Korean J. Chem. Eng., 39(6), 1507-1516 (2022)
DOI: 10.1007/s11814-021-1022-y

pISSN: 0256-1115
eISSN: 1975-7220

Kinetics of pentachlorophenol co-metabolism removal by micro-aeration sequencing batch reactor process

Jianhui Wang*, Guolong Xie*, Xin Qi*, Ruifeng Ming*, Bin Zhang*, and Hai Lu,†**

*Key Laboratory of Songliao Aquatic Environment, Ministry of Education, Jilin Jianzhu University, Changchun City, Jilin Province, P. R. China
**College of Civil Engineering and Architecture, Changchun Sci-Tech University, Changchun City, Jilin Province, P. R. China
(*Received 20 August 2021 • Revised 18 November 2021 • Accepted 20 November 2021*)

Abstract−Four carbon sources (including trehalose, glucose, acetic acid, and yeast extract) were used as the co-metabolic matrix of pentachlorophenol (PCP). The effect of the carbon sources on the process of acclimatization and degradation of PCP was investigated. The acclimatization rate of carbon sources with different substrates, the activities of microbial enzymes in the co-metabolism process, and the control of co-metabolism reaction conditions were evaluated. The kinetic model of co-metabolic degradation of PCP in micro aerated sequencing batch reactor (SBR) was established based on the Monod equation. The model was applied to fit the operating conditions of the micro aerated SBR process in this study. The experimental results showed that the type and concentration of metabolic matrix greatly influenced the degradation rate of PCP, and its trehalose, glucose, and acetic acid enhanced the degradation of PCP. In particular, the strengthening effect of trehalose was pronounced. When trehalose was used as a co-metabolic carbon source, the time required for PCP degradation to a predetermined degree was shortened to one-fifth of the original, PCP removal rate exceeded 95%. At the same time, yeast extract inhibited the biodegradation of PCP when it was used as an additional matrix carbon source. After the co-metabolism carbon source was added to the system, the proliferation rate of the microorganism was increased, and the key enzymes of PCP degradation were induced in the system. When the co-metabolic carbon source concentration was high, it accelerated active enzymes' induction and maintained high activity; 2,3,5-triphenyltetrazolium chloride-electron transport system (TTC-ETS) activity reached about 7.6 mgTF/(gTSS·H), and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl Tetrazolium chloride-electron transport system (INT-ETS) activity reached 63.5 mgINTF/(gTSS·H). When the concentration of co-metabolism carbon source was extremely high, the co-degradation of toxic organic compounds was inhibited, leading to a decrease in the co-degradation rate. The kinetic model optimized the co-metabolism substrate. The degradation rate of PCP was increased by 54.9% by micro-aeration-co-metabolism. The kinetic model was used to fit the microaerobic reaction process of micro aeration SBR. The relevant result was in agreement with the experimental result by 97.6%.

Keywords: Micro Aeration, Sequencing Batch Reactor, Co-metabolism, Pentachlorophenol, Dynamics

INTRODUCTION

Microbial co-metabolism, also known as synergistic oxidation, is the phenomenon that microorganisms metabolize unusable substances in the presence of the sole available carbon source. For many refractory organics, microorganisms can change their chemical structure through metabolism but cannot be degraded as a carbon source. Therefore, other substrates must provide metabolic carbon sources and energy for microorganisms to proliferate, metabolize, and degrade the refractory organics. These external supplies for the microbial substrate carbon source and energy sources, called primary matrices, are used for the proliferation of microorganisms and provide energy for metabolism. Conversely, these degraded refractory organics are secondary matrices and have no apparent biological effect [1,2].

Co-metabolism exists in the aerobic, anaerobic, and autotrophic microorganisms in the biological wastewater treatment process. Many compounds can be used as the first matrix in co-metabolism, such as simple proteins, polysaccharides, and inorganic substances. The degradable second matrix is also diverse, mostly high concentration, refractory, and highly toxic substances. These substances include polycyclic aromatic hydrocarbons, nitro compounds, and organochlorine pesticides [3]. Studies have shown that organics initially considered challenging to biodegrade, such as nitrobenzene and PCP, are co-metabolized and degraded by adding suitable matrix carbon sources. For example, most chlorophenol degrading engineering bacteria can degrade trichlorophenol (TCP) by using phenol as a co-metabolic substrate, and the growth mechanism of microorganisms has been studied under co-metabolism [4,5]. The results showed that the synergistic effect between substrates could reduce the toxicity of toxic substances to microorganisms. Some researchers studied the co-metabolic degradation of Tetrachlorophenol (TeCP) with isoamyl alcohol and phenol as matrix carbon sources, respectively. They concluded that the adaptability of micro-

†To whom correspondence should be addressed.
E-mail: haimm110@126.com

organisms to highly toxic organics varied with different matrix carbon sources [6,7].

In China, highly toxic and refractory organic compounds such as PCP have been quite profound. Due to long-term use, PCP has existed in the natural environment for a long time [8,9]. In 1968, the United Nations Environment Programme (UNEP) conducted a global survey on PCP pollution. Through this investigation, people realized that PCP's environmental pollution sources spread across all aspects of daily life. In summary, they were mainly summed in the following six aspects: (1) Spatial diffusion when pesticides, fungicides and herbicides are used; (2) Infiltration and volatilization diffusion during brushing as a preservative; (3) Chlorine disinfection in water treatment and diffusion of water and atmosphere in the process of chlorine oxidation; (4) Water and atmospheric diffusion in the production process of PCP compounds; (5) Degradation, metabolism and derivation of similar chemical insecticides and herbicides; and (6) Discharge of wastewater from tannery and pulping plants. Thus, PCP exists in almost all environmental media, including human beings, due to its mass production and wide use worldwide, as well as its non-degradability and enrichment [10]. According to the comprehensive comparison of the data reported by environmental protection departments to the UNEP, the highest amount of PCP was found in soil (about 95.5%), followed by water (about 2.5%), and then in sediment (about 1%). The lowest amount of PCP was the particulate matter in the atmosphere (less than 1%). PCP in the environment outside the water environment medium finally enters the water environment medium with the effects of material exchange, metabolic degradation, transfer and enrichment between environmental media, seriously affecting the water environment quality and causing severe pollution and damage to the water environment [11].

