Pol. J. Environ. Stud. Vol. 32, No. 2 (2023), 1813-1820
DOI: *10.15244/pjoes/157657* ONLINE PUBLICATION DATE: 2023-02-13

Original Research

The Preparation of a Microbial Inoculum Based on Phoxim- Degrading Bacteria and Multicomponent Carrier for The Enhancement of Phoxim Degradation in Activated Sludge Reactor

Jianshe Tang[1,2]*, Xiaomei Zhang[1], Li Xiang[1]

[1]Department of Environmental and Energy Engineering, Anhui Jianzhu University, Ziyun Road 292, Hefei 230601, China
[2]Anhui Provincial Key Laboratory of Environmental Pollution Control and Resource Reuse, Hefei 230601, China

Received: 22 July 2022
Accepted: 19 December 2022

Abstract

Biochemical treatment of pesticide wastewater is difficult accompanied by nonviable microorganisms and low treatment efficiency due to the unique characteristics of the complex components and high biological toxicity of pesticide wastewater. Herein, a highly efficient inoculum was prepared for the degradation of phoxim. The application of inoculum on the treatment of phoxim pesticide wastewater was investigated. A highly efficient phoxim-degrading strain was obtained through the acclimation of phoxim-specific strain in an inorganic salt medium containing 400 mg/L phoxim and 1.0 g/L glucose. The degradation rates of phoxim with initial concentrations of 21.5 mg/L and 50.5 mg/L reached 75.84% and 73.36%, respectively. Inoculum was prepared using the mixture of wheat bran, sawdust and diatomite at the ratio of 80:10:10 (mass ratio) as carrier. The inoculum has the bifunctional advantage of adsorption and degradation to phoxim. The degradation kinetics showed that the degradation rates of phoxim at the initial concentration of 200 mg/L and 400 mg/L reached 75.84% and 73.36% within 10 hours, respectively. The good effect on the removal of phoxim and the wastewater quality purification can be realized when the inoculum was used in sequencing batch reactor activated sludge process to treat pesticide wastewater. This study provides technical means and theoretical foundation for the biological treatment of phoxim pesticide wastewater using a microbial inoculum.

Keywords: wastewater treatment, biological treatment technology, microbial inoculum, pesticide, aerobic oxidation

*e-mail: tjs28@ahjzu.edu.cn

Introduction

Although the serious environmental problems from the extensive application of pesticide, the yield and quality of crop is improved significantly due to contribution of pesticide [1]. Thus, the production and consumption of pesticides is increasing continuously presently [2]. Global pesticide use has grown over the past 20 years to 3.5 billion kilogram per year [3]. Pesticide is an essential agricultural chemical, and its application is imperative. However, nearly 60 % of the synthetic intermediates exist in the pesticide wastewater as pollutants with high concentration due to the complex process of pesticide production and low utilization rate of raw materials [4]. In addition, the operation of purification and equipment cleaning in the pesticide production process discharges wastewater. The discharge of untreated pesticide wastewater into water bodies can cause severe damages to the balance of aquatic ecosystems. Toxic pollutant in pesticide wastewater, i.e., phenol, can pose a serious threaten to human health through the food chain [5]. Therefore, it is very important to control the discharge of toxic pollutants from pesticide wastewater.

Pesticide wastewater treatment is one of the main industrial wastewater treatment problems because of the unique pesticide wastewater characteristics of high organic load, complex components, large water volume fluctuation, as well as many microbicidal pesticide and their analogues [6]. Therefore, more and more attention has been paid to the research on the wastewater treatment procedures and strengthening strategy for the enhancement water purification capacity during biological treatment process of pesticide wastewater [7].

Currently, the treatment technology of pesticide wastewater mainly includes physical, chemical and biological treatment methods [8]. Physical processes are commonly used as pretreatment processes. Adsorption strategy is used as a physical method commonly for the preliminary treatment of wastewater. Nevertheless, the physical wastewater treatment process is implementing essentially the transferring instead of the decomposition process of pollutant. Chemical method is often used as pretreatment process of biochemical treatment to improve the biodegradability of pesticide wastewater. It is not an ultimate mean for pesticide wastewater elimination. Biological method is generally considered as an ideal wastewater treatment technology due to its properties of low energy consumption, high degradation efficiency and stable effluent. However, the toxicity of industrial wastewater limits the application of the biological method [9]. The attachment of microorganisms to solid phase carriers is a mean to effectively enhance the anti-pollution load of sewage biological treatment system and alleviate the toxicity of wastewater on microorganisms [10].

