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Studies on Runoff Wastewater Remediation Technology Based on Immobilized Microorganisms

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ABSTRACT

To study the impact of immobilized microorganisms on runoff wastewater treatment, the immobilized microbial activity pellets have been prepared in the paper by using polyvinyl alcohol (PVA) as the embedding agent, the sodium alginate, silicon dioxide (SiO_2), calcium carbonate, attapulgite or activated carbon as the additive and the Limon microbial bacteria as the microbial agent. Through the orthogonal experiment, the optimal embedding condition of microbial immobilization is determined by taking the removal rate of overlying waterbody COD and NH_4^+-N as the assessment index, reaching up to 71.87% and 92.65% respectively. The correlation analysis shows that the simultaneous nitrification and denitrification reactions are carried out in the removal process of overlying waterbody NH_4^+-N.

INTRODUCTION

River pollution is one of the most prevalent and prominent environmental problems that are difficult to be solved worldwide. The river sediment is an important part of the river ecosystem, which is not only the central part of all kinds of material recycling in the river, but also the main gathering base of such substances. The sediment is the main "pollutant source" and "pollutant base" in the water (Zhang et al. 2010). Therefore, the research and governance of river sediment pollution is an important part of the comprehensive improvement of river pollution as well as one important way to fundamentally solve the aforesaid problem.

There are three main remediation methods for river sediment, i.e. physical remediation, chemical remediation and bioremediation technologies (Wang et al. 2008), wherein the physical and chemical remediation technologies are not the ideal methods with the characteristics of larger cost, slower effect and potential eco-destructiveness. Currently, the most practical approach is the immobilized microbial method, which employs certain techniques (including adsorption, embedding, crosslinking and closure method etc.) to allow microorganisms growing immobilized and not suspending in the water, but still maintaining their biological activity and repeated usage (Sanjeev et al. 2004). Embedding method adopts a particular polymer material as the carrier to immobilize the microorganisms in the carrier network structure, and it takes advantage of the carrier's own structural characteristics to enable the free access of matters with small molecules, and the interaction of those matters with internal microorganisms to achieve the effect of removing contaminants.

The application of immobilized microbial technology can degrade the degradation-resistant heavy metal wastewater, organic wastewater and ammonia wastewater etc. effectively (Li et al. 2005, Mata et al. 2007, Liu et al. 2010, Kazuichi et al. 2007, Xiao et al. 2009), but the technology has not yet been involved in the research and application of ecosystem remediation of riverbed sediment. In this paper, the optimal embedding condition of microbial immobilization is researched by using the sodium alginate, SiO_2, calcium carbonate, attapulgite or activated carbon as the additive, PVA as the embedding agent and the Limon microbial bacteria as the microbial agent.

MATERIALS AND METHODS

Chemicals and materials: Chemicals include anhydrous calcium chloride ($CaCl_2$), boric acid (H_3BO_3), calcium carbonate ($CaCO_3$), anhydrous sodium chloride (NaCl), sodium alginate ($C_5H_7O_4COONa$), polyvinyl alcohol ($[C_2H_4O]_n$), and SiO_2; all chemicals were analytically pure.

Microbial agents are Limon microbial bacteria S-1, which include *Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis*, fiber *Aeromonas*, *Pseudomonas* denitrification, *Rhodopseudomonas palustris*, dinitrogen *Cellulomonas*, and Shi *Pseudomonas*, etc. Limon microbial bacteria N-1, include the nitrification bacteria, and so on.

Attapulgite: 300 mesh; Activated carbon powder: 300 mesh.

Experimental device: Plexiglass container with dimensions of 35cm × 35cm × 50cm was used as the reaction equipment to simulate the repair experiment of polluted sediments in the river. And the pretreated 10cm polluted sediments were evenly tiled in the reaction equipment. The contaminated sediment and waterbody applied in the experiment were taken from Taihu Wangyu River (the east coast of Wangyu River to the east, the east coast of Xicheng Canal to the west, the Yangtze River to the north, and the Taihu lake to the south.)