The existing physical, chemical, and biological treatment technologies have certain technical disadvantages. Although the treatment effect obtained by physical and chemical methods is relatively good, chemical agents or physical treatment equipment need to be added in the use process. So the long-term investment after is significant, the treatment cost is relatively high, and the power consumption is large. In addition, it is challenging to remove PCP by physicochemical method completely but only convert it into other pollutants. At the same time, there is a risk that the adopted chemicals will react to produce new pollutants, or the adopted physical treatment method will lead to the release of PCP, which makes it difficult to popularize. Furthermore, when using the traditional biological treatment process to treat PCP, due to its high toxicity and non-degradability, there are often problems such as slow degradation rate, low degree of mineralization, complex detoxification degradation and concentration accumulation of intermediate products. Therefore, the effluent of many biological treatment processes cannot meet the design standards [10-13].

Therefore, we sought to profoundly and systematically study the theory and process of biological co-metabolism and apply it to practical projects to reduce the total discharge of refractory organic wastewater. Thus, it is of great practical significance to promote the improvement and sustainable development of the water environment in China.

In addition, the micro aeration SBR process based on co-metabolism studied in this paper is also a popular research topic in the field of wastewater biological enhanced treatment. Its content involves the cultivation and domestication of micro-aerobic granular sludge, the optimization of process operation conditions, the dynamic analysis of microbial flora, the construction of kinetics and many other aspects. These theoretical and technological studies will further improve the PCP biological co-metabolism system and contribute to the whole field of bioaugmentation of extreme environmental compounds (high cold, high temperature, high toxicity and acid-base compounds). Therefore, this study has paramount scientific significance.

EXPERIMENTAL MATERIALS AND METHODS

1. Water Quality and Apparatus

In the experiment, four carbon sources, including trehalose, glucose, acetic acid and yeast extract, were selected as co-metabolic substrates for PCP domestication of micro-aerobic granular sludge. In the experiment, the cultivation and domestication of micro-aerobic granular sludge adopted the asynchronous inoculation method. The flocculent sludge from the secondary sedimentation tank of an urban sewage treatment plant in Changchun was inoculated. The amount of inoculated sludge was 30% of the effective volume of the reactor, the mixed liquor suspended solids (MLSS) was 8,600 mg/L, and the volatile suspended solids (VSS)/suspended solid (SS) value characterizing sludge activity was 0.52. The simulated PCP wastewater was used as the investigation object. According to the principle of C : N : P=100 : 5 : 1, trehalose and PCP-Na were used as chemical oxygen demand (COD) sources, and $(NH_4)_2SO_4$ and KH_2PO_4 were added as nutrient elements of granular sludge microorganisms. The addition of $MgSO_4$ and $CaCl_2$ met Mg^{2+} and Ca^{2+} requirements in the sludge granulation process. Thus, microelement solution was added to meet the nutritional requirements of granular sludge's microbial growth and reproduction. The addition amount of $NaHCO_3$ depended on the operation, and the pH value inside the reactor was maintained between 6.8 and 7.5. The water quality is shown in Table 1. The water quality was measured according to parameters including COD_{Cr}, biochemical oxygen demand (BOD), (NH_4^+-N, NO_3-N, total nitrogen (TN), total phosphorus (TP), Alkalinity ($CaCO_3$), as shown in Table 1.

The test apparatus is shown in Fig. 1. The reactor was made of plexiglass, 80 cm high, 30 cm diameter, and the total effective volume was 40 L. A microporous aeration pipe aerated it; a rotameter adjusted the aeration intensity. According to engineering experience, the range of aeration intensity was 4.0-5.5 $m^3/(m^2 \cdot h)$. Generally, it is not suitable to be less than 4.0 $m^3/(m^2 \cdot h)$, so the aeration intensity selected in this study was 4.0 $m^3/(m^2 \cdot h)$, 4.8 $m^3/(m^2 \cdot h)$ and 5.5 $m^3/(m^2 \cdot h)$, respectively. The temperature controller adjusted the water temperature. The water pump fed the inlet water, and the drainage sampling port discharged the drainage. During the test process, various detection equipment was connected as required. The operation cycle of the reactor was 6.5 h, i.e., water inflow for 10 min, aeration for 6 h, static sedimentation for 10 min, decanting for 10 min, and operation for three cycles per day. Thus, the culture's acclimation to enrichment and degradation test lasted about 400 days.

Table 1. Experimental simulated wastewater composition and water quality characteristics

Configuration of pharmaceuticals	Dosage (g/L)	Composition of microelement solution	Concentration (g/L)	Water quality index	Concentration (mg/L)
Trehalose	0-0.01	$FeCl_3$	0.78	COD_{Cr}	900±30
PCP-Na	0-0.05	H_3BO_4	0.08	BOD	455±8
$(NH_4)_2SO_4$	0.125	$CoCl_2{\cdot}7H_2O$	0.15	NH_4^+-N	23-26
KH_2PO_4	0.015	$CuSO_4{\cdot}5H_2O$	0.03	NO_3^--N	<1
$CaCl_2$	0.03	KI	0.18	TN	23.5-27
$MgSO_4{\cdot}7H_2O$	0.06	$MnSO_4$	0.01	TP	3-5
Microelement solution	1 mL/L	$ZnCl_2$	0.06	Alkalinity ($CaCO_3$)	0-1,400
		$Na_2MoO_4{\cdot}2H_2O$	0.035		

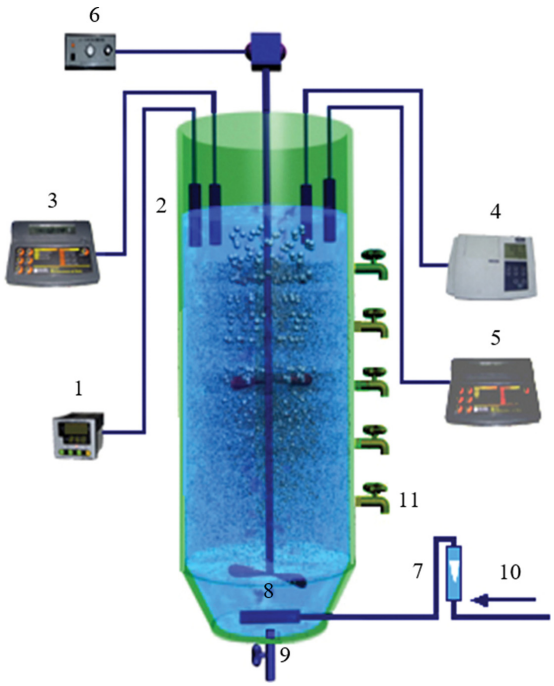


Fig. 1. Schematic diagram of micro aeration SBR reactor.

