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INFLUENCE OF SPREADING CULTIVATION WITH FOOD WASTE CULTURE MEDIUM ON THE DEGRADATION OF ORGANOPHOSPHORUS PESTICIDES BY A MICROBIAL CONSORTIUM OP-1

A microbial consortium OP-1 was enriched from a pesticide production plant and spread cultivated on various food waste (FW) culture media. Results indicated that OP-1 can efficiently degrade organophosphorus pesticides (OPs): isocarbophos, dimethoate and dichlorvos (DDVP). The microbial consortium obtained from the spreading cultivation using FW culture maintained certain degradation capacity of OPs. However, it was decreased after spreading cultivation, probably due to the change of microbial community structure. The finding of this study provided scientific supporting for the development of microbial degradation technique for the environmental remediation.

1. INTRODUCTION

Organophosphorus pesticides (OPs) have been widely used in agriculture for crop protection and pest control since 1937 [1]. However, the continuous and excessive use of OPs has become a growing global environmental pollution and health issue because of the toxicity of OPs on the inhibition of neurotransmitter acetylcholine breakdown [2, 3]. Although OPs are considered less persistent in the environment compared with organochlorine pesticides [4], some OPs can still be retained in soils for a long time and contaminate surrounding surface and ground water environment via runoff and leaching [5, 6]. Therefore, efficient methods for the remediation of OP contamination must be developed.

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Among the remediation technologies, the utilization of microbial degradation is a viable and environmental friendly approach. Since 1973, many strains of OP-degrading bacteria, fungi, and actinomycetes have been isolated and studied [7–10]. However, the complete degradation of OPs may need the corporation of co-existing microorganisms. For instance, chlorpyrifos could be degraded into 3,5,6-trichloro-2-pyridinol (TCP) by many degraders, but further degradation of TCP can only be performed by certain microbes, such as *Pseudomonas* sp. [11-12]. In addition, different types of OPs are simultaneously used to control agricultural diseases and pests, suggesting that field contamination usually comes from mixed OPs. Thus, the development of mixed microbial consortium that can simultaneously effectively degrade various OPs is necessary.

The degradation of OPs by microorganisms is influenced by complex environmental conditions, including various carbon sources [13, 14]. Although microorganisms usually degrade several types of OPs, the co-existence of various OPs in field may lead to different performance of microbial degradation rather than the degradation for single OP [15]. In addition, some microorganisms degrade OPs via the co-metabolic pathway, which requires other organic compounds (i.e., glucose and acetate) as carbon source [16, 17]. For instance, Xie et al. [18] found that the addition of succinate and acetate promotes the growth and degradation performance of a malathion degrader. However, Yañez-Ocampo et al. [19] found that the degradation of methylparathion and tetrachlorvinphos is decreased with glucose supply. Thus, the variety and distribution of carbon source is a key factor that controls the performance of OP degraders.

In addition, the spreading cultivation of microbial consortium is an important issue when using microbial technology. Municipal solid waste (MSW) such as food waste (FW) contains high level of organic constituents, which can serve as the carbon and energy source of microorganisms. In China, FW contributes from 37% to 62% of MSW, in which the daily discharge of FW is higher than 20 000 ton [20]. The use of FW for the spreading cultivation of microbial inoculum will provide additional economic value during waste management. However, spreading cultivation has no specific target because of the lack of OPs in FW. Therefore, OP degradation may be influenced by spreading cultivation parameters, including FW culture medium composition and cultivation period.

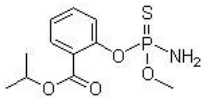
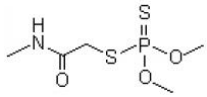
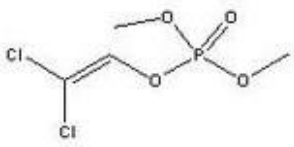
In this study, an OP degrading-microbial consortium (OP-1) was obtained from a pesticide production plant via enrichment culture. Then, a series of experiments was conducted to evaluate the degradation capacity of microbial consortium for three typical OPs (isocarbophos, dimethoate, and dichlorvos (DDVP)) in China. These OPs have caused serious contamination of soil and water environment. A pilot spreading cultivation of microbial consortium using FW culture was conducted, and then changes in the degradation capacity and microbial community structure of the inoculum were investigated. This study aims to improve the combination of the environment remediation and the reuse of municipal organic waste, providing useful information for the decontamination of OP pollution.