To address the logic antinomy of the microbial treatment system to eliminate industrial wastewater with microbial hazards property, immobilization of highly efficient degradation bacteria against pesticide on the surface of carrier is a good means to solve the problem that microorganisms survive in the pesticide wastewater environment and perform their decomposition functions. The insight on the efficiency of incubation, pesticide wastewater treatment can be strengthened by combining the physical adsorption with biological degradation in the presence of incubation.

To immobilize phoxim-specific degrading strain, the solid phase carrier of wheat bran : sawdust : diatomite was adopted to prepared a microbial inoculum, which was used for pesticide wastewater treatment in sequencing batch reactor (SBR) (Fig. 1). The method can not only overcome the problem of pollutant incomplete removal in physical adsorption, but also effectively alleviate the toxicity of pesticide wastewater on microorganism in a wastewater treatment system. One possibility is that the microbial inoculum could provide the carbon source for sewage treatment system under the technical requirements of nitrogen and phosphorus removal [11].

Experimental

Strain and Reagents

Phoxim degrading strain X-02766 was purchased from Agricultural Culture Collection of China. Phoxim (1000 mg/L) was purchased from the Institute of Environmental Protection, Ministry of Agriculture (Tianjin, China). Protein, beef extract, sodium chloride, magnesium heptahydrate sulfate, ammonium sulfate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and agar were purchased from Sinopharm Chemical Reagent Co. LTD (Shanghai, China). Methanol and acetonitrile (HPLC reagent grade), and acetone (analytical reagent grade) were purchased from Merck & Co Inc (Shanghai, China). Corn straw flour, rice husk flour, wheat bran flour, wood chip flour, diatomite, and kaolin were purchased from Taobao merchants. The water used for all the experiments was purified using a Milli-Q system (Millipore, Bedford, MA, USA). All experiments were carried out in tipples.

Optimum Glucose Content

Glucose solution was added to of inorganic salt medium (phoxim: 21.5 and 50.5 mg/L) to obtain the final glucose concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 g/L with the last volume of 100 mL. Then, 1 % of bacterial solution was added and incubated with the continuing shaking (160 r/min) at 30ºC under the thermostatic shaker (Jintan Instrument Equipment Co., Ltd, Tianjing, China) for 24 h. Samples were taken at 3 hours intervals. OD values of bacterial solution were measured using a UV-visible spectrophotometer (Evolution 220, Thermo Scientific, USA. The concentration of phoxim was determined synchronous by the high-performance liquid

Fig. 1 The pesticide wastewater aeration biological treatment device with the application of the prepared microbial inoculum (insert: Physical map of the prepared microbial inoculum).

chromatography (HPLC) (Beijing Innovation Tongheng Technology Co., LTD, Beijing China).

Acclimation of X-02766

Prepared inorganic salt medium containing 50, 100, 200 and 400 mg/L phoxim. Then equivalent X-02766 bacteria suspension was put and incubated at 30ºC for 2-3 days. Further cultivation was carried out in solid inorganic medium containing 100 mg/L phoxim. The obtained acclimatizable strain for phoxim degradation were noted as X-50, X-100, X-200 and X-400.

Degradation Performance of the Acclimatizable Strain

The bacterial solution was put into the basic salt medium containing 200 mg/L or 400 mg/L of phoxim, and incubated with shaking at 150 r/min for 24 hours. 12 mL of the mixed solution was taken and centrifuged with the speed of 8000 r/min for 10 minutes. The supernatant was filtered through an organic phase filter in 0.22 μm pore size. Phoxim was extracted by added 6 mL of acetone in triple. The extracted phoxim was mixed and condensed into 3 mL, and then determined by an HPLC equipment.

Preparation of Microbial Inoculum

50 mL of the acclimatizable X-400 was mixed with the carriers and the adsorption process of the strain on the surface of carriers was performed at 30ºC for 24 hours. Then the mixture was centrifuged at 4000 r/min for 10 minutes. 20 mL of distilled water was added and centrifuged again. The two supernatants were mixed to evaluate the ability of the carrier to load bacteria. The adsorption capacity of the carriers was determined by dilution plate count method.