1. Temperature control instrument
2. Temperature sensor
3. ORP measuring instrument
4. DO measuring instrument
5. pH measuring instrument
6. Water pump
7. Air rotor flow meter
8. Micropore aeration pipe
9. Sludge discharging pipe
10. Compressed air
11. Water outlet

2. Analytical Method

The analysis test and detection method of the conventional indicators in the test mainly refer to the "Standard Methods for the Examination of Water and Wastewater (22nd Edition)" issued by the American Public Health Association [14]. In the unconventional analysis items and detection methods, PCP and its intermediate products were determined by gas chromatography/mass spectrometry [15]. The activity of sludge was determined by the absorbance method as previously [16]. The specific techniques are shown in Table 2.

RESULTS AND DISCUSSION

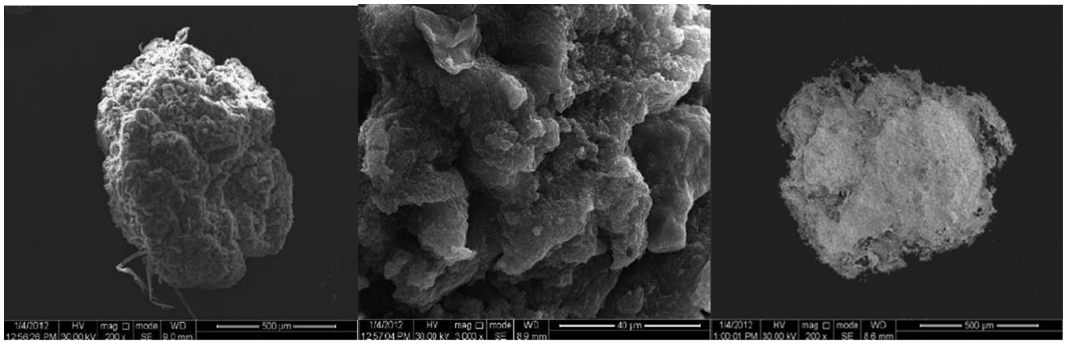
1. Culture and Characterization of Micro-aerobic Granular Sludge

The inoculated activated sludge was the sludge from the secondary sedimentation tank of the urban sewage treatment plant, which was dark grey. Microscopic observation found that it was flocculent and loose in structure, mainly composed of dense bacterial micelles of different sizes. Furthermore, it was found that its settlement performance was poor when it was treated by closed aeration. To further investigate micro-aerobic granular sludge's spatial structure and microbial population, the mature micro-aerobic granular sludge was observed by scanning electron microscope (SEM), as shown in Fig. 2.

Fig. 2(a), (b) and (c) are SEM images of the overall structure, surface and section of cultured mature micro-aerobic granular sludge, respectively. SEM observation showed that micro-aerobic granular sludge had high biomass and an affluent population structure.

Table 2. Routine index analysis test and analysis method

Test index	Method
COD_{Cr}	High-temperature digestion rapid determination
pH value	Glass electrode method
DO	Diaphragm electrode method
ORP	Glass electrode method
Temperature	Thermometer method
Determination of PCP and its intermediates	Gas chromatography/mass spectrometry
ETS activity assay	Spectrophotometry
Morphological observation of sludge	Optical microscopy and scanning electron microscopy



(a) Overall structure×200 (b) Surface ×3000 (c) Section×200

Fig. 2. SEM micrographs of mature micro-aerobic particles in the reactor.

Brevibacterium and globular bacteria were on the sludge surface, and protozoa were also found on the sludge surface. From the appearance point of view, the mature micro-aerobic granular sludge had precise apparent contour, compact structure and high strength. Meanwhile, the stability of micro-aerobic granular sludge pretreated by SEM was still strong and large particle size. Most granular sludges were ellipsoidal or spherical, and the particle surface was uneven with various folds or ditches. Granular sludge had many external pores but few internal pores, compact structure, and evident stratification from outside to inside.

Through the analysis of the morphological changes of the sludge during the cultivation process and combined with the results of SEM pictures, we can assume the granulation process of micro-aerobic granular sludge is as follows: the inorganic particles and various microorganisms in the initial flocculent sludge are adsorbed, bonded and aggregated under the action of appropriate water flow shear force. Under the inertial shear force of micro aeration, the mycelium of filamentous bacteria curls and interweaves into a network frame structure. The network structure captures Brevibacterium and globular bacteria free in the reactor so that the bacteria are attached to the net structure to grow, and the initial granular sludge is formed. However, the granular sludge structure in this state was extremely unstable. Once the water flow shear force was too high, it disintegrated and dissolved. Therefore, the aeration intensity should be strictly controlled in the culture process. Under the influence of inertial shear force scouring, lack of nutrients and pH value, the metabolic activity and quantity of filamentous bacteria forming a network were reduced. However, the attached and growing Brevibacterium and globular bacteria had occupied an advantage in the system and became the main microbial flora.

2. PCP Co-metabolism

2-1. Analysis of PCP Degradation Process in Microbial Co-matrix Metabolism

Figs. 3-6 show the biodegradation of PCP under four co-metabolic matrix carbon sources, thus, trehalose, glucose, yeast extract, and acetic acid.