Preparation of immobilized microbial activity pellets

1. Preparation of PVA gel: A certain amount of PVA is weighed as the requirement of concentration and added to 100mL water. After the infiltration for 24h, the PVA was completely dissolved in the thermostat water bath at 85°C, then a certain amount of additives (sodium alginate, SiO_2, $CaCO_3$, attapulgite power or activated carbon power) was added to stir uniformly and cool for 4h to room temperature, and finally a certain amount of activated Limon microbial bacteria was added (S-1:N-1 = 1:1).
2. Preparation of crosslinking agent: A certain amount of boric acid and anhydrous calcium chloride were weighed and dissolved in 200mL water to prepare the boric acid solution saturated by 2% calcium chloride, and pH adjusted by sodium carbonate to 7.5.
3. The uniformly mixed PVA gel was added dropwise to the boric acid solution saturated by 2% calcium chloride through a peristaltic pump, the spherical particles of immobilized microbial activity with the diameter of about 3-5mm were obtained through the continuous stirring using a magnetic stirrer, and then the particles were cross-linked for a fixed time when placing in a refrigerator at 4-8°C, and at last, the normal saline was used for rinsing and stand-by application after the removal. The process for preparing the particles of immobilized microbial activity pellets is shown in Fig. 1.

OPTIMAL EMBEDDING CONDITIONS FOR MICROBIAL IMMOBILIZATION

Selection for Main Parameters of Embedding Conditions of Immobilizing Process

In the preparation process of immobilized microbial activity pellets, the influencing factors for pollutants degraded by immobilized pellets are mainly the concentration of PVA embedded agents, additives, microbial bacteria as well as the crosslinking time (Zhang et al. 2006).

Concentration of PVA embedded agents: The research of (Sun et al. 2009) finds the appropriate PVA concentration in immobilized microbial technology of 7.5 % to 10 %. The overly low PVA concentration results in the insufficient hardness of particles and the hard formation, easily breaking; while the overly high PVA concentration results in the dense gel and the increased mass transfer resistance, causing the ineffective treatment result. In summary, the 8% and 10% PVA concentrations are selected.

Concentration of the additives: The research of (Huang Chuan et al. 2008) shows that in the process of embedding immobilized microorganisms, both the permeability and mechanical strength of PVA particles have improved to some extent after the proportionate addition of small amount of sodium alginate, SiO_2 and calcium carbonate. And both the mass transferability and the mechanical strength of microbial pellet are enhanced after the addition of activated carbon power or attapulgite power. On the basis of previous experiments, the alginate concentrations of 0.5 % and 1 %, calcium carbonate concentrations of 0.2 % and 0.4 %, SiO_2 concentrations of 2.5 % and 3.5 %, and the concentration of activated carbon power or attapulgite power of 0.5 % have been selected.

Concentration of microbial bacteria: Concentration of microbial bacteria determines the content of microorganisms in immobilized microbial pellets, having a direct impact on the removal of pollutants. The overly low concentration of microbial bacteria results in the incomplete removal of contaminants; while the overly high concentration of microbial bacteria results in the easy precipitation of microorganisms. On the basis of previous experiments, the microbial bacterial concentrations of 5 % and 10 % have been selected.

Crosslinking time: Crosslinking time refers to the forming time of immobilized microbial pellets in the saturated boric acid solution. The overly short crosslinking time results in the inadequate pellet strength; while the overly long crosslinking time results in the reduction of cell activity due

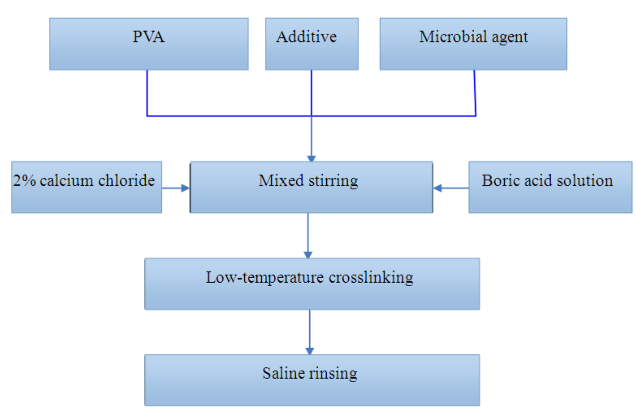

Fig. 1: Process for preparing the particle of immobilized microbial activity pellets.