The carrier of wheat bran, sawdust and diatomite with various mass ratio were mixed and sterilized for 30 min, cooled, and inoculated with 10 mL of bacterial suspension, fermented by ventilation at 30ºC for 48 hours and dried by ventilation at 30ºC for 1 hour. Took 10.0 g of crushed microbial inoculum, added 100 mL of sterile water, shaken at 150 r/min for 1 hour. The retention performance of carrier was evaluated by dilution coating plate count method. The prepared microbial inoculum was shown in the Fig. 1.

HPLC Analysis Conditions

Phoxim was quantified by an HPLC system, equipped with C_{18} column and an ultraviolet detector. The column temperature was kept at 30ºC , and the wavelength of detector was set at 254 nm. The mobile phase was comprised of methanol and 0.1% phosphoric acid aqueous solution (V:V = 70:30). The flow rate of mobile phase was 1 mL/min.

Pesticide Wastewater Treatment

The experimental setup was made of Plexiglas, and the reaction cell is a cylinder with an inner diameter and a height of 15 cm and 35 cm, respectively (Fig. 1). The biological reaction process was performed under the condition of blast aeration. The synthetic pesticide wastewater was obtained from the mixture of sewage with glucose, ammonium chloride, and potassium dihydrogen phosphate as the carbon, nitrogen, and phosphorus source, respectively. The ratio of CODcr : N : P in concentration was 100 : 5 : 1. The final concentration of pesticide was approximate 59 mg/L by the addition of phoxim. The reactor was run in 2 cycles per day.

Results and Discussion

Carbon Source

Glucose as one of the nutrients affects the growth rate of bacteria, it can typically affect the decomposition of target pollutants by microorganisms [12]. When the initial phoxim concentrations was 21.5 mg/L, there was no correlation between the degradation rate of phoxim and the content of glucose in the medium (Fig. 2a). However, with the increase of glucose content in the medium, the degradation rate of phoxim was promoted obviously when the increase of phoxim concentration (50.5 mg/L) increased the pressure of microbial growth (Fig. 2b). This phenomenon could be explained by insufficient carbon source supply capacity from the small amount of glucose in the medium [13]. As shown in Fig. 2, the addition of 1.0 g/L glucose to the inorganic salt culture medium can effectively promote the degradation of phoxim with the significant degradation rate of phoxim of 56.34% and 63.24%

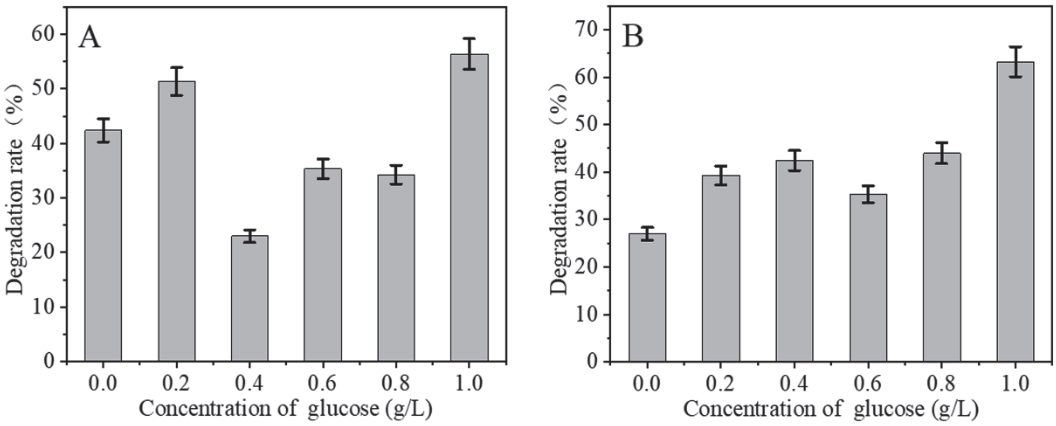

Fig. 2. Degradation rate of phoxim under the condition of the various glucose content in the medium. The initial concentrations of phoxim were 21.5 mg/L a) and 50.5 mg/L b).

for the initial phoxim concentrations of 21.5 mg/L and 50.5 mg/L, respectively.