The degradation rate of PCP was significantly affected by the type and concentration of the metabolic matrix. From Fig. 3 and Fig. 4, it can be deduced that trehalose and glucose enhanced the degradation of PCP, and trehalose had a more apparent strengthening effect. When trehalose was a co-metabolism carbon source, the time required for PCP degradation to a predetermined level was shortened to about one-fifth of the original time. Other studies confirmed that the co-metabolism removal rate of toxic substances was

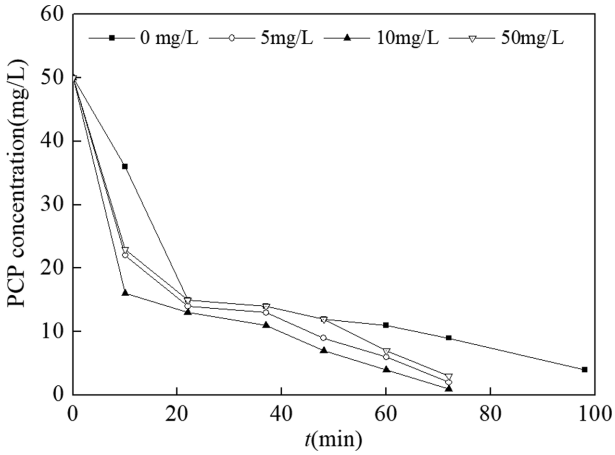


Fig. 3. PCP degradation curve under different initial concentrations of trehalose.

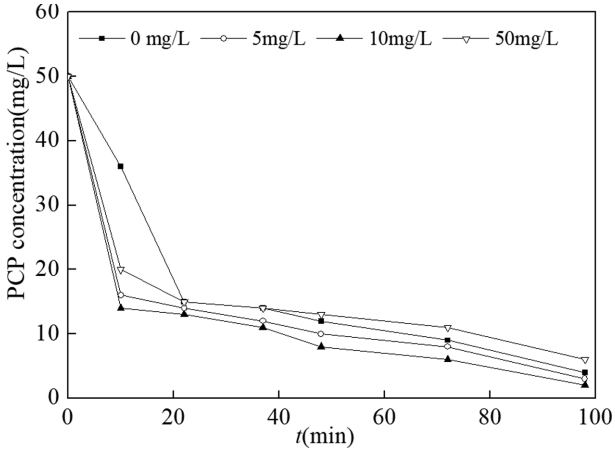


Fig. 4. PCP degradation curves under different initial concentrations of glucose.

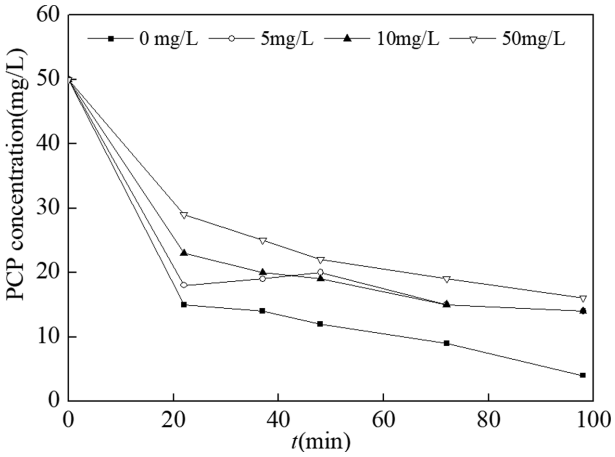


Fig. 5. PCP degradation curve under different initial concentrations of yeast extract.

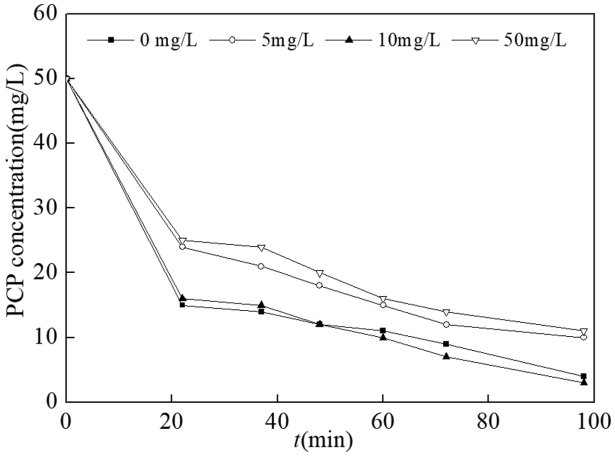


Fig. 6. PCP degradation curve under different initial concentrations of acetic acid.

mainly determined by the level of enzymes and reduced coenzymes and product toxins, so the critical step of co-metabolism degradation was to obtain reductant from the first-order substrate [17-20]. Both trehalose and glucose enhanced the biodegradation of PCP, which may be attributed to them being easy to degrade carbon sources and were easy to degrade and utilized by microorganisms, which provided sufficient carbon source and energy for microbial proliferation. However, when the concentration of trehalose and glucose was different, the effects on PCP degradation were also different. Fig. 2 and Fig. 3 again show that the PCP degradation was the most obvious when the trehalose concentration was 10 mg/L, followed by 5 mg/L and 50 mg/L. It may be that when the dosage concentration was 5 mg/L, the concentration of matrix carbon source in the system increased, which alleviated the growth lag of microorganisms to a certain extent and promoted the biodegradation of PCP. When the concentration was 50 mg/L, the concentration of matrix carbon source was far beyond the energy substance needed for microbial proliferation. Therefore, the mass concentration of the matrix carbon source was no longer the limiting factor for microbial growth. For the dominant microorganism degrading PCP, the mixed matrix formed by the excess of primary matrix trehalose and glucose and secondary matrix PCP restrained the proliferation of predominant bacteria. The process of PCP degradation was inhibited, the removal efficiency was reduced.

It can be seen from Fig. 5 that yeast extract inhibited the biodegradation of PCP when it was used as an additional matrix carbon source, and the greater the dosage of yeast extract, the more pronounced the inhibition effect. Thus, it was mainly due to the metabolic pathway difference between yeast extract and PCP. However, it was also due to the synthetic factors, including the low microbial utilization of yeast extract, the difficulty of providing carbon source and energy for microbial proliferation, the difficulty of maintaining biomass in the system, and the difficulty of inducing the synthesis of critical enzyme used for PCP biodegradation.

It can be seen from Fig. 6 that although acetic acid also had acid inhibition on microorganisms, because the process of acetic acid affecting PCP biodegradation was related to its content, the appropriate concentration of acetic acid did not inhibit the degradation of PCP in micro-aerobic granular sludge. On the contrary, PCP degradation was significantly improved when the acetic acid dosage was 10 mg/L. When under the condition of proper concentration, the existence of acetic acid will not compete with PCP. This was because, on the one hand, acetic acid can be used as a growth substrate which can be easily used to provide a necessary carbon source for the initial stage of biodegradation. On the other hand, acetic acid helped to produce the enzyme system and improve the biodegradation of PCP. However, the co-metabolic degradation of PCP was inhibited when the dosage of acetic acid was 5 mg/L and 50 mg/L. When the acetic acid was insufficient, it could not provide enough carbon sources for the biodegradation process.