2. MATERIALS AND METHODS

Chemicals and media. Isocarbophos (20% purity) and DDVP (77.5% purity) were purchased from Guomei Chemical Plant, Hebei, China. Dimethoate (40% purity) was purchased from Xianrong Inc., Hubei, China. Details of the chemical properties of three OPs are given in Table 1. Acetone and dichloromethane of high-pressure liquid chromatography (HPLC) gradient grade were purchased from TEDIA Company Inc.. All other reagents used in this study were of analytical reagent grade.

Table 1

Properties of isocarbophos, dimethoate, and DDVP

Compound	Isocarbophos	Dimethoate	DDVP
	O-methy O-[2-(isopropoxy-carbonyl)phenyl] phosphoramidothioate	Phosphorodithioic acid, O,O-dimethyl S-[2-(methylamino) -2-oxoethyl] ester	Dichlorvos, O,O-dimethyl -O-2,2-dichlorovinyl -phosphate
Molecular formula[a]	$C_{11}H_{16}NO_4PS$	$C_5H_{12}NO_3PS_2$	$C_4H_7Cl_2O_4P$
Molecular weight ($g \cdot mol^{-1}$)	289.29	229.26	220.98
Molecular structure			
Water solubility and volatility	insoluble	slightly soluble (23.5 $g \cdot dm^{-3}$)	slightly soluble (1 $g \cdot dm^{-3}$), high volatility
LD_{50} ($mg \cdot kg^{-1}$)	50	387	25
Toxicity level	moderately toxic	moderately toxic	highly toxic

Molecular structure and solubility data were obtained from Sigma-Aldrich Inc. LD_{50} obtained from Sigma-Aldrich Inc.. was the medium lethal dose for rats. The toxicity was classified based on the classification scheme of world health organization (WHO).

The mineral salt medium (MSM) used in this study contained 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot 2H_2O$, 0.001 g of $FeSO_4 \cdot 7H_2O$, 1.5 g of $Na_2HPO_4 \cdot 12H_2O$, and 1.5 g of KH_2PO_4 per 1 dm^3 of deionized water [15]. The final pH was adjusted to 7.0. In some experiments, 3.6 $g \cdot dm^{-3}$ of glucose was added into medium (MSM-Glu).

Enrichment of microbial consortium OP-1. The OP-contaminated soils were collected from a pesticide production plant in Xiamen, Fujian Province, China. The enrichment was performed in a brown jar with a rubber stopper and an aeration device. Three subsamples of soils (10 g) were added into brown jars with 2 dm^3 of MSM. Then, 1 dm^3 of OP solution containing 1 $g \cdot dm^{-3}$ of isocarbophos, dimethoate, or DDVP was added into one of the brown jars to enrich the OP degraders for 2 months. Afterward, the same volume of suspensions was collected from the jars and then mixed. The suspension mixture was centrifuged at 10 000 rpm for 10 min at 4 °C and then washed twice with 0.05 $mol \cdot dm^{-3}$ phosphate-buffered saline (PBS, pH 7.0). Afterward, the microbial consortium OP-1 was obtained by the dispersion of the precipitates in MSM.

Evaluation of the degradation capacity of microbial consortium OP-1. The OP degradation capacity of microbial consortium OP-1 was evaluated by adding 45 cm^3 of culture medium with OPs and 5 cm^3 of microbial consortium into a 100 cm^3 triangular flask. After covered with ventilate sealing film, the degradation of OPs was performed by shaking at 120 rpm at 25 °C in the dark for 12 days. Two types of culture medium were used: MSM with OPs and MSM-Glu with OPs. Control treatments without the microbial consortium were also prepared. The concentrations of isocarbophos, dimethoate, and DDVP in the medium were 25, 30, and 150 $mg \cdot dm^{-3}$, respectively. Triplicate samples were prepared for each treatment.

OPs degradation in culture media of various compositions. In this experiment, 27 cm^3 of culture medium with OPs and 3 cm^3 of microbial consortium were added into a 60 cm^3 serum bottle, leaving 30 cm^3 of airspace to maintain aerobic conditions. Then, the serum bottles were closed with rubber stoppers and aluminum caps before shaking at 150 rpm at 25 °C in the dark for 5 days. Two types of culture medium were used: MSM with OPs and MSM-Glu with OPs. Control treatments without microbial consortium were also prepared. The concentrations of isocarbophos, dimethoate, and DDVP in the medium were 25, 15, and 20 $mg \cdot dm^{-3}$, respectively. Triplicate samples were prepared for each treatment.