Table 1: Factor level table of orthogonal experiment.

Factor	A_factor	B_factor	C_factor	D_factor	E_factor	F_factor	G_factor
Level_1	0.2%	2.5%	Activated Carbon 0.5%	0.5%	24h	5%	8%
Level_2	0.4%	3.5%	attapulgite 0.5%	1%	36h	10%	10%

Table 2: Combination table of orthogonal experiment.

No.	A_factor	B_factor	C_factor	D_factor	E_factor	F_factor	G_factor
1	1	1	1	1	1	1	1
2	1	1	1	2	2	2	2
3	1	2	2	1	1	2	2
4	1	2	2	2	2	1	1
5	2	1	2	1	2	1	2
6	2	1	2	2	1	2	1
7	2	2	1	1	2	2	1
8	2	2	1	2	1	1	2

to the toxicity of boric acid to microorganisms. On the basis of previous experiments, the crosslinking time of 24h and 36h has been selected.

Calcium carbonate concentration (A_factor), SiO_2 concentration (B_factor), concentration of activated carbon powder or attapulgite powder (C_factor), sodium alginate concentration (D_factor), crosslinking time (E_factor), microbial bacterial concentration (F_factor) and PVA concentration (G_factor) are taken as the affecting factors; then two levels are selected for each factor, and L8 (2^7) orthogonal table is adopted to conduct orthogonal experiment so as to determine the optimal embedding conditions. The factor levels of orthogonal experiment are given in Table 1, and the combination of orthogonal experiment is given in Table 2.

Determination for Optimal Embedding Conditions of Microbial Immobilization

The comparative experiment of sediment remediation is conducted for microspheres under the above 8 kinds of immobilized conditions. The reaction equipment of 8 plexiglasses is numbered from 1-8 respectively, and 300g of immobilized microsphere under different embedding conditions are added in corresponding devices. The comparative experiment lasts for 45 days, and the removal rate of overlying waterbody COD and NH_4^+-N is taken as the assessment index to determine the optimal embedding conditions of microbial immobilization.

Determination of optimal embedding conditions by the COD removal rate as the indicator: Fig. 2 shows the changes of overlying waterbody COD concentration of eight sets of equipment over time during the experiment.

All of the eight sets of equipment have experienced the process of increase first and decrease then as to the overlying waterbody COD values in the initial experiments. As microbial bacteria contain certain organic nutrients, the overlying waterbody COD values increase rapidly along with the addition of immobilized microbial pellets, which reach the peak value in the 5th to 7th day of the experiment with the its peak concentration of between 268.66mg/L and 349.43mg/L. Till the 8th to 12th day, the remediation bacteria in microbial bacteria become the dominant species enabling a large degradation of organic matter, and the COD concentration keeps declining with the lowest concentration of between 180.26mg/L and 245.93mg/L. During the 13th to 15th day, the COD concentration increased sharply to the highest concentration of between 405.28mg/L and 472.94mg/L. In the later 14th to 18th day, COD concentration fluctuates in a higher concentration range and keeps up since the loose disintegration is occurred in some pellets at this stage, and the entrance of a lot of PVA organics with macromolecules into the overlying waterbody results in an increase of COD concentration. Although microbial bacteria are of certain degradation role for organic pollutants at this stage, their degradation rate is far less than the dissolution rate of PVA molecules. From the 23rd day on, the immobilized pellets tend to stabilize, PVA stops dissolution, and the remediation bacteria in microbial bacteria play its role to sustain the rapid degradation of organic matters till the eventual stabilization, with the final COD concentration of between 43.95mg/L and 121.48mg/L, and COD removal rate of between 22.24% and 71.87 %.

The result of orthogonal experiment is given in Table 3 by taking COD removal rate as the assessment index. The intuitive analysis made from the orthogonal experiment data shows that the selected seven factors present the following descending impacts for COD removal rate of immobilized embedded pellets: media concentration > crosslinking time

Table 3: Orthogonal experiment table by the COD removal rate as the indicator.