Acclimation of Strain

The strain was tamed to degrade high concentrations of phoxim. As shown in Fig. 3, it is worth to note that the strain domesticated by low concentration phoxim had high degradation rate of phoxim without glucose. However, the strain under high concentration phoxim stress need additional carbon source in the process of phoxim degradation. The degradation rates of X-400 in inorganic salt medium (containing 1.0 g/L glucose) were 73.63 % and 87.41 % for the initial phoxim concentrations of 21.5 mg/L and 50.5 mg/L, respectively. The degradation rates of phoxim by X-400 in the present of glucose were 3.8 and 2.8 times of those in the absence of glucose with the initial phoxim at 21.5 mg/L and 50.5 mg/L, respectively. Strain Yw12 for the degradation of organophosphorus pesticide was isolated from activated sludge. It can be used to completely degrade and utilize methyl parathion as the sole carbon within 2-6 hours [14]. However, in our experimental results, the external carbon source can greatly promote the degradation efficiency of phoxim.

Growth of Strain X-400

In the inorganic salt medium with glucose, the strain (X-400) grew rapidly because glucose provided sufficient carbon source (Fig. 4). Under inhibition by phoxim in inorganic salt medium, the strain X-400 grew well at the phoxim concentrations of 21.5 mg/L in 9 hours. No strain X-400 growth inhibition was found in the continuous growth process after 9 hours because of the moderate pressure of lower phoxim concentration (Fig. 4a). The growth of the strain at 50.5 mg/L phoxim was inhibited after 18 hours, and the OD_{600} value gradually decreased with the cultivation continues (Fig. 4b). It was noteworthy that growth of the strain in the higher phoxim concentration was better than that in the lower phoxim concentration, probably because the strain

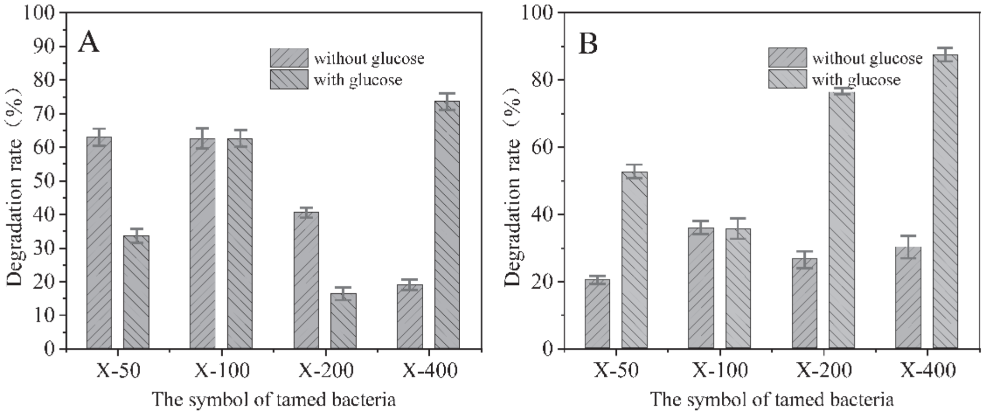

Fig. 3. Degradation rates of phoxim with/without glucose by the strains tamed in various concentration. The initial concentrations of phoxim were 21.5 mg/L a) and 50.5 mg/L b).

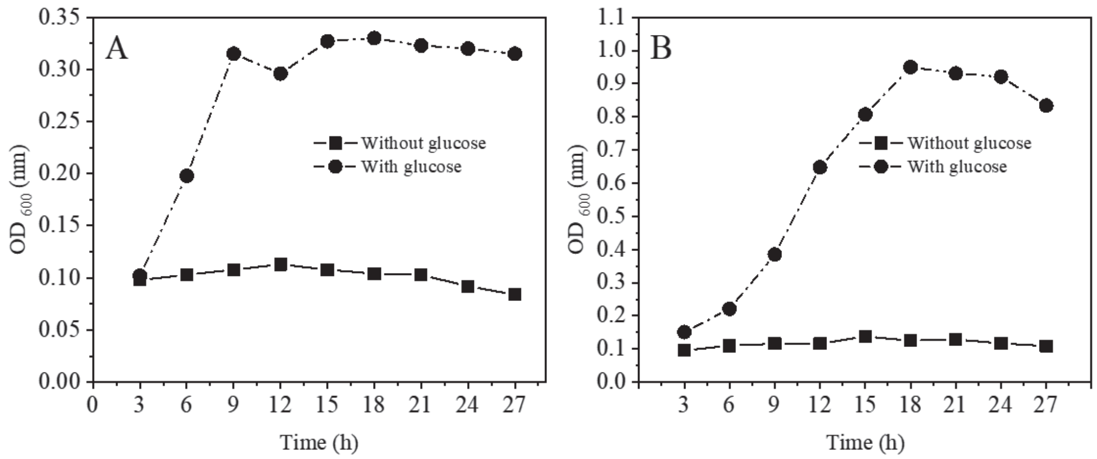

Fig.. 4 Growth curves of X-400 at phoxim concentration of 21.5mg/L (a) and 50.5mg/L b).