Degradation of OPs by microbial consortium from spreading cultivation with FW culture. In the spreading cultivation, 5 cm^3 of the microbial consortium OP-1 was added into 50 cm^3 of the FW culture and then cultivated on a rotator at 30 °C in the dark for 2 days. Approximately 10 cm^3 of the suspension was centrifuged, washed twice with 0.05 $mol \cdot dm^{-3}$ of PBS (pH 7.0), and then dispersed in 10 cm^3 of MSM to obtain the microbial consortium. Three types of FW culture were used, in which the solid concentrations of FW were 0.5%, 1%, and 5%, producing consortia OP-A, OP-B, and OP-C, respectively. To prepare the artificial FW, 100 g of cabbage, 12.5 g of rice, 12.5 g of noodle, 25 g of potato, 10 g of pork, 1 g of salt, and 18 g of bean oil were mixed and crushed. Considering the water content in FW (especially for cabbage), 0.5 dm^3 of water

was added to obtain 5% FW culture, in which the concentrations of total solid and ash were 55.4 $mg \cdot g^{-1}$ and 19.5 $mg \cdot g^{-1}$, respectively. In addition, the contents of protein, starch and fat in 5% FW culture were 0.88%, 0.74%, and 2.1%, respectively. Then, the 5% FW culture was diluted to 0.5% and 1% solid concentration with sterilized water according to the set-up of solid-holdup. Then, the artificial FW was boiled for 10 min. After cooling to room temperature, the artificial FW was used as culture medium of the spreading cultivation.

Then the degradation experiment was conducted in 20 cm^3 serum bottles as mentioned above. In short, 1 cm^3 of microbial consortium was added into 9 cm^3 of MSM with mixed OPs. The serum bottles were shaken at 150 rpm at 25 °C. At time intervals, the concentrations of OPs in the medium were analyzed. One treatment with original microbial consortium OP-1 and one control treatment without any consortium were also prepared. Triplicate samples were prepared for each treatment.

Analysis. The OPs were extracted from the medium by mixing with dichloromethane in the volume ratio of 1:1. After shaking for 5 min and equilibrating for 2 min, the concentrations of OPs in the dichloromethane phase were measured using a 7900A gas chromatograph equipped with a flame photometric detection system (Shanghai Tianmei, Inc., Shanghai, China). The capillary column was a TM-5 (30 m×0.25 mm i.d. with 0.25 μm). Nitrogen gas was used as a carrier gas at a flow-rate of 35 $cm^3 \cdot min^{-1}$. Injector and detector temperatures were 220 °C and 250 °C, respectively. Oven temperature was programmed as follows: 120 °C for 1 min, raised to 180 °C at 25 °C min^{-1}, and then raised to 200 °C (20 $°C \cdot min^{-1}$) for 5 min. The sample (1 μL) was injected in the splitless mode (1 min). The recovery of OPs by dichloromethane extraction ranged from approximately 94.8% to 104.5%.

3. RESULTS AND DISCUSSION

3.1. DEGRADATION OF OPS BY MICROBIAL CONSORTIUM OP-1

The concentration of isocarbophos in MSM medium rapidly decreased after incubation with microbial consortium OP-1 (Fig. 1a). The concentration of isocarbophos in MSM medium decreased to a nondetectable level within 3 days. By contrast, the concentrations of isocarbophos in the control treatment was stable (ca. 20 $mg \cdot dm^{-3}$) during the incubation. Similarly, the concentration of dimethoate in MSM medium decreased during the incubation with microbial consortium, whereas the concentration of the control treatment showed minimal changes (Fig. 1b). After 12 days of degradation, dimethoate almost completely disappeared from the medium. Meanwhile, regarding DDVP, the decreasing rate of DDVP with OP microbial consortium was higher than that with the control treatment. On day 12, the concentrations of DDVP with OP microbial consortium was 1.85 $mg \cdot dm^{-3}$ in MSM medium compared to 13.70 $mg \cdot dm^{-3}$ in the control treatments without OP microbial consortium.