Meanwhile, acetic acid and PCP inhibited PCP degradation when the concentration of acetic acid was relatively high by competing for a limited number of inducible enzymes. Furthermore, when the concentration of acetic acid exceeded 10 mg/L, the acidic toxicological properties of acetic acid became more prominent, and the inhibition of PCP biodegradation became more pronounced. As a result, the promotion of acetic acid as a carbon source and energy was partially cancelled, so the effect of co-metabolism degradation was decreased.

2-2. Analysis of Microbial Enzyme Activity During PCP Co-metabolism

Domestication culture is often used to achieve the induction synthesis of key enzymes. Fig. 7 shows the changes in key enzyme activities during the low biomass induction process. It can be seen from Fig. 7 that after the co-metabolism carbon source was added to the system, the microbial proliferation rate was significantly faster, and the key enzymes of degradation of PCP were also induced in the system. The sensitivity and synthesis rate of the key enzymes corresponded to the length of the microbial tolerance period. Supposing co-metabolism carbon source was degraded entirely, the microorganism would enter the endogenous respiration period. As a result, the key enzyme activity synthesized in the body would be gradually reduced, and the content would also be progressively reduced. Therefore, to maintain the activity and content of the inducible enzyme, a specific concentration of matrix carbon source was added to the reaction system that had been domesticated and

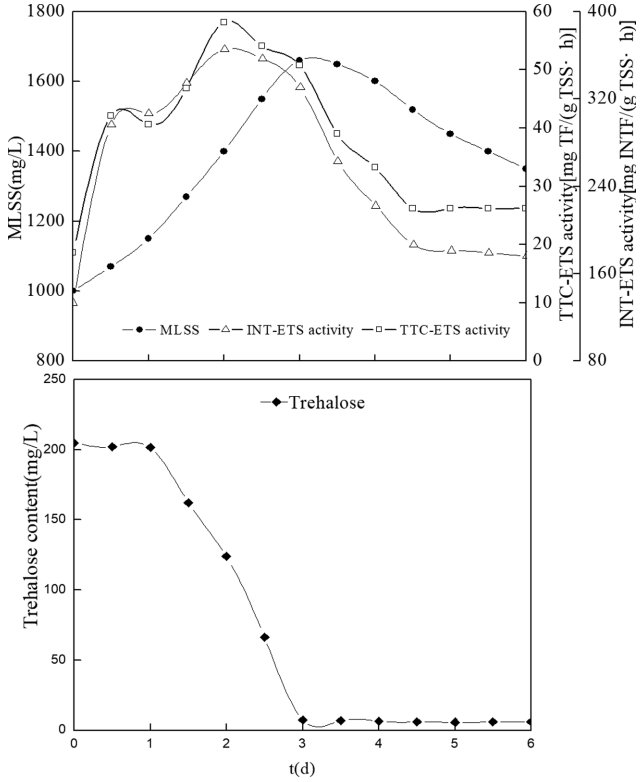


Fig. 7. Activity changes of key enzymes under low biomass conditions.

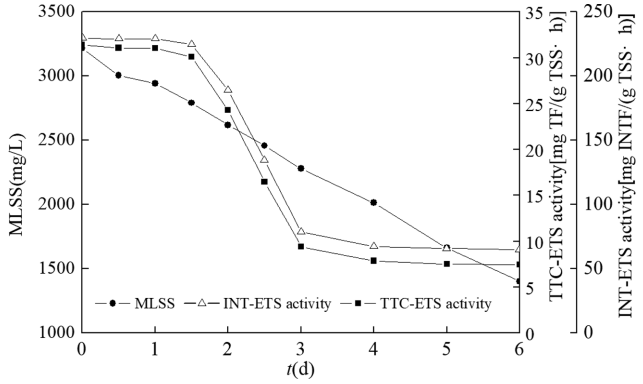


Fig. 8. Activity changes of key enzymes under high biomass conditions.

mature. The presence of a lower concentration of co-metabolic carbon source helped induce the synthetic active enzyme and activate its activity (see Fig. 8).

Meanwhile, when the concentration of co-metabolic carbon source was high, it accelerated active enzymes' induction and maintained high activity. However, many studies on co-degradation techniques have shown that co-degradation of toxic organics is inhibited when co-metabolic carbon source concentrations are too high, resulting in a reduction in co-degradation rates [21-26]. The reason may be competitive inhibition, the competition between the two substrates for key enzyme active positions. Fig. 9 shows competitive inhibition of co-degradation processes. For example, it can be seen from

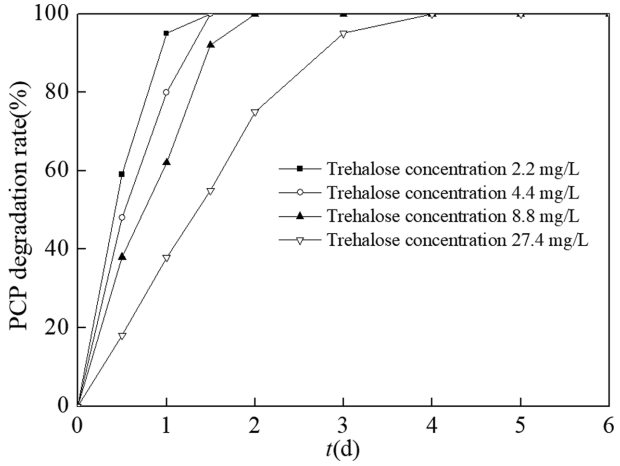


Fig. 9. Competitive inhibition during co-degradation under high biomass conditions.