No.	A_factor	B_factor	C_factor	D_factor	E_factor	F_factor	G_factor	COD Removal rate (%)
1	1	1	1	1	1	1	1	30.70
2	1	1	1	2	2	2	2	49.86
3	1	2	2	1	1	2	2	49.24
4	1	2	2	2	2	1	1	69.71
5	2	1	2	1	2	1	2	71.87
6	2	1	2	2	1	2	1	69.78
7	2	2	1	1	2	2	1	62.35
8	2	2	1	2	1	1	2	22.24
K_1	199.51	222.21	165.15	214.16	171.96	194.52	232.54	
K_2	226.24	203.54	260.6	211.59	253.79	231.23	193.21	
k_1	49.88	55.55	41.29	53.54	42.99	48.63	58.14	
K_2	56.56	50.89	65.15	52.90	63.45	57.81	48.30	
Range R	6.68	4.66	23.86	0.64	20.46	9.18	9.84	
Primary and secondary sequence	C_factor > E_factor > G_factor > F_factor > A_factor > B_factor > D_factor							
Excellent level	A_factor-2	B_factor-1	C_factor-2	D_factor-1	E_factor-2	F-_factor-2	G_factor-1	
Excellent combination	A_factor-2	B_factor-1	C_factor-2	D_factor-1	E_factor-2	F-_factor-2	G_factor-1	

Table 4: Orthogonal experiment table by the NH_4^+-N removal rate as the indicator.

No.	A_factor	B_factor	C_factor	D_factor	E_factor	F_factor	G_factor	NH_4^+-N removal rate (%)
1	1	1	1	1	1	1	1	77.00
2	1	1	1	2	2	2	2	85.30
3	1	2	2	1	1	2	2	90.10
4	1	2	2	2	2	1	1	92.65
5	2	1	2	1	2	1	2	89.62
6	2	1	2	2	1	2	1	90.73
7	2	2	1	1	2	2	1	86.90
8	2	2	1	2	1	1	2	81.79
K_1	345.05	342.65	330.99	343.62	339.62	341.06	347.28	
K_2	349.04	351.44	363.1	350.47	354.47	353.03	346.81	
k_1	86.26	85.66	82.75	85.91	84.91	85.27	86.82	
K_2	87.26	87.86	90.78	87.62	88.62	88.26	86.70	
Range R	1	2.2	8.03	1.71	3.71	2.99	0.12	
Primary and secondary order	C_factor > E_factor > F_factor > B_factor > D_factor > A_factor > G_factor							
Excellent level	A_factor-2	B_factor-2	C_factor-2	D_factor-2	E_factor-2	F_factor-2	G_factor-1	
Excellent combination	A_factor-2	B_factor-2	C_factor-2	D_factor-2	E_factor-2	F_factor-2	G_factor-1	

> PVA concentration > microbial bacterial concentration > calcium carbonate concentration > SiO_2 concentration > sodium alginate concentration. The optimal conditions for the immobilization are 0.5 % of attapulgite concentration, 36h of crosslinking time, 8% of PVA concentration, 10% of microbial bacterial concentration, 0.4% of calcium carbonate concentration, 2.5% of SiO_2 concentration and 0.5 % of sodium alginate concentration.

Determination of optimal embedding conditions by the NH_4^+-N removal rate as the indicator: Fig. 3 shows the changes of overlying waterbody NH_4^+-N concentration of eight sets of equipment over time during the experiment. It can be seen from the Fig. 3 that there is sudden rise of overlying waterbody NH_4^+-N concentration in eight sets of equipment first, then the continuous declination, and the final stabilization at a lower range. In the initial experiments, the organic nitrogen in sediment is degraded by microorganisms to generate the ammonia nitrogen in the reaction and come into the overlying water, causing the overlying water NH_4^+-N concentration to increase to the peak of between 7.59mg/L and 9.35 m /L. From the 5^{th} day on, the nitrifying bacteria in microbial bacteria adapt to the environment and become the dominant species; the nitration reaction speed change from fast to slow, and the NH_4^+-N concentration shows an overall declining trend till the eventual stabilization, with the NH_4^+-N concentration of between 0.46 mg/L and 1.44

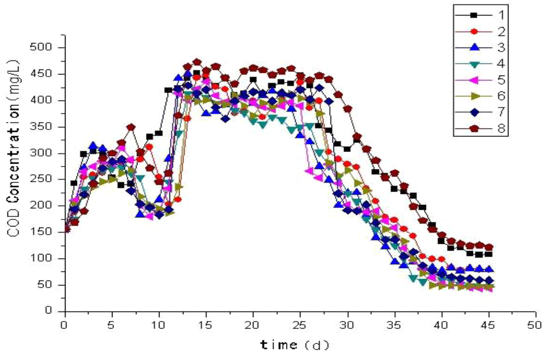

Fig. 2: Changes of overlying waterbody COD concentration over time.

