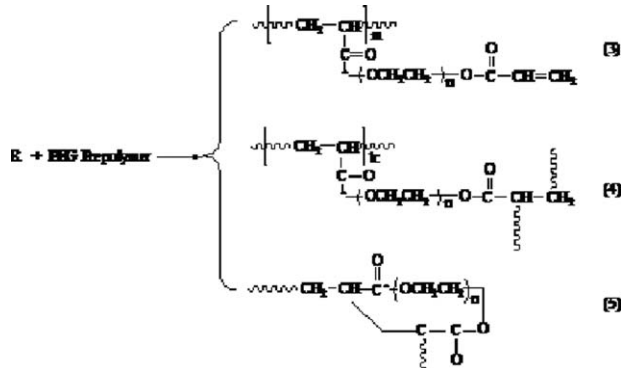


Figure 4. Photo-polymerization pathways of PEG prepolymer.

Table II. Influence of Solution Depth and O₂ on the Immobilization of Activated Sludge

Depth of reaction mixture (mm)		2	5	10	15
Outer appearance of resulting matrix	in N ₂	Elastic matrix	Thick liquid	Liquid	liquid
	in the air	Elastic matrix	Thick liquid	Liquid	liquid

C_{PEG prepolymer}: 12%; C_{concentrated activated sludge}: 8%; irradiation time: 5 min; temperature: 25°C; initiator: DBK.

were much less reactive towards double bonds, thereby reducing the initiating efficiency.²⁰ However, in our study, the change of atmosphere (in N₂ and air) did not have much influence on the immobilization of activated sludge (see data in Table II). This indicated that oxygen did not play an important role in the photo-immobilization. This may owe to the following reasons: from one side, PEG dimethyl-acrylates were two-functional prepolymer, whose reaction rate with free radicals being much higher than that between O₂ and free radicals,²¹ making photo-polymerization and crosslinking of PEG prepolymer preferentially take place; from another side, the irradiation time was long enough, making the initiator consume oxygen faster than the ingress of oxygen from atmosphere, so oxygen inhibition was less prominent.

Development of High Efficient Photo-Initiator System

From above analysis, it can be deduced that in order to successfully immobilize the activated sludge in large scale, there are two methods can be taken. One is let the light penetrate the whole system, making more free radicals produced. Obviously, it is impossible to realize as aggregates or particles in the opaque system would absorb, reflect, and screen the light. The other method is to develop a photo-initiator system, making free radicals diffuse rapidly across the whole system, ensuring the smoothly proceeding of the polymerization.

Persulfate salt is a kind of mild hydrophilic oxidizing agent. It is readily dissolved into water and easy to diffuse long distance in a short time, so it is expected that persulfate salt was a suitable photo-initiator candidate for immobilization. However, its main drawbacks are slow oxidizing speed and poor initiating efficiency under UV irradiation, for its sensitivity to atmospheric oxygen.²² In our experiment, variation in KPS concentration (1–5%) did not affect the immobilization of activated sludge: the formulation (10-mm depth) remained liquid even for 10 min UV irradiation (data not shown). In order to improve its oxidizing efficiency and simultaneously eliminate negative effect of O₂ on the immobilization, a tertiary amine, TEMED, was added to the reaction media. The influence of TEMED concentration on properties of the immobilized activated sludge was demonstrated in Table III (KPS concentration was fixed at 0.01M).

Table III. The Influence of Formulation Depth on the Properties of the Immobilized Activated Sludge

Formulation depth (mm)	5	10	20	40	60
W _e (%)	91	90	92	91	91
f (%)	21	20	20	22	20

C_{KPS} = 0.01M, C_{TEMED} = 0.02M.

We can see that with the addition of TEMED, the immobilized activated sludge were not only successfully prepared, but also not influenced by solution depth and the presence of O₂ (see data in Table III). The optimizing concentration for TEMED was 0.02M.

In order to evaluate the role of UV irradiation played in the immobilization, we thereafter prepared the immobilized cells initiated by the combination of potassium persulfate (KPS) with *N, N, N', N'*-tetramethylethylenediamine (TMEDA) without UV irradiation, and compared them with those with UV irradiation (see data in Table IV), finding that mechanical stability of the immobilized cells correspondingly decreased. This indicated that UV irradiation could accelerate the production of free radicals for KPS plus TMEDA initiator system, thereby increased the mechanical strength of the immobilized cells.