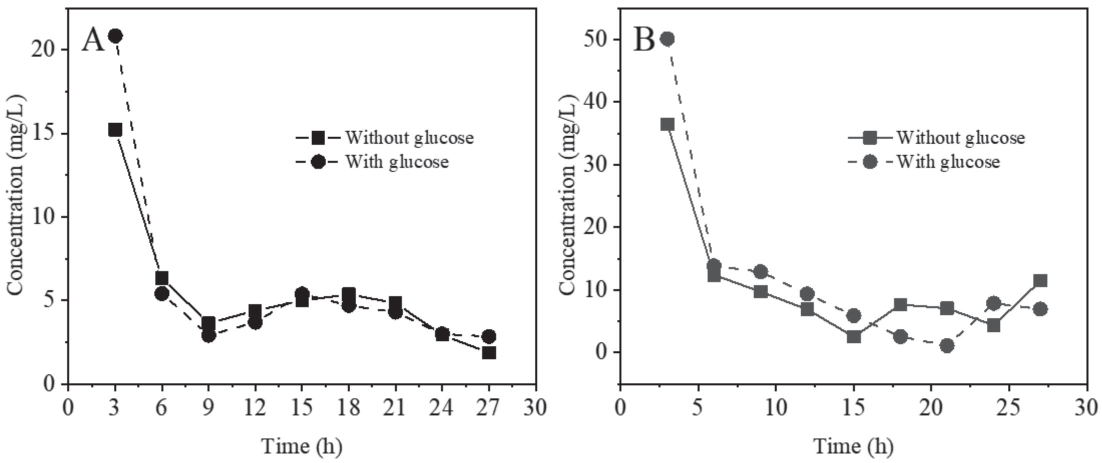

Fig. 5 The degradation curves with/without glucose in the initial phoxim concentrations of 21.5 mg/L a) and 50.5 mg/L b).

could make full use of phoxim as the carbon source to obtain growth conditions in the late growth stage.

Degradation of Phoxim

In the inorganic salt medium containing phoxim, the early degradation rate (before 6 h) was rapid due to the large growth of the strain X-400. The continuous decline of degradation rate in the later stage may be related to the inhibition of bacterial growth. The degradation rates of the initial phoxim concentrations of 21.5 mg/L and 50.5 mg/L were 75.84% and 73.36% in 24 hours in the present of glucose, respectively (Fig. 5).

Table 1. Comparison of the survival organism loaded on the various carriers.

Carrier	Number of microorganisms (10^9 CFU/g)	Carrier		Number of microorganisms (10^9 CFU/g)
Rice husk	0.92	Wheat bran: Sawdust: Diatomite	75:20:5	5.4
Wheat bran	2.77		75:15:10	4.8
Sawdust	1.96		80:10:10	7.2
Corn straw powder	0.68		80:15:5	5.0
Kaoline	0.99		85:10:5	3.7
Diatomite	1.33			