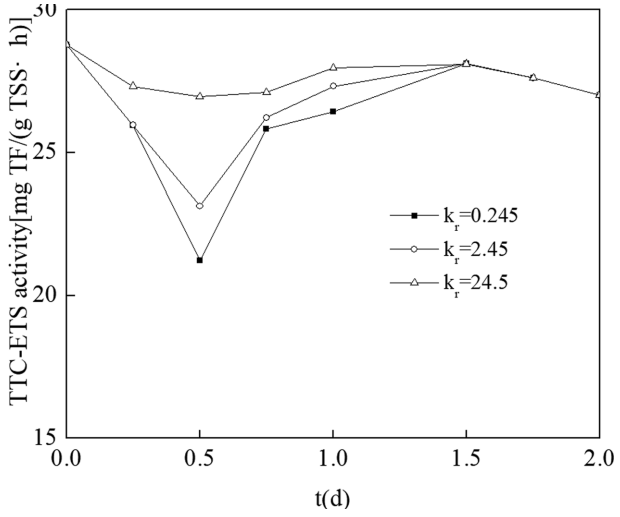


Fig. 10. Toxicity inhibition and self-recovery during co-degradation (TTC-ETS).

Fig. 9 that co-degradation rate of the second matrix PCP decreased as the concentration of the first matrix trehalose increased from 2.2 mg/L to 27.4 mg/L.

In co-degradation, it is difficult for the intermediate metabolic products of toxic organic matter to enter the metabolism of co-metabolic carbon sources; that is, the co-metabolized products are challenging to be used by microorganisms for anabolism. On the other hand, most co-metabolites are toxic substances, which tend to inhibit the active enzyme, cause its inactivation, and reduce the removal rate. To eliminate the toxicity inhibition of co-metabolites, microorganisms initiate a self-recovery function, which is usually measured by a specific recovery coefficient, k,. The toxicity inhibition occurring during the co-metabolism of organisms and the self-recovery process of microorganisms are fitted. Fig. 10 shows the toxicity inhibition and self-recovery during co-degradation for TTC-ETS, while Fig. 11 shows that for INT-ETS. The fitting results were highly consistent with the processes occurring in the biological treatment process. It should be emphasized that self-recovery

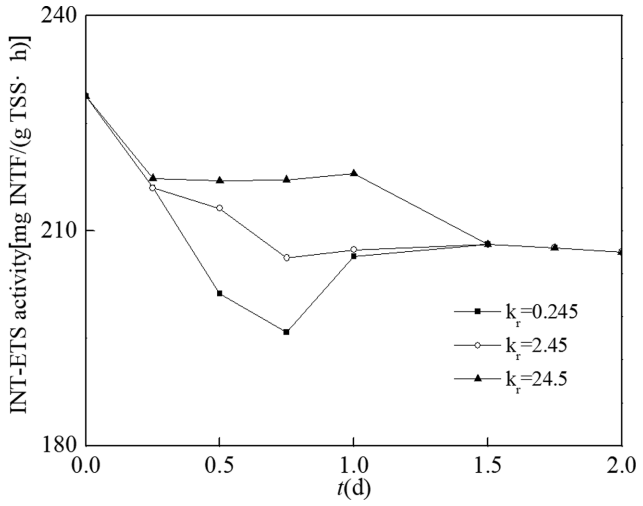


Fig. 11. Toxicity inhibition and self-recovery during co-degradation (INT-ETS).

and toxicity inhibition co-occur in the process of being impacted by toxic substances, and they must be considered simultaneously in the process of kinetic analysis.

In addition, from the perspective of energy metabolism, the co-metabolism process of secondary matrix requires high energy, and this part of energy comes from the degradation process of primary matrix carbon source, that is, the metabolism of secondary matrix extensively consumes the energy in cells [27-31]. If that required energy is significant, the metabolic rate of the second matrix might decrease even if a large number of key enzymes with high activity is present in the system. Therefore, to promote the co-metabolism of the secondary matrix, an energy matrix should be added to the system. However, the concentration of the energy matrix should be moderate. Otherwise, it will accelerate the metabolic rhythm of cells. A too fast intracellular biochemical reaction will considerably consume intracellular energy, resulting in the attenuation and failure of metabolic enzyme activity and even cell inactivation and death. Therefore, the dosage of the primary matrix should not be too high.

3. Co-metabolism Kinetic Model for PCP Removal by Micro-aerated SBR Process

The kinetic degradation model of micro-aerobic granular sludge mainly includes the following parts: (1) Biochemical reaction kinetics of matrix degradation and microbial proliferation; (2) Physicochemical reaction process in sewage; (3) Mass transfer and diffusion process of oxygenated pollutants in granular sludge; (4) Mass transfer and diffusion process of the liquid body [32]. To simplify the complexity of the model, reasonable assumptions were made for the reaction process, mainly including: (1) There were only acidogenic bacteria, methanogens and sulfate-reducing bacteria in the anaerobic zone of micro-aerobic granular sludge; (2) Microorganisms in micro-aerobic granular sludge were in a dynamic equilibrium state of proliferation and decay; (3) The degradation rate of the substrate was equal to the utilization rate of microorganism; (4) The influent did not contain microorganisms, and the free microorganisms in the reactor were ignored; (5) The reactor operated stably under the best working conditions.

3-1. The Modified Formula of Monod Equation in the Presence of Toxic and Harmful Substances

One of the Michaelis-Menten relations based on the enzymatic reaction which was put forward after using a single matrix to culture pure microorganisms, that is, the Monod equation is:

$$\mu = \frac{\mu_{max} S}{K_S + S} \tag{1}$$

In the formula, μ - the specific growth rate of microbial (d^{-1}); μ_{max} - the maximum specific growth rate of microorganisms in the saturated concentration (d^{-1}); K_S - saturation constant, which is the matrix concentration when (mg/L); S - matrix concentration (mg/L).

The Monod equation generally used is only applicable to non-toxic substrates. If there are toxic substances and the concentration reaches a certain level, the growth of microorganisms will be inhibited. Andrews proposed the following modified formula in 1968 based on Haldane's inhibition of high-substrate-concentration enzyme:

$$\mu = \frac{\mu_{max} S}{1 + K_S + \frac{S^2}{K_i}} \tag{2}$$

where K_i - inhibition coefficient.

The inhibition of microbial growth is mainly due to 1) some intermediate products or metabolites produced by the biochemical reaction in the treatment system, such as volatile acid produced in the anaerobic digestion fermentation process. When the concentration of this acid is low, it can be absorbed and used as food by methanogens. But when high, it will inhibit the growth of methane bacteria. 2) For the presence of some industrial wastewater, for example, in aerobic treatment, low concentrations of phenol can be used as a bacterial diet, but high concentrations will play an inhibitory role.