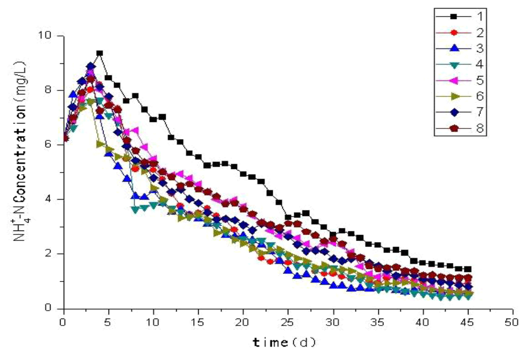

Fig.3: Changes of overlying waterbody NH_4^+-N concentration over time.

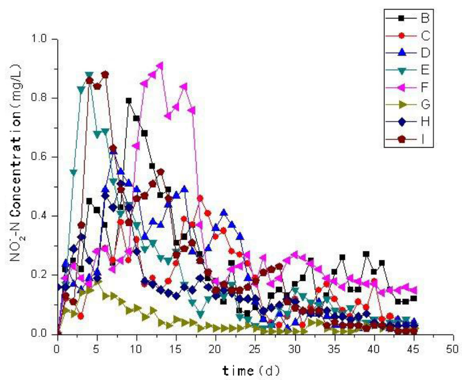

Fig. 4: Changes of overlying waterbody NO_2-N concentration over time.

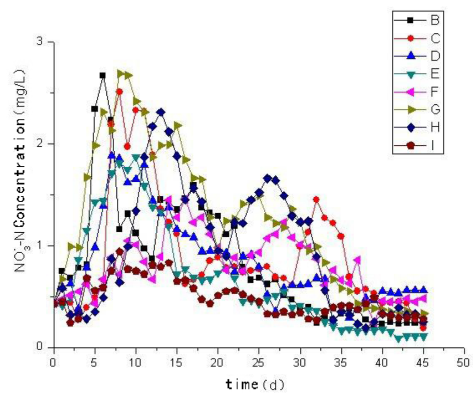

Fig.5: Changes of overlying waterbody NO_3-N concentration over time.

mg/L, and NH_4^+-N removal rate of between 77.00% and 92.65 %.

The result of orthogonal experiment is shown in Table 4 by taking NH_4^+-N removal rate as the assessment index. The intuitive analysis made from the orthogonal experiment data shows that the selected seven factors present the following descending impacts for NH_4^+-N removal rate of immobilized embedded pellets: media concentration > crosslinking time > microbial bacterial concentration > SiO_2 concentration > sodium alginate concentration > calcium carbonate concentration > PVA concentration. The optimal conditions for the immobilization are 0.5 % of attapulgite concentration, 36h of crosslinking time, 10% of microbial bacterial concentration, 3.5% of SiO_2 concentration, 1% of sodium alginate concentration, 0.4% of calcium carbonate concentration and 8% of PVA concentration.

Changes and analysis of overlying waterbody nitrite-N and nitrate-N concentrations: Figs. 4 and 5 show the changes of overlying waterbody NO_2^--N and NO_3^--N concentrations of eight sets of equipment over time during the experiment. As seen from the figure, NO_2^--N and NO_3^--N concentrations maintain at a lower level, and only during the 5th to 8th day, when the nitration reaction is faster, does the NO_2^--N and NO_3^--N concentrations rise, with NO_2^--N concentration between 0.11mg/L and 0.91mg/L while NO_3^--N concentration between 0.11mg/L and 2.69mg/L.

