Applications of Biological Enhancement in Carbon/Sand Filter

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Efficiently making use of granular activated carbon (GAC) to reduce pollution is always a golden goal for water industry. We developed ultrasonication collection method for determination of the biomass and microbial attachment and growth in activated carbon filter. Based on this method, we found that using hydraulic cycle to immobilize cells onto GAC can greatly improve the attachment of inoculated microbial cells. The immobilization parameters of immobilization time and flow rate were optimized to assure the dominant growth of inoculated cells on GAC. To keep the consistency of the surrounding biological environment, sand layer and backwash were employed to dilute the locally enriched amino acids and cells due to the inoculation. Although the backwash can cause some cell detachment on GAC, this is of little significance compared with cells staying on GAC. Optimized hydraulic cycle immobilization (HCI) can accelerate the biodegradation process, thus extend the life of GAC, but has little effect on the stability of the surrounding biological environment.

Key words:
Granular activated carbon, ultrasonic, hydraulic cycle immobilization, bacteria biomass

Introduction

Granular activated carbon (GAC) is applied in the potable water industry to eliminate organic materials as a measure of pretreatments for its great absorption surface. The high absorption capacity of GAC causes the bacterial colonization on the rough porous GAC surface to establish biofilms, which subsequently form biologically activated carbon (BAC) filter. The active biofilms can degrade organic material efficiently and thus reduce the loading of GAC and extend its life.

The first step of biofilm formation is the attachment of microorganisms to the GAC surface. As the microorganisms grow to a certain thickness and density, biofilm forms. This process is however time-consuming due to the low level of biodegradable substrate, which serves as the nutrition for bacteria. Only bacteria that fit to the environment of poor nutrition can survive,[1] and by the time the biofilm has formed, the capacity of GAC absorption has been partially depleted. Therefore, the sooner the biofilm forms and biodegradation takes effect, the longer GAC life will be extended.

To make the biodegradation operate quickly, two kinds of technology for cell immobilizing have been developed to inoculate cells on GAC surface. Chemical technology fastens cells to the carrier with a higher attaching strength by cross-link medicaments or chemical bonds. A disadvantage of this technology is the cell toxicity caused by the medicaments during the cross-linked process. Furthermore, the water quality may also be affected. On the other hand, physical mobilization technology makes use of absorbing and preserving the ability of the carrier to mobilize microorganisms, which is simple and with no effect to microbial activity, though the link between carrier and cells is weaker.[2]

The chemical immobilization technology, though with the higher fixation strength of cells on GAC, will eventually achieve the equivalent effect as the physical technology does for the detachment of cells from the carrier due to the microbial metabolism and the shearing force of the liquid. Some of the detached cells and bacteria in influent can still attach to GAC surface, and finally develop to natural BAC filter similar as using the physical immobilizing technology.

Therefore, in this paper we used physical techniques to inoculate diluted microbial cells onto GAC filter, in which hydraulic cycle immobilization (HCI) system was applied and optimized. Our data suggest that this system can accelerate biodegradation and thus extend the GAC life.

Materials and methods

Water samples were collected from a pilot-scale plant. The pilot plant was operated at a volume flow rate of Q = 5 m^3 h^{-1}. The treatment

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stream consisted of dissolved air floatation, anthracite/sand filter and pre-ozonation, followed by GAC/sand filter operating at $Q = 1.25$ m^3 h^{-1}. GAC unit was constructed of steel materials with the total height of $h = 1.5$ m and diameter of $d = 0.6$ m. Diluted cells solution (10^5 CFU mL^{-1}) was cycled by peristaltic pump. Empty bed contact time (EBCT) 400 min, backwashed after 24-hour cultivation (backwash intensity of air: $q_G = 9.8$ L s^{-1} m^{-2} for 3 min; backwash intensity of water: $q_L = 7.9$ L s^{-1} m^{-2} for 10 min). GAC samples were collected from the height of 0 m, 0.75 m and 1.5 m in the GAC filter to obtained carbon particles with attachments of microorganisms for analysis. The bench plant was manufactured geometrically according to the pilot plant. The diameter of tube reactor was $D = 9$ cm, GAC lay height was $H = 22.5$ cm. Diluted cells solution (10^5 CFU mL^{-1}) was cycled by peristaltic pump. Empty bed contact time (EBCT) 20 min, unbackwashed, cultivated for 48 hours.