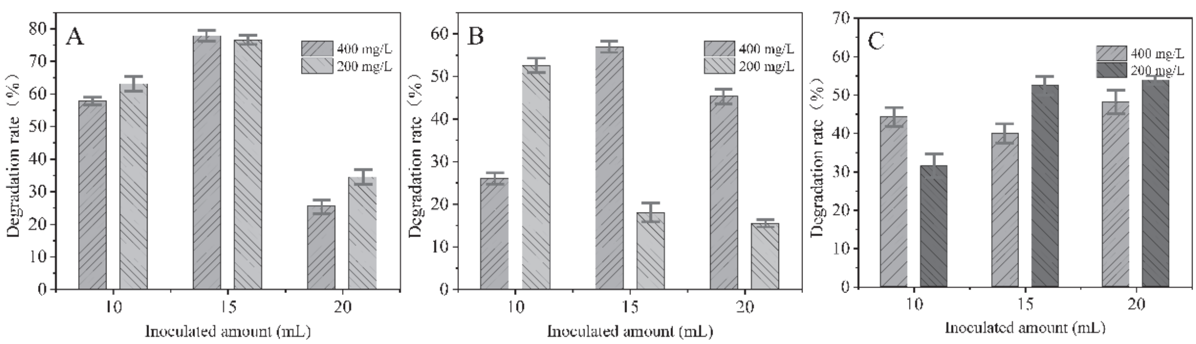

Fig. 6 Degradation rates of phoxim by the inoculum prepared at drying temperatures of 20ºC a), 30ºC b) and 40ºC c) with different strain amounts.

The Preparation of Inoculum

As a global waste stemmed from wood processing, sawdust has tremendous potential to be used for wastewater treatment. The inherent cellulose component of sawdust increased its strength when it was used as a carrier. Sawdust powder as carriers had less microorganism adsorption [15]. Much literatures reported the approaches on sawdust-biomass-based material for the adsorptive removal of different pollutants from an aqueous solution [16]. Wheat bran had higher microorganism adsorption [17]. Therefore, the carrier with multiple substances was a good option to obtain the inoculum with high loading capacity. Considering microbial growth, wheat bran can provide sufficient nutrients for microorganisms [18]. Wheat bran was a source of high-quality protein with low cost. Moreover, superfine bran protein hydrolysate exhibited the highest antioxidant activity and starch-digestive enzyme inhibitory activity that could facilitate the application of bran as a multifunctional nutrient [19]. Considering the aeration of the carrier, sawdust and wheat bran could make the carrier loose, which was benefit for the growth of microorganisms. However, the small density of the two substances makes the carrier suspended in the reactor, which is extremely unfavorable for sewage treatment. Diatomite could capture nitrogen, phosphorus, potassium and other nutrients to provide a stable nutrient environment for the microorganism [20]. In addition, the 3 microbial support materials above-mentioned were selected on the basis of the investigation of the ability of single carrier to carry microorganisms (Table 1). When the ratio of wheat bran: sawdust: diatomite was 80:10:10, the carrier provided a suitable growth environment for the bacteria and the maximum number of live bacteria could be obtained as 7.2×10^9 CFU/g (Table 1).

Mixture of wheat bran : sawdust : diatomite in the ratio of 80 : 10 : 10 has good degradation efficiency when the strain amount was 15 % (15 mL). Too little strain amount would reduce the immobilized microorganisms, thus reducing the degradation rate. The drying temperature in the preparation process of inoculum has a serious impact on the activity of microorganisms on the inoculum [21]. Fig.6 shows the phoxim degradation rates by the prepared inoculum dried at the different temperature. The low degradation rates with the inoculum dried at 20ºC indicated that the incomplete drying of the inoculum have great interference with the stability of microorganisms. On the contrary, the high drying temperature accelerated the senescence and death of the microorganisms, thus led to the lower degradation rate of phoxim. The degradation rates of phoxim at initial concentrations

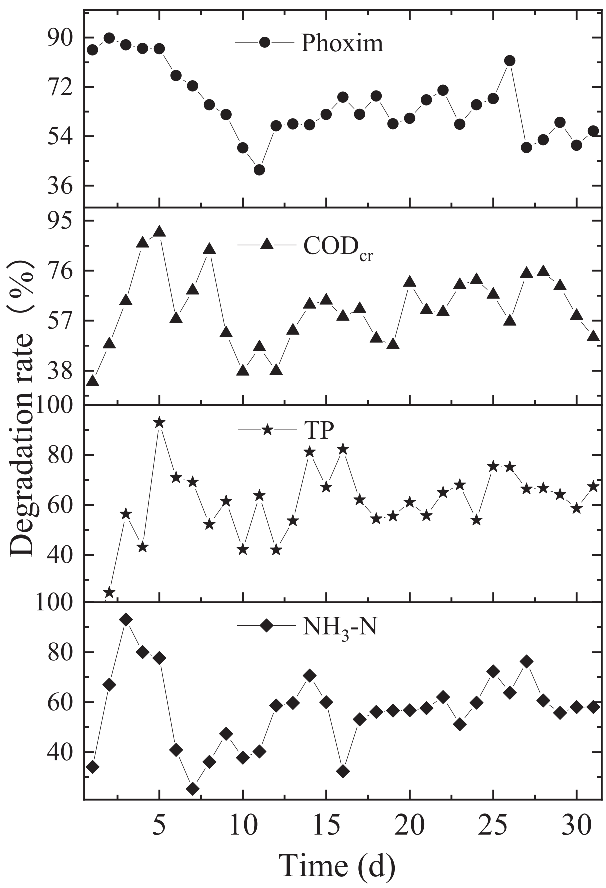

Fig. 7 Time courses of the concentration of phoxim, COD_{cr}, TP and NH_3-N in the effluent.