However, the obtained immobilized matrix had low mechanical strength (see Tables III and IV), with swelling ratio being up to 95%. Low mechanical strength meant low crosslinking density. So, if enough free radicals were generated, crosslinking density and mechanical stability of the immobilized cells would be improved.

From above discussion, we can see that DBK had the highest efficiency in initiating the immobilization of activated sludge, so we tried to combine DBK with KPS plus TMEDA, and tested initiating efficiency of the complex system. Gel strength and swelling behavior of the obtained PEG hydrogels were studied (see Table V).

With the addition of DBK, mechanical strength of the resulting immobilized hydrogel was improved greatly, e.g., the fracture ratio of the immobilized matrix decreased with an increase in DBK concentration, with minimum value of *f* being 14 at 0.03M DBK. Furthermore, swelling ratio of the obtained immobilized cells also decreased. The optimizing concentration of DBK in this complex photo-initiator system was 0.03M.

Table IV. The Influence of TEMED on the Properties of the Immobilized Activated Sludge in Ambient Air Under UV Rays

TEMED content (M)	0.005	0.01	0.02	0.03	0.05
W _e (%)	95	94	91	93	94
f (%)	32	28	21	26	27

(C_{KPS} = 0.01M, formulation depth: 5 mm). *W_e and *f* of the immobilized cells initiated by combination of KPS and TEMED without UV irradiation was 93 and 25, respectively (C_{KPS} = 0.01M, C_{TEMED} = 0.02M).

Table V. The Influence of the Addition of DBK to KPS Plus TEMED on the Properties of the Prepared Immobilization Cells (KPS and TEMED Were kept at 0.01M and 0.02M, Respectively)

DBK concentration (M)	0.005	0.01	0.03	0.05
W_e (%)	93	90	88	87
f (%)	18	16	14	15

* W_e and f of the immobilized cells initiated by combination of KPS/TEMED/DBK without UV irradiation was 93 and 26, respectively ($C_{KPS} = 0.01M$, $C_{TEMED} = 0.02M$, $C_{DBK} = 0.03M$).

In addition, W_e and f of the immobilized cells initiated by combination of KPS/TEMED/DBK (the photo-initiator system has been applied for patent) without UV irradiation was also demonstrated (see *data below Table V), their value being 93 and 26, respectively, almost same to those obtained by KPS plus TEMED without UV irradiation. This fact demonstrated that UV also played an important role in the production of free radicals from the combination initiator of KPS/TEMED/DBK or KPS/TEMED.

Moreover, formulation depth did not affect properties of the prepared immobilized cells: their swelling ratio and fracture frequency did not varied much with the depth (data not shown). These facts indicated that synergistic effect existed among DBK, KPS, and TEMED: much more free radicals were generated by the complex initiator system under UV irradiation and they could be transferred rapidly to the whole system, initiating the PEG prepolymer to immobilize the concentrated activated sludge.

Reactions (6)–(9) in Figure 5 showed conventional oxidation route of aqueous persulfate salt, which could finally lead to the formation of hydroxyl, carbonyl etc. groups in the reaction mixture. In this route, the sulfate anion free radical ($SO_4^{\cdot-}$) produced by the decomposition of $S_2O_8^{2-}$ [reaction (6)] may attack water molecule to give out $HO\cdot$ (reaction (7)) or abstract hydrogen atom from C–H bonds of polymer surface. As both $SO_4^{\cdot-}$ and $HO\cdot$ were able to abstract hydrogen atom from a polymer molecule forming macro-free radical (reaction (8)), polymer chain growth thus took place. Carbonyl group [reaction (9)] came from the participation of O_2 during the polymerization. In the present experimental setup, there was little difference in polarity between persulfate salt aqueous solution and aqueous formulation, so the initiator and free radicals diffused rapidly across the water reaction solution.

From above discussion, it can be deduced that the presence of DBK in the co-initiator system may have the following

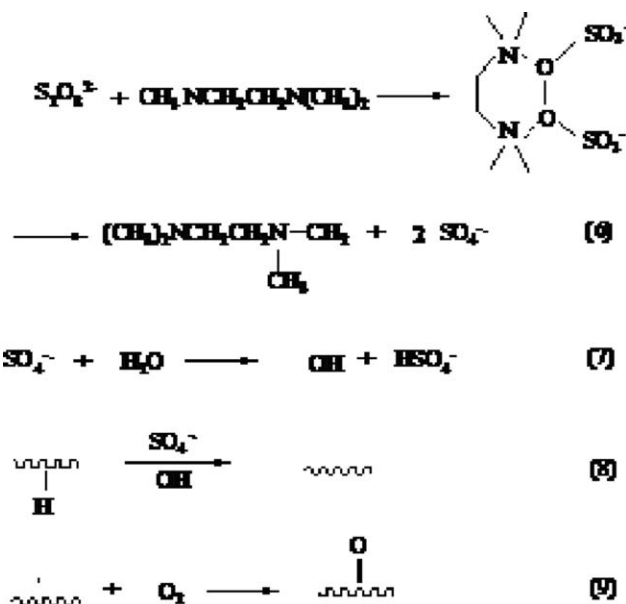


Figure 5. Initiating mechanism of KPS plus TEMED.