The Pearson correlation coefficients for overlying water NH_4^+-N and NO_2^--N concentrations, NH_4^+-N and NO_3^--N concentrations, and NO_2^--N and NO_3^--N concentrations of eight sets of equipment are calculated respectively through using SPSS software as given in Table 5.

As can be seen from Table 5, the correlation between NH_4^+-N and NO_2^--N concentrations, NH_4^+-N and NO_3^-N concentrations, and NO_2^--N and NO_3^--N concentrations is small, and the lowest Pearson correlation coefficients of these three are 0.269, 0.020 and 0.049, respectively, which suggests the inconsistency between the experimental result and the conventional nitrification and denitrification processes of NH_4^+-N-NO_2^--N-NO_3^--N. Combined with Fig. 3, NO_2^--N and NO_3^--N concentrations are analysed of no significant accumulation during the decreasing period of NH_4^+-N concentration, and only during the 5th to 8th day when the nitration reaction is faster, does the NO_2^--N and NO_3^--N concentrations rise to certain extent because the immobilized microbial pellets have successively formed the anaerobic zone, anoxic zone and aerobic zone from the inside to the outside and the concurrent anaerobic aerobic environment; the denitrifying bacteria and aerobic bacteria grow and reproduce in their own

Table 5: Pearson correlation coefficient table.

No.	NH_4^+-N and NO_2^--N	NH_4^+-N and NO_3^--N	NO_2^--N and NO_3^--N	No.	NH_4^+-N and NO_2^--N	NH_4^+-N and NO_3^--N	NO_2^--N and NO_3^--N
1	0.583	0.534	0.474	5	0.269	0.091	0.514
2	0.280	0.153	0.338	6	0.573	0.531	0.554
3	0.469	0.364	0.589	7	0.555	0.020	0.049
4	0.529	0.626	0.685	8	0.634	0.478	0.496

suitable environments to conduct simultaneous nitrification and denitrification.

RESULTS AND CONCLUSIONS

1. The overlying water COD concentration of eight sets of equipment has all experienced the rise and fall processes twice with the final concentrations of between 43.95mg/L and 121.48mg/L, and the removal rate of between 22.24% and 71.87 %. The COD removal rate is taken as the assessment index for the determination of the optimal embedding conditions of microbial immobilization, and the optimal conditions are determined as 0.5% of attapulgite concentration, 36h of crosslinking time, 8% of PVA concentration, 10% of microbial bacterial concentration, 0.4% of calcium carbonate concentration, 2.5% of SiO_2 concentration, and 0.5% of sodium alginate concentration. The seven factors present the following descending impacts for COD removal rate of immobilized embedded pellets: media concentration > crosslinking time > PVA concentration > microbial bacterial concentration > calcium carbonate concentration > SiO_2 concentration > sodium alginate concentration.
2. Overlying waterbody NH_4^+-N concentration in eight sets of equipment shows the sudden rise first, then the continuous declination, and the final stabilization at a lower range, with the final NH_4^+-N concentration of between 0.46 mg/L and 1.44 mg/L, and NH_4^+-N removal rate of between 77.00% and 92.65 %. The NH_4^+-N removal rate is taken as the assessment index for the determination of the optimal embedding conditions of microbial immobilization, and the optimal conditions are determined as 0.5 % of attapulgite concentration, 36h of crosslinking time, 10% of microbial bacterial concentration, 3.5% of SiO_2 concentration, 1% of sodium alginate concentration, 0.4% of calcium carbonate concentration and 8% of PVA concentration. The seven factors present the following descending impacts for NH_4^+-N removal rate of immobilized embedded pellets: media concentration > crosslinking time > microbial bacterial concentration > SiO_2 concentration > sodium alginate concentration > calcium carbonate concentration > PVA concentration.
3. The inconsistency is shown between the removal process of underlying waterbody and the conventional nitrification and denitrification processes of NH_4^+-N-NO_2^--N-NO_3^--N, and the NO_2^-N and NO_3^-N concentrations show no obvious accumulation during the decreasing period of NH_4^+-N concentration because the immobilized microbial pellets have successively formed the anaerobic zone, anoxic zone and aerobic zone from the inside to the outside and conducted the simultaneous nitrification and denitrification.

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