Ultrasonication collection method was used for determination of the biomass and microbial attachment and growth in activated carbon filter.[3,4] A total of two to five grams of GAC sample added to 20 mL of autoclaved tap water (pH 7.5) in a 150 mL borosilicate flask. Borosilicate flask was placed in the sonication chamber with 200 mL of autoclaved tap water. Sonication was applied for 12 min with an ultrasonic clearance (AutoScience company) at a constant frequency of 40 kHz and a constant power output of 60 W. A volume of 1 mL of the obtained suspension was collected every 2 min for examination, and 1 mL of autoclaved tap water was added back to maintain power density.

Bacteria colonies count method was applied to evaluate the number of cells. 1 mL of serial of decimal dilutions of microbial suspensions collected from GAC were spread in triplicate over the surface of nutrition agar plates comprising beef ointment, peptone and NaCl. Plates were then incubated for $t = 24$ h at $\theta = 37 \pm 1$ °C. Bacteria colonies were counted as the colony forming units normalized by the milligram of activated carbon (CFU mg^{-1}).

Results and discussion

Determination time points for collection of microorganisms

As shown in Fig. 1, collected microorganisms reach peak smoothly at $t = 10$ min of sonication. This suggests that most microorganisms are detached from GAC by sonication of 10 min. After that, the discharged GAC may reabsorb the released (free) microorganisms, making them hard to maintain in a dissociation state. It was also found that the peak for detachment of microorganisms from GAC all occurred around 10 min of sonication, regardless of the amount of biomass. The oscillation after 10 min appeared likely to result from the activation of carbon that led to the repeated adsorption and breaking off of microorganisms.

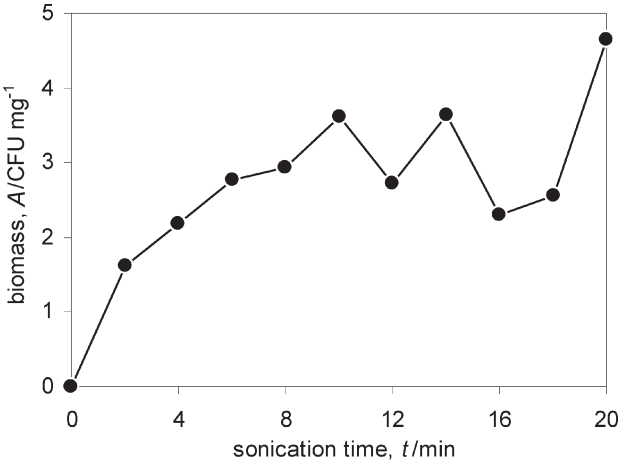


Fig. 1 – *Tendency of cumulative biomass with sonication time*

Effect of sonication on inactivation of microorganism

Because of the inactivation of microorganism by sonication,[3,4] the real amount of biomass on GAC would be higher than the calculation according to the counts of CFU. To evaluate this negative effect, we investigated the relationship of the microbial inactivation with sonication duration. As shown by Fig. 2, inactivation of microorganism exhibit first order behavior, which suggests that the inactivation effect by sonication is linear to sonication time given constant power and frequency of ultrasonication. Compared to sonolytic inactivation,[3] the damage of microorganisms caused by sonication surging in our experiment is quite weaker.

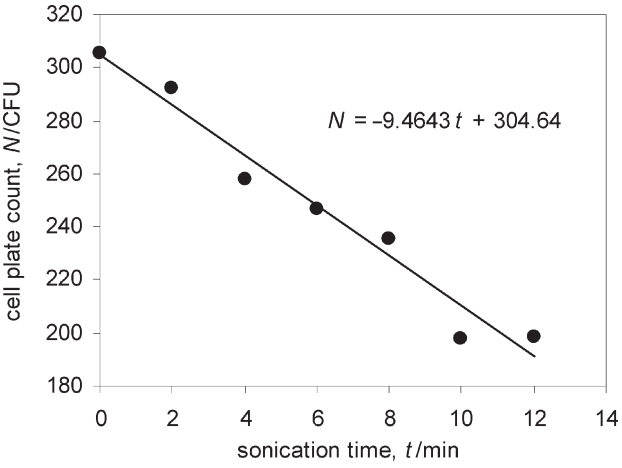


Fig. 2 – *Inactive effect of sonication on cultured bacteria*

Effect of GAC absorption on microorganism biomass

Sonication is applied to solutions containing cultivated cells and GAC with different adsorption ability for comparison. As shown in Fig. 3, except for unloaded GAC preserving nearly all suspension cells, the tendency of recovery rate of suspension cells in solution containing preloaded GAC is similar with blank solution, which suggests that GAC had no adsorption effect on suspension cells after preserving enough cells corresponding to its adsorption potential.

$$A(t) = A(t-2) + b(t) - c(t) - d(t) \qquad (1)$$

where $A(t)$ is microorganism biomass at sonication time (t), $b(t)$ is microorganism biomass detached from GAC in sonication surging, $c(t)$ is inactivated microorganism biomass due to sonication which is correlative to sonication time (t) as shown by Fig. 2, $d(t)$ is microorganism biomass due to GAC absorption which changes with sonication time. $d(t)$ is constantly 0 before sonication time t_p at peak, but variable after sonication time t_p at peak, (as shown in Fig. 1). It is supposed that GAC had no adsorption effect on the suspension cells after preserving enough cells before t_p as shown by Fig. 3.