functions: (1) accelerated the consumption of O_2 , making more free radical alive; (2) Radicals formed from DBK (most on the surface) under UV irradiation not only initiated the PEG prepolymer to polymerize, but also abstracted H from TEMED. Radical of TEMED move from surface to far away to initiate the polymerization of PEG pre-polymer; (3) act as H donor: $SO_4^{\cdot-}$ or $HO\cdot$ produced by KPS rapidly diffused and attracted H atoms from DBK molecule far from the UV source, generating more free radicals (see Figure 6). The free radicals generated were distributed evenly in the whole system, accelerating the polymerization and crosslinking of PEG chains, and finally compact and high mechanical hydrogels came into being.

To sum up, photo-immobilization of activated sludge in PEG prepolymer solution initiated by DBK+KPS+TEMED can be separated into three events: (1) KPS, TEMED, and DBK adsorbed UV light and got excited in the upper part of the formulation, generating free radicals; (2) some of them make polymer chain grow and crosslinked, while the others rapidly transferred to a certain distance, and attracted H atoms from DBK or TEMED far from UV source, generating some more reactive free radicals; (3) these active species in dark area ensured the smooth photo-polymerization and crosslinking of PEG prepolymer, resulting in the formation of high mechanical strength immobilized cells.

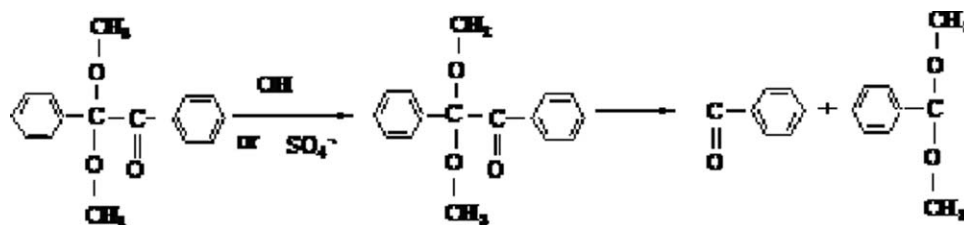


Figure 6. Free radicals generation of DBK molecule attacked by $OH\cdot$ and $SO_4^{\cdot-}$ far from UV source.

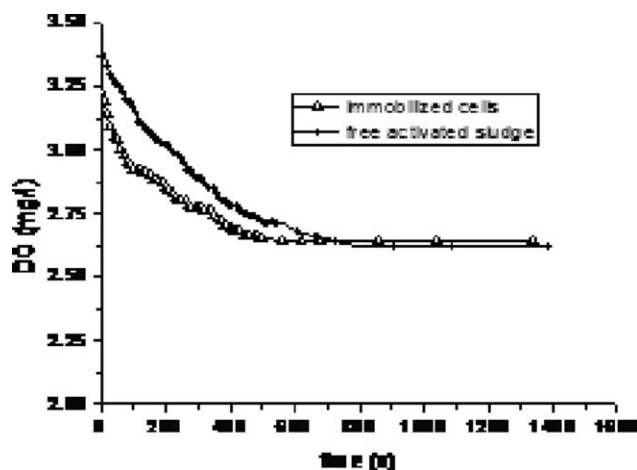


Figure 7. Variation of DO with time.

We also examined the effect of photo-immobilization on respiration activity of bacteria, and compared respiration activity of the immobilized cells with that of free activated sludge (see Figure 7).

Data in Figure 7 demonstrated that there was no big difference in respiration activity between the immobilized cells and free activated sludge. Respiratory activity of the concentrated activated sludge before immobilization was measured to be $10.8 \text{ mg O}_2 \text{ g}^{-1} \text{ VSS h}^{-1}$, while that of the immobilized cell was $9.5 \text{ mg O}_2 \text{ g}^{-1} \text{ gel h}^{-1}$, thereby around 88% of an activity yield was obtained for bacteria after immobilization. The high activity yield indicated that our proposed photo-immobilization of activated sludge from PEG was mild and UV polymerization was an efficient method for maintaining bacteria activity during immobilization.