of 400 mg/L and 200 mg/L were 77.80% and 76.52%, respectively, under the conditions of drying temperature of 30ºC (Fig. 6).

Application of Inoculum in Pesticide Wastewater Treatment

Fig. 7 shows the time course of the phoxim, CODcr, TP and NH_3-N concentration of the effluent. During early run time (10 days), the phoxim, CODcr, TP and NH_3-N concentration of the effluent wastewater fluctuated violent. With the progress of sewage treatment operation, the dynamic balance of continuous growth and extinction of microorganisms was established, and the effluent quality was becoming more and more stable. In terms of process performance, the prepared inoculum has achieved good results in the removal of phoxim, COD_{Cr}, TP and NH_3-N in the treatment of pesticide wastewater. The mean removal efficiencies for phoxim, COD_{Cr}, TP and NH_3-N reached up to 60.57%, 65.15%, 68.85%, and 59.29% in 30 days of operation, respectively, which claims efficient candidature of the prepared inoculum used in pesticide wastewater treatment process.

Conclusions

In this paper, we have domesticated the bacterium X-02766 with the performance of degrading phoxim. The optimized bacterial growth conditions for the degradation of phoxim was investigated. The degradation rates of the domesticated bacteria X-400 in medium (containing 1.0 g/L glucose) were 73.63% and 87.41% for the initial concentration of 21.5 mg/L and 50.5 mg/L of phoxim, respectively. The optimal carrier for inoculum was discovered by single-factor experiment as wheat bran : sawdust : diatomite = 80 :10 :10. The inoculum was prepared by adding 15 mL of bacterial solution into the carrier and drying at 30ºC. The degradation rates of phoxim by inoculum for 24 hours at the initial concentration of phoxim of 200 mg/L and 400 mg/L were 75.84% and 73.36%, respectively. The prepared inoculum was used to treat the pesticide wastewater in continuous bioreactor. The mean removal efficiencies for phoxim, COD_{Cr}, TP and NH_3-N reached up to 60.57 %, 65.15 %, 68.85 %, and 59.29 % in 30 days, respectively.

Acknowledgments

This work was supported by the Natural Science Foundation of Anhui Province (Grant Number 2108085ME184), the Collaborative Innovation Project of Anhui Provincial Department of Education (Grant Number GXXT-2021-057), and the Doctoral Scientific Research Startup Foundation of Anhui Jianzhu university (Grant Number 2020QDZ36).

Conflict of Interest

The authors declare that they have no known competing inancial interests or personal relationships that could have influenced the work reported in this paper.