3-2. Construction of PCP Model for Matrix Co-metabolism Degradation

Biodegradation is a comprehensive process of metabolic activity of multiple microorganisms. At present, the biodegradation mechanism of a single pollutant component has been relatively straightforward [33,34]. However, the complexity is significantly increased for numerous pollutants due to the superposition between pollutants and the mutual inhibition process of co-degradation. The following formula can express the biochemical process in which the pollutant is a single component:

$$B_w^l = -\mu_{max} X \left(\frac{C_{w,l}}{K_{S,l} + C_{w,l}}\right)\left(\frac{C_{w,O_2}}{K_{S,O_2} + C_{w,O_2}}\right) \tag{3}$$

$$B_w^{O_2} = -\mu_{max} F X \left(\frac{C_{w,l}}{K_{S,l} + C_{w,l}}\right)\left(\frac{C_{w,O_2}}{K_{S,O_2} + C_{w,O_2}}\right) \tag{4}$$

$$\frac{dX}{dt} = Y\mu_{max} X \left(\frac{C_{w,l}}{K_{S,l} + C_{w,l}}\right) - k_d X \tag{5}$$

where: μ_{max} - the maximum specific growth rate of matrix carbon source (1/T); Y - matrix sludge yield (M/M); $C_{w,l}$ - concentration of matrix in water (M/L^3); C_{w,O_2} - concentration of dissolved oxy-

gen (DO) in water (M/L^3); X - microbial mass concentration (M/L^3); $K_{S,l}$ - semi saturated velocity constant of the matrix (M/L); K_{S,O_2} - semi saturated velocity constant of oxygen (M/L); F - the mass ratio of oxygen required for microbial growth to electron donor; k_d - microbial endogenous respiratory hormone rate (1/T).

There is independent inhibition of microbial substrates for the dual matrix biodegradation system and accumulation inhibition between substrates. Therefore, to describe the competitive inhibition between substrates, the influence factors are introduced and expressed by the following formula:

$$\frac{1}{X}\frac{dS}{dt}=\frac{\mu_{max,g}S}{K_{Sg}\left(1+\frac{I}{K_{SI}}\right)+S}\left(\frac{A}{K_{SA}+A}\right)\left(\frac{R}{K_{SR}+R}\right) \quad (6)$$

$$\frac{1}{X}\frac{dI}{dt}=\frac{\mu_{max,I}I}{K_{SI}\left(1+\frac{S}{K_{Sg}}\right)+I}\left(\frac{A}{K_{SA}+A}\right)\left(\frac{R}{K_{SR}+R}\right) \quad (7)$$

where: S - mass concentration of growth matrix (mg/L); I - mass concentration of non-growing substrate (mg/L); X - Microbial concentration for co-metabolism (mg/L); A - DO concentration (mg/L); R - restrictive nutrients (mg/L); $\mu_{max,g}$ - maximum specific proliferation rate of microorganisms undergrowth substrate (1/h); $\mu_{max,I}$ - maximum specific proliferation rate of microorganisms under non-growth substrate (1/h); K_{Sg} - growth matrix semi saturation velocity constant (mg/L); K_{SI} - semi saturated velocity constant of the non-growth matrix (mg/L); K_{SA} - dissolved oxygen semi saturation velocity constant (mg/L); K_{SR} - semi saturated velocity constant of restrictive nutrients (mg/L).

The microbial concentration can be expressed by formula (8):

$$\frac{\partial X}{\partial t}=\left\{\mu_{max,1}Y_1\left(\frac{S}{K_{Sg}+S}\right)+\mu_{max,2}Y_2\left(\frac{I}{K_{SA}+I}\right)+\mu_{max,3}Y_3\left(\frac{R}{K_{SR}+R}\right)-k_d\right\}\cdot X \quad (8)$$

Assuming that oxygen is a non-limiting factor and that nutrients are sufficient, the equation can be simplified as follows:

$$\frac{1}{X}\frac{dS}{dt}=\frac{\mu_{max,g}S}{K_{Sg}\left(1+\frac{I}{K_{SI}}\right)+S} \quad (9)$$

$$\frac{1}{X}\frac{dI}{dt}=\frac{\mu_{max,I}I}{K_{SI}\left(1+\frac{S}{K_{Sg}}\right)+I} \quad (10)$$

3-3. Numerical Solution of the Model

We obtained the numerical solution using the classical 4th-order Runge-Kutta method's theoretical analysis and calculation method. The selection process should keep the comparability or compatibility between the coefficients, as shown in Table 3. For ordinary differential equations:

$$y'=f(x, y_1, y_2, \ldots, y_m) \qquad y(x_0)=y_0 \quad (11)$$

The formula of the classical R. K method is as follows:

$$y_{n+1}=y_n+\frac{h}{6}(k_1+2k_2+2k_3+k_4) \quad (12)$$

Table 3. Parameter values of PCP co-metabolism model

Co-metabolic matrix	Co-metabolic matrix concentration (mg/L)	μ_{max} (1/h)	K_{Sg} (mg/L)	$K_{S,l}$ (mg/L)
Trehalose	0	0.299	24.091	/
	5	0.408	20.038	2.996
	10	0.405	19.700	0.413
	50	0.452	12.433	7.567
Glucose	0	0.299	24.091	/
	5	0.479	17.499	2.441
	10	0.361	17.069	2.932
	50	0.349	10.089	9.911
Acetic acid	0	0.299	24.091	/
	5	0.041	19.378	0.040
	10	0.390	12.841	7.159
	50	0.070	4.599	15.401
Yeast extract	0	0.299	24.091	/
	5	0.049	19.078	0.019
	10	0.029	18.169	1.841
	50	0.080	18.170	1.848

$$k_1=f(x_n, y_n) \quad (13)$$

$$k_2=f\left(x_n+\frac{h}{2}, y_n+\frac{h}{2}k_1\right) \quad (14)$$

$$k_3=f\left(x_n+\frac{h}{2}, y_n+\frac{h}{2}k_2\right) \quad (15)$$

$$k_4=f(x_n+h, y_n+hk_3) \quad (16)$$

4. Dynamic Fitting and Verification of the Model for the Actual Operation of the Reactor

Based on the above theoretical model and its basic parameters, the change law of PCP degradation by micro-aerobic granular sludge was fitted under different initial concentrations of co-metabolic substrate. Figs. 12-15 show the fitting results of measured and fitted values of degradation of trehalose, glucose, acetic acid and yeast extract at different initial concentrations.