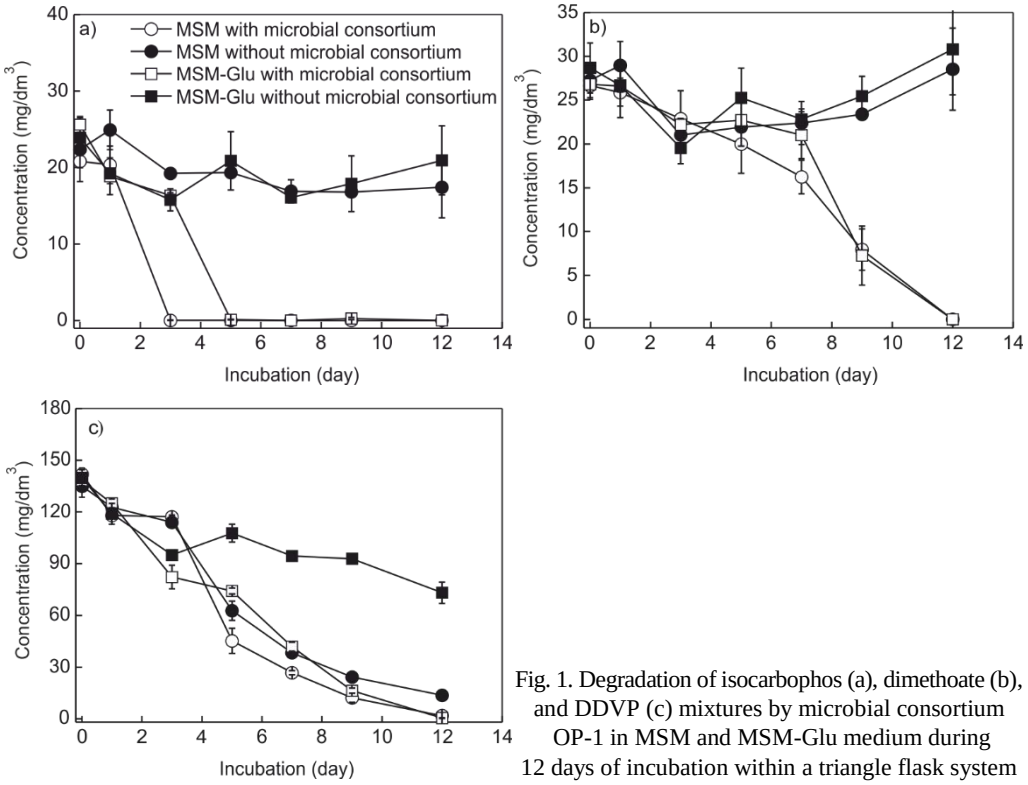


Fig. 1. Degradation of isocarbophos (a), dimethoate (b), and DDVP (c) mixtures by microbial consortium OP-1 in MSM and MSM-Glu medium during 12 days of incubation within a triangle flask system

These results demonstrated that the microbial consortium enriched from the soils of the pesticide production plant could efficiently degrade the isocarbophos, dimethoate, and DDVP. According to the analysis of microbial community structure (Fig. 2), the bands affiliated to *Flavobacteriaceae* and *Xanthomonadaceae* were presented in the microbial consortium OP-1, which had been previously reported as efficient OP degraders with broad substrate specificity [21–23]. Thus, the microbial consortium OP-1 could present the degradation capacity of OPs due to the existence of such OP degraders. Among three types of OPs, the isocarbophos was preferentially degraded rather than that for dimethoate (Fig. 1). Wang et al. [24] found similar results. They attributed the harder degradation of dimethoate to its specific molecular structure and properties. The different molecular structures of OPs lead to higher hydrophobicity and hence slower advective-dispersive transport of dimethoate in the environment compared with isocarbophos, resulting in the lower bioavailability of dimethoate for OP degraders than isocarbophos [25]. Regarding DDVP, the addition of OP microbial consortium decreased DDVP concentration compared with the treatment without OP microbial consortium. However, in the control treatment without addition of OP microbial consortium, the decrease of DDVP concentration was also observed. So, the high volatility of

DDVP also partially contributed to its decrease in concentration regardless of the absence or presence of the microbial consortium [26].