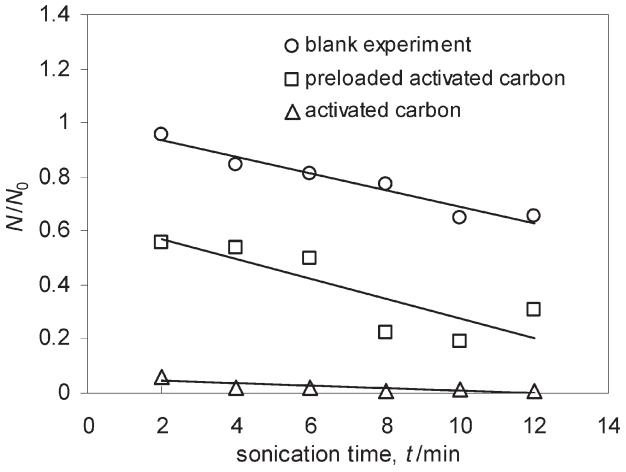


Fig. 3 – *Recovery of cell in different cases*

As $d(t) = 0$, $t \in (0, t_p)$, $A(t)$ is determined by $b(t)$ and $c(t)$, so most microorganism biomass on GAC surface is equal to the sum of detected microorganism biomass in solution and inactivated microorganism biomass due to sonication. Microorganism biomass detected at t is only determined by the attaching affinity of cells on GAC surface. t_p is a good indication of affinity of cells attaching to GAC matrices. According to the tendency of microorganism biomass curve, peak value and peak time (t_p), it is possible to determine the amount of microorganism biomass on GAC surface and its attaching stability.

Analysis of biomass on GAC surface from layers of different height under steady-state

As microorganism biomass and attaching stability of cells varies from layer to layer, microorganism biomass peak value and t_p are also different among layers. As shown in Fig. 4, microorganism biomass curves of GAC from filter height 1.5 m and 0.75 m are smoother than 0 m before peak; microorganism peak value of GAC from filter height 1.5 m is obviously the biggest of all, microorganism peak value of GAC from filter height 0.75 m are slightly bigger than 0 m; from 1.5 m to 0 m, t_p is shortened gradually.

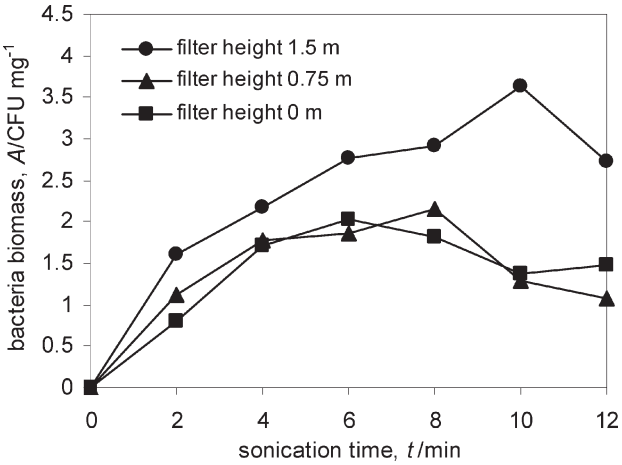


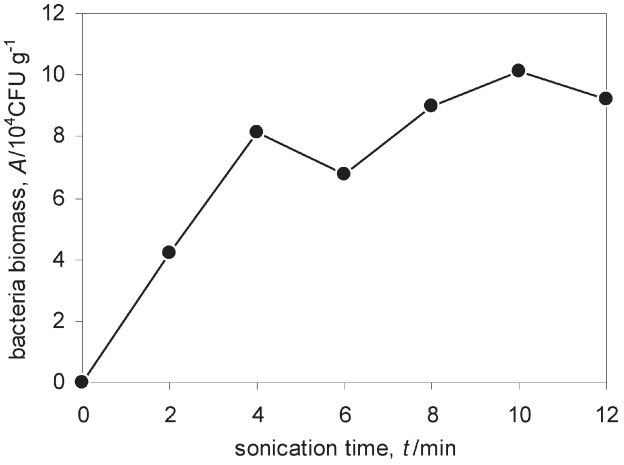
Fig. 4 – *Biomass on activated carbon in different carbon layer height of GAC unit*

Because the level of biodegradable substrates decreases gradually as water flows down through GAC layer, microorganism biomass will certainly decrease, too. As the GAC reactor runs, some cells will come off GAC surface and move into the flowing liquid. Loading rate of detaching cells increases gradually and attached cells become easier to detach as water flows down.