CONCLUSIONS

DBK was an efficient photo-initiator in the immobilization of concentrated activated sludge in poly(ethylene glycol) dimethylacrylate aqueous solution. However, solution depth greatly affected the immobilization effect, making activated sludge immobilization fail. KPS plus TMEDA system could successfully photo-initiate PEG prepolymer to immobilize the concentrated activated sludge, not influenced by solution depth. But its drawback was low mechanical strength of the obtained PEG pellet beads. The addition of DBK to the KPS plus TMEDA initiator system could solve the problem. They accelerated the production of more free radicals, leading to the formation of compact and highly crosslinked PEG carriers. Respiratory measurement showed that about 88% activity of concentrated activated sludge was maintained during the photo-immobilization. These results indicated that photo-polymerization was a mild and efficient technique, suitable for application in micro-organism immobilization.

ACKNOWLEDGMENTS

The authors thank the financial support of National Science Foundation of China by a grant No. of 50708058.

REFERENCES

1. Haque, M. A.; Kurokawa, T.; Gong, J. P. *Polymer* **2012**, *53*, 1805.
2. Meunier, C. F.; Rooke, J. C.; Hajdu, K.; Cutsem, P. V.; Cambier, P.; Leonard, A.; Su, B. L. *Langmuir* **2010**, *26*, 6568.
3. Bashan, L. E.; Bashan, Y. *Water Res.* **2004**, *38*, 4222.
4. Elgren, T. E.; Zadvorny, O. A.; Brecht, E.; Douglas, T.; Zorin, N. A.; Maroney, M. J.; Peters, J. W. *Nano. Lett.* **2005**, *5*, 2085.
5. Ignacio, M. G. *Bioresour. Technol.* **2008**, *99*, 3949.
6. Bianco, G.; Gehlen, M. H. *J. Photochem. Photobiol. A* **2002**, *149*, 115.
7. Boyer, C.; Bulmus, V.; Davis, T. P.; Admiral, V.; Liu, J. Q.; Perrier, S. *Chem. Rev.* **2009**, *109*, 5402.
8. Chen, J. P.; Lin, Y. S. *Process Biochem.* **2007**, *42*, 934.
9. Martinez, A.; Sergio, D.; Bustos, T. Y. *Biodegradation* **2009**, *20*, 709.
10. Li, L.; Lee, L. *J. Polymer* **2005**, *46*, 11540.
11. Totsuka, R.; Yamashita, H.; Chino, Y. Enzyme or microorganisms immobilization carrier for e.g. bioreactor comprises polymerizable unsaturated group-containing resin, polymerization initiator, water-soluble polymer polysaccharide, and aqueous liquid-like composition. JP2012070678-A.
12. Mishra, S.; Scarano, F. J.; Calvert, P. J. *Biomed. Mater. Res. Part A* **2012**, *100*, 2829.
13. Mishima, K.; Watanabe, A.; Endo, K. Jpn Patent No. Jp1216688, **1986**.
14. Nilsson, I.; Ohlson, S.; Häggström, L.; Molin, N.; Molin, K.; Mosbach, K. *Appl. Microbiol. Biotechnol.* **1980**, *10*, 261.
15. Reyes, N.; Rivas-Ruiz, I.; Dominguez-Espinosa, R.; Solis, S. *Biochem. Eng. J.* **2006**, *32*, 43.
16. Chen, K. C.; Lee, S. C.; Chin, S. C.; Houng, J. Y. *Enzyme Microb. Technol.* **1998**, *23*, 311.
17. Fouassier, J. P., Ed. *Photo-Initiating, Photo-Polymerization and Photo-Curing*, London: Hanser Publisher, **1995**. p 125.
18. Andrzejewska, E. *Prog. Polym. Sci.* **2001**, *26*, 605.
19. Yang, W.; Benct, R. *J. Appl. Polym. Sci.* **1996**, *62*, 545.
20. Deng, J. P.; Yang, W. T.; Rånby, B. *J. Appl. Polym. Sci.* **2000**, *77*, 1513.
21. Lee, T. Y.; Guymon, C. A.; Jönsson, E. S.; Hoyle, C. E. *Polymer* **2004**, *45*, 6155.
22. Yang, P.; Deng, J. Y.; Yang, W. T. *Polymer* **2003**, *44*, 7157.