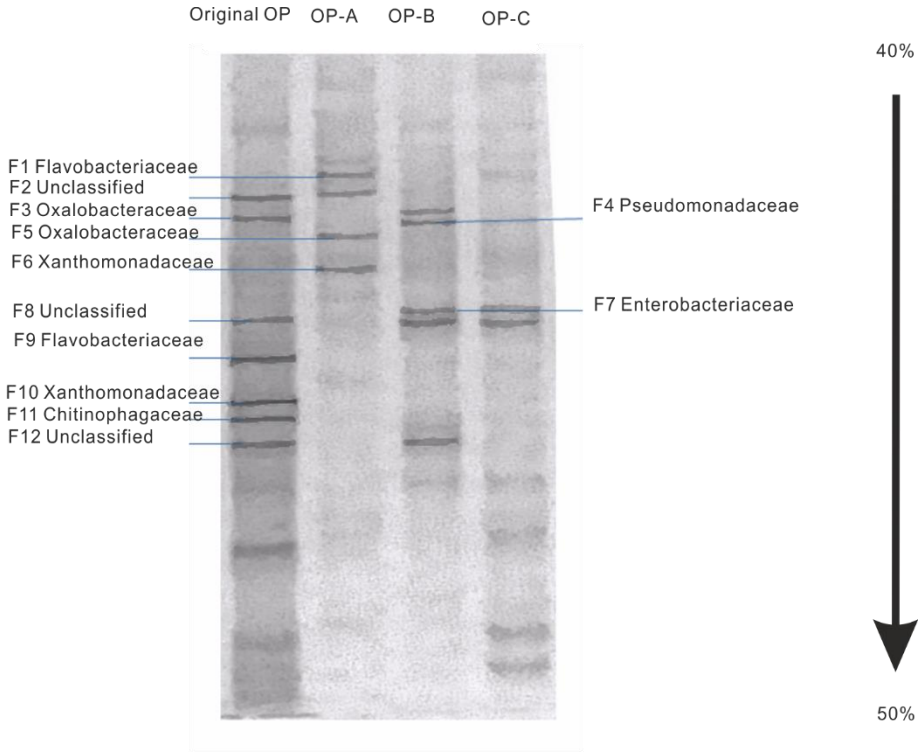


Fig. 2. Denaturing gradient gel electrophoresis (DGGE) analysis of partial 16S rRNA sequences of microorganisms in samples of microbial consortium OP-1 and its spreading cultivation with food waste medium. 40% and 50% indicate the gradient denaturant level

In addition, the existence of other carbon sources (glucose) could influence the degradation capacity of microbial consortium. As shown in Fig. 1, the degradation of isocarbophos in the MSM-Glu medium needed longer time (5 days) to reach the maximum compared to that in the MSM medium. Similarly, the rate of degradation of dimethoate or DDVP in the MSM-Glu medium was higher than that in MSM medium, leading to the higher concentrations detected on days 5 and 7. These results suggest that the addition of glucose decreased the biodegradation of OPs. Similar results were obtained by Xie et al. [18], who pointed out that the addition of glucose and fructose prompts the cell growth of *Acinetobacter* but inhibits the degradation of malathion [18]. A possible reason was that the OP degraders would preferentially utilize the more easily utilized carbon sources, including glucose, rather than the OPs, resulting in the suppression of OP degradation, especially for the prior degradable isocarbophos.

3.2. INFLUENCE OF THE CO-EXISTENCE OF OPs ON THE DEGRADATION

Further experiments were conducted in the capped serum bottle system to minimize the volatilization of OPs. Since the degradation of isocarbophos was preferential in the treatment with OP-1, the co-existence of OPs showed limited influence on the degradation of isocarbophos. The degradation of isocarbophos in MSM medium with microbial