Optimization immobilizing parameters

Like special species of inoculating cells, such as nitrobacteria,[5] immobilizing cells from mixed bacteria communities living under poor nutrition were inoculated. Cells free for carcinogenesis by Ames test undergo immobilization by hydraulic cycling.

As shown in Fig. 5, though the high absorption capacity of unloaded GAC causes oscillation of detected microorganism biomass, it is obvious that microorganism biomass increases rapidly to peak value. t_p is comparable to the GAC t_p at steady-state operation. After culture, a fraction of cells were immobilized firmly on GAC surface, but most cells at-

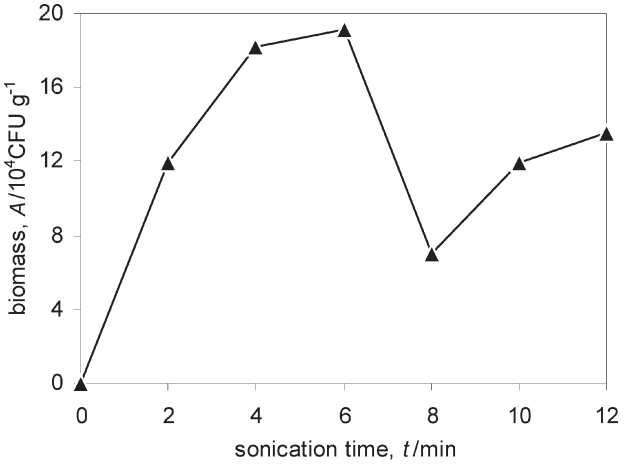


F i g . 5 – *Tendency of biomass with sonication durations without backwash*

tached unsteadily. Considering the instability in the beginning phase of reactor running, cells should be immobilized as much as possible to maintain their predominance and adapt to the new environment.

After a period of time, most cells were absorbed by GAC, but there were still many cells left free in the solution. Because of amino acids from the inoculation in the solution, chlorine added into solution reacted with aminophenol (amino acids) and lowered the efficiency of the disinfection by chlorine. Furthermore, the production of chloramines from the reaction might be harmful. Therefore, backwashing is necessary to discharge cells and chloramines from the reactor to avoid their effect on disinfection.

As shown in Fig. 6, though many cells were discharged upon backwash, microorganism biomass under EBCT 400 min and immobilizing time 10 h was more than EBCT 20 min and immobilizing time 6 hours. The big change of the curve indicates that most cells detach easily and are reabsorbed back by GAC. Backwash may be the important reason for this phenomenon, which was also observed during steady-state operation.[6] To yield biologically stable water, sand is employed under GAC layer to withhold bacteria leaks.

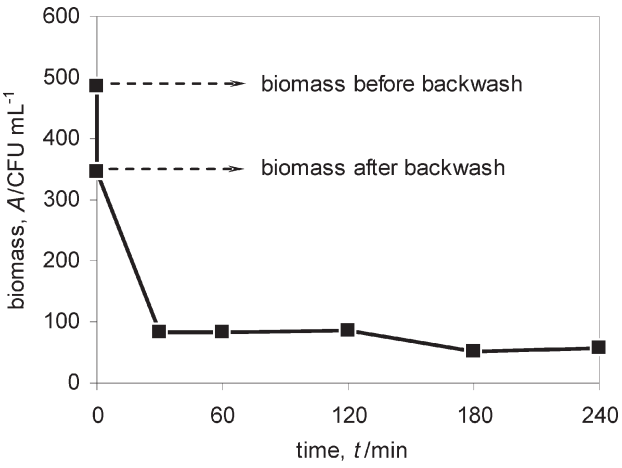


F i g . 6 – *Tendency of biomass with sonication durations upon backwash*

Biological stability in effluent at selected immobilizing parameters

Albeit the advantage of biological reaction technology, microorganisms in the reactor also bring the problem of bacteria breakthrough, especially GAC filter located in the tail end of the potable water treatment stream, the leak of microorganisms exposes to disinfection directly without any pretreatment. Most investigations focus on residual biodegradable dissolved organic matter[7] and assimilable organic matter,[8] however, it is more harmful if the residual bacteria after immobilization get into the water and escape from disinfection. Especially, those immobilized bacteria are collected from the tap water network, which previously achieved certain resistance to industrial disinfection.[9] After reentering into the drink water, they may reproduce and propagate very fast.