The co-metabolic parameters obtained by fitting calculation are

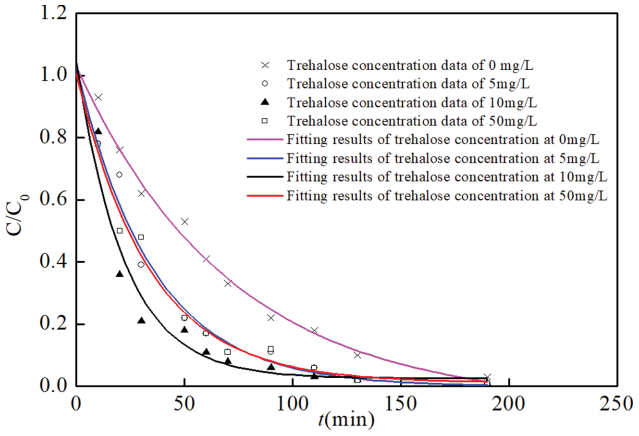


Fig. 12. Fitting results of measured and fitted PCP degradation values under different initial concentrations of trehalose.

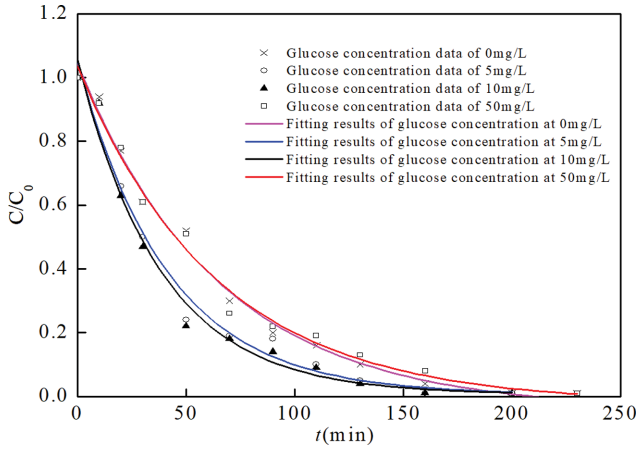


Fig. 13. Fitting results between measured and fitted values of PCP degradation under different initial concentrations of glucose.

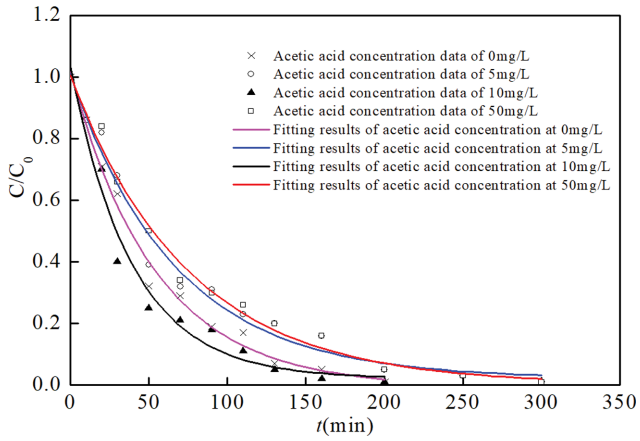


Fig. 14. Fitting results between measured and fitted values of PCP degradation under the different initial concentrations of acetic acid.

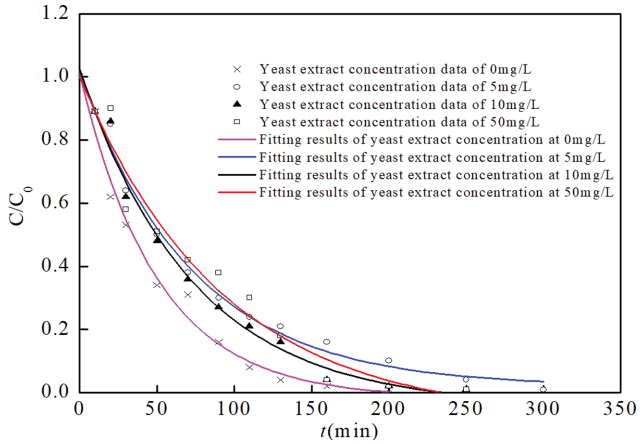


Fig. 15. Fitting results of measured and fitted PCP degradation values under the different initial concentrations of yeast extract.

listed in Table 3. The origin fitting results showed that the fitting values were in good agreement with the measured values. It can be seen from Table 3 that the preferred co-metabolic matrix and micro aeration co-metabolism increased the degradation rate of PCP by 54.9%.

CONCLUSION

Acclimated granular sludge was successfully cultivated in a micro-aerated SBR co-metabolism system. The PCP and its intermediates were removed based on the simultaneous aerobic-anaerobic biodegradation process. The degradation rate of PCP was significantly affected by the type and concentration of the metabolic matrix. Trehalose was added into the system as a co-metabolized matrix, and the growth rate of microorganisms was significantly increased. Higher active biomass was obtained. When the concentration of co-metabolic carbon source was high, it accelerated the induction of active enzymes and maintained high activity. When the co-metabolic carbon source concentration was too high, the co-degradation of toxic organic compounds was inhibited, resulting in decreased co-degradation rate.

The co-metabolic degradation model of PCP in micro aeration SBR and the general kinetic model of micro-aerobic granular sludge were established. The co-metabolic matrix was optimized by solving the model. The degradation rate of PCP increased by 54.9% by micro aeration co-metabolism. The kinetic model was used to fit the microaerobic reaction process of micro-aerated SBR. The coincidence between the theoretical fitting results and the experimental results was as high as 97.6%. The study on the theory and methodology of bio-co-metabolism has a positive effect on improving the bio-metabolism efficiency of refractory organic compounds. It can be applied in practical engineering and is very beneficial to alleviate the pollution of refractory organic wastewater to the water environment.

ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of China (No. 51678272, No. 52170034) and the Department of Science and Technology of Jilin Province (No. 20190303085SF).

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