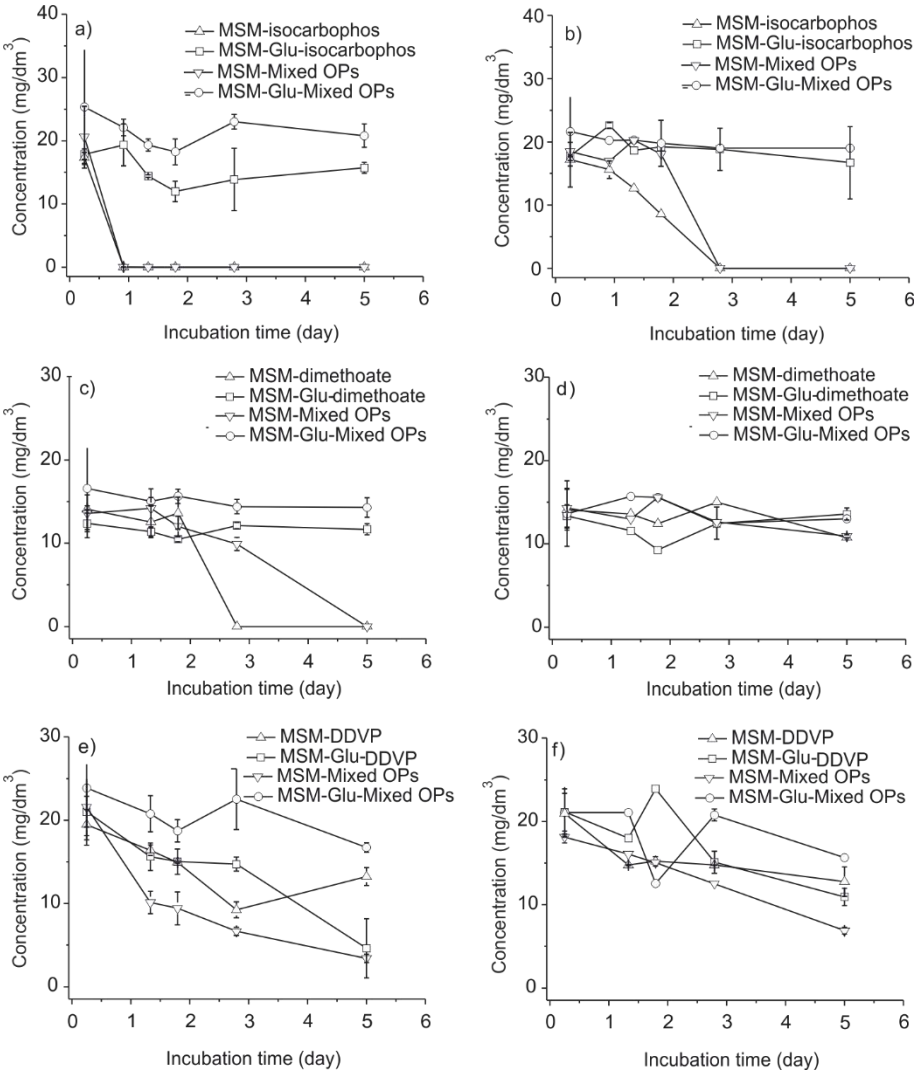


Fig. 3. Degradation of single and mixed isocarbophos (a, b), dimethoate (c, d) and DDVP (e, f) by microbial consortium OP-1 (with OP-1 – a, c. e and without OP-1 – b, d, f) in MSM and MSM-Glu medium during 5 days of incubation within a capped serum bottle system

consortium OP-1was almost completed within 1 day either in the single OP treatment or in the mixed OPs treatment (Fig. 3a), whereas the concentrations of isocarbophos in the treatments without microbial consortium decreased to non-detectable levels within 3 days (Fig. 3b). By contrast, the concentrations of isocarbophos in the MSM-Glu medium showed minimal changes, demonstrating independence on either the co-existence of mixed OPs or the addition of microbial consortium.

For dimethoate, the degradation of single dimethoate in MSM medium was completed within 3 days, whereas that in mixed OPs treatment was completed within 5 days (Fig. 3c). Without the incubation with microbial consortium, the concentrations of dimethoate showed minimal changes. The concentrations of dimethoate in MSM-Glu medium were stable (ca. 15 $mg \cdot dm^{-3}$) in single dimethoate or mixed OP treatments whether or not microbial consortium was added. This result suggested that the co-existence of OPs suppressed the degradation of dimethoate. The possible reason was that the persistence of dimethoate resulted in the prior degradation of labile OPs, including isocarbophos, thereby decreasing dimethoate degradation [24, 25].

By contrast, the concentrations of DDVP slowly decreased either in the single OP treatment or in the mixed OPs treatment (Fig. 3e). After 5 days of incubation, the concentration of DDVP was still higher than 5 $mg \cdot dm^{-3}$. Neither the co-existence of mixed OPs nor the addition of glucose influenced the degradation of DDVP. Attributed to the high volatility and low biodegradation affinity for microbial consortium OP-1, the co-existence of OPs showed limited influence on DDVP biodegradation.