ORCID

Jianshe Tang http://orcid.org/0000-0003-2370-2091

References

1. POPP J., PETO K., NAGY J. Pesticide productivity and food security. A review. Agron. Sustain. Dev. **33** (1), 243, **2013**.
2. PANDISELVAM R., KAAVYA R., JAYANATH Y., VEENUTTRANON K., LUEPRASITSAKUL P., DIVYA V., KOTHAKOTA A., RAMESH SV. Ozone as a novel emerging technology for the dissipation of pesticide residues in foods-a review. Trends. Food Sci. Tech. **97**, 38, **2020**.
3. PRETTY J., BHARUCHA Z.P. Integrated pest management for sustainable intensification of agriculture in Asia and Africa. Insects. **6** (1), 152, **2015**.
4. NIE J., SUN Y., ZHOU Y., KUMAR M., USMAN M., LI J., SHAO J., WANG L., TSANG DCW. Bioremediation of water containing pesticides by microalgae: Mechanisms., methods., and prospects for future research. Sci. Total Environ. **707**, **2020**.
5. SINGH A.K., CHANDRA R. Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. Aquat. Toxicol. **211**, 202, **2019**.
6. DE OLIVEIRA M., FARIAS FRIHLING B.E., VELASQUES J., CORREA MAGALHAES FILHO F.J., CAVALHERI P.S., MIGLIOLO L. Pharmaceuticals residues and xenobiotics contaminants: Occurrence., analytical techniques and sustainable alternatives for wastewater treatment. Sci. Total Environ. **705**, **2020**.
7. NAKANO Y., MIYAZAKI A., YOSHIDA T., ONO K., INOUE T. A study on pesticide runoff from paddy fields to a river in rural region - 1: Field survey of pesticide runoff in the kozakura river., Japan. Water Res. **38** (13), 3017, **2004**.
8. BHATIA S.K., MEHARIYA S., BHATIA R.K., KUMAR M., PUGAZHENDHI A., AWASTHI M.K., ATABANI A.E., KUMAR G., KIM W., SEO S-O. Wastewater based microalgal biorefinery for bioenergy production: Progress and challenges. Sci. Total Environ. **751**, **2021**.
9. PUNZI M., ANBALAGAN A., BORNER R.A., SVENSSON B.M., JONSTRUP M., MATTIASSON B. Degradation of a textile azo dye using biological treatment followed by photo-Fenton oxidation: Evaluation of toxicity and microbial community structure. Chem. Eng. J. **270**, 290, **2015**.
10. ZHUANG L.L., AZIMI Y., YU D., WANG W.L., WU Y.H., DAO G.H., HU H.Y. Enhanced attached growth of microalgae *scenedesmus. Lx1* through ambient bacterial pre-coating of cotton fiber carriers. Bioresource Technol. **218**, 643, **2016**.

11. ZHANG M., JI B., LIU Y. Microalgal-bacterial granular sludge process: A game changer of future municipal wastewater treatment? Sci. Total Environ. **752**, **2021**.
12. REISCHKE S., ROUSK J., BAATH E. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biol. Biochem. **70**, 88, **2014**.
13. BOLLOK M., RECZEY K. Cellulase enzyme production by various fungal strains on different carbon sources. Acta Aliment. **29** (2), 155, **2000**.
14. WANG S., ZHANG C., YAN Y. Biodegradation of methyl parathion and p-nitrophenol by a newly isolated agrobacterium *sp* strain *yw12*. Biodegradation. **23** (1), 107, **2012**.
15. MENEZES BDS., ROSSI D.M., SQUINA F., AYUB MAZ. Xylooligosaccharides production by fungi cultivations in rice husk and their application as substrate for lactic acid bacteria growth. Bioresource Tech. Reps. **2**, 100, **2018**.
16. ADEGOKEA K.A., ADESINA O.O., OKON-AKAN O.A.O., ADEGOKE O.R., OLABINTAN A.B., AJALA O.A., OLAGOKE H., MAXAKATO N.W., BELLO O.S. Sawdust-biomass based materials for sequestration of organic and inorganic pollutants and potential for engineering applications. Curr. Res. Green Sustain. Chem. **5**, 100274, **2022**,
17. SANTOS PDS., ABATI K., MENDOZA NVR., MASCARIN GM., JUNIOR ID. Nutritional impact of low-cost substrates on biphasic fermentation for conidia production of the fungal biopesticide metarhizium anisopliae. Bioresource Tech. Reps. **13**, 100619, **2021**.
18. ONILUDE A.A., FADAUNSI I.F., GARUBA E.O. Inulinase production by sp in solid state fermentation using wheat bran as substrate. Ann. Microbol. **62** (2), 843, **2012**.
19. LI Y., WANG H., WANG L.J., QIU J,. LI Z,. WANG L.L. Milling of wheat bran: Influence on digestibility, hydrolysis and nutritional properties of bran protein during in vitro digestion. Food Chem. **404** (15), 134559, **2023**.
20. WANG X., ZHENG J., HAN Z., CHEN H. Bioremediation of crude oil-contaminated soil by hydrocarbon-degrading microorganisms immobilized on humic acid-modified biofuel ash. J. Chem. Technol. Biot. **94** (6), 1904, **2019**.
21. VALENTE S., MELONI G.R., PRENCIPE S., SPIGOLON N., SOMENZI M., FONTANA M., GULLINO M.L., SPADARO D. Effect of drying temperatures and exposure times on aspergillus flavus growth and aflatoxin production on artificially inoculated hazelnuts. J. Food Protect. **83** (7), 1241, **2020**.