Here biological stability refers to the highest potentials of bio-degradable organic matters to support microorganism growth in drinking water when organic compounds become the limiting factor to microorganisms. Tendency of bacteria biomass in effluent is shown in Fig. 7. Bacteria biomass in effluent is under 100 CFU mL^{-1} and decreases gradually, which is under standard levels of bacteria estimation after disinfection as prescribed by potable water providers in China. After operating at EBCT 20 min for 1 year, bacteria biomass detection and disinfection test were applied. Bacteria biomass in GAC filter effluent increased to 10^3 CFU mL^{-1}. Since the minimum residual chlorine concentration was 1.0 mg L^{-1}, and contact 15 min was sufficient to reduce bacteria biomass in effluent. Furthermore, bacteria biomass in effluent



F i g . 7 – *Tendency of bacteria biomass in effluent*

Table 1 – *Statistical analysis of bacteria biomass in effluent of different processes (detected per 6 hours)*

	Mean	Median	SD	Valid-N	Min	Max	–25th %	–75th %
O_3	6	3	10	85	0	54	0	9
GAC	410	158	491	85	19	2244	88	552
BAC	466	217	569	85	15	3416	72	650

O_3 stands for pre-zonation; GAC stands for GAC filter; BAC stands for immobilized BAC filter.

of GAC filter without immobilized cells was comparable to the immobilized biologically activated carbon filter (as shown by Table 1).

Effect of biodegradation

Biodegradation is effective to ammonia removal, but GAC adsorption has little effect on ammonia removal.[10] Using the removal rate of ammonia as an indicator, biodegradation is measured. After cell immobilization, NH_4Cl was added into influent of filter with concentration of $\gamma = 0.2$ mg L^{-1}. Removal rate of ammonia reached 75 % (data now shown). Judging by the low level of residual NH_4Cl, biodegradation had played an important role in ammonia removal after cell immobilization.

Conclusions

In this paper, we studied bacteria biomass from activated carbon using ultrasonic surging collection method. The resulted peak value of bacteria biomass, sonication time at peak, t_p, and microbial growth state was used to analyze operation state of biological reactor. After immobilization with hydraulic cycle, a great number of incubated cells were attached steadily in activated carbon due to absorption of activated carbon. The stability is equal to biologically activated carbon filter, in which biofilm is naturally formed.

The objective of cells immobilization is to inoculate enough cells to assure their predominance. At the same time, it should be considered that bacteria and other by-products (e. g. by-products from microbial metabolisms) from immobilizing process might have a negative effect on biological stability in effluent. Appropriate immobilizing parameters were optimized. It was found that EBCT 300 min and immobilizing 10 h is more effective to assure enough cells in activated carbon than EBCT 20 min and immobilizing 6 h. EBCT 300 min and immobilizing 10 h stands for diluted cells solution cycling 2 times, 300 min per cycle; EBCT 20 min and immobilizing time 6 hours stands for diluted cells solution cycling 18 times, 20 min per cycle, as filter operation as usual. To restrict the leaks of cells into effluent, sand layer is employed to withhold.

Although most of the cells attached in activated carbon, residual diluted cells in solution should be backwashed from filter for aminophenol reacts with the disinfectant chlorine to lower its efficiency, and residual immobilized cells could resist disinfecting action.

Bacteria biomass seems no changes after backwash, but it decreases rapidly as filter operation. It is no doubt that residual diluted cells solution is discharged absolutely from filter, but partial attached cells are also detached from activated carbon due to backwash.

Analysis of ammonia consumption by bacteria indicated that immobilizing with hydraulic cycle could accelerate bio-degradation.

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List of symbols

A – microorganism biomass, CFU mg^{-1}
b – microorganism biomass detached from GAC in sonication surging
c – inactivated microorganism biomass due to sonication
d – microorganism biomass due to GAC absorption which is changing as sonication time
d, D – diameter, m
h, H – height, m
N – cell plate count, CFU
Q – volume flow rate, m^3 h^{-1}
t – time, min
q_G – gas flux, L s^{-1} m^{-2}
q_L – liquid flux, L s^{-1} m^{-2}
t_p – sonication time
γ – mass concentration, mg L^{-1}
θ – temperature, °C

List of abbreviations

GAC – granular activated carbon
HCI – hydraulic cycle immobilization
BAC – biologically activated carbon
EBCT – empty bed contact time
CFU – colony-forming units

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