3.3. INFLUENCE OF THE SPREADING CULTIVATION ON OPS DEGRADATION

The culture with FW of various solid concentrations was used to spread cultivate the microbial consortium OP-1. The concentrations of isocarbophos, dimethoate, and DDVP in the treatments with microbial consortium from spreading cultivation were higher than those in the treatments with original microbial consortium OP-1 after 2 hours (Fig. 4). In addition, the concentrations of OPs decreased with the increase in FW solid concentration in the spreading culture. The concentrations of isocarbophos in the treatment with either inoculum decreased after 2 days compared with those after 2 hours, whereas the concentration in the treatment without inoculum showed minimal changes. The concentrations of isocarbophos in various treatments followed the order of with original microbial consortium OP-1 > with OP-A consortium (obtained from 0.5% FW culture) > with OP-B consortium (obtained from 1% FW culture) > with OP-C consortium (obtained from 5% FW culture). By contrast, the concentrations of dimethoate after 2 days were not significantly different from those sampled after 2 hours. The concentration of dimethoate in the treatment with original OP-1 consortium was still the lowest, whereas the concentrations were slightly different among the treatments with OP-A, OP-B, and OP-C consortium. The concentrations of DDVP after 2 days were lower than

those after 2 hours, in which the concentrations followed the order of with OP-C consortium < with original OP-1 consortium < with OP-B consortium < with OP-A consortium < without inoculum.

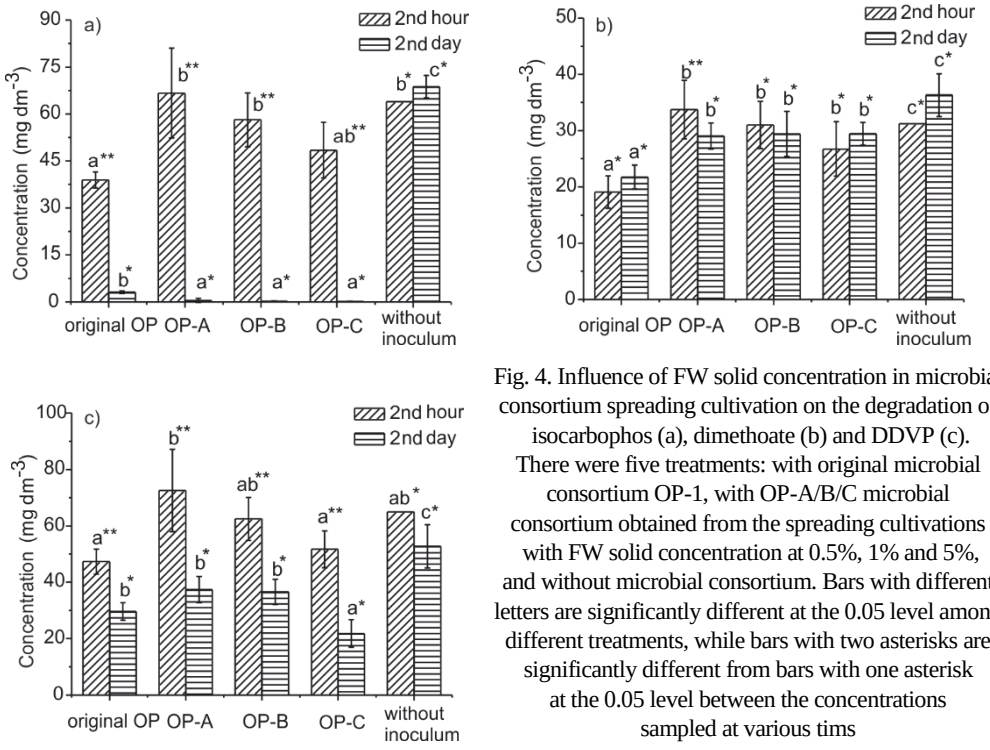


Fig. 4. Influence of FW solid concentration in microbial consortium spreading cultivation on the degradation of isocarbophos (a), dimethoate (b) and DDVP (c). There were five treatments: with original microbial consortium OP-1, with OP-A/B/C microbial consortium obtained from the spreading cultivations with FW solid concentration at 0.5%, 1% and 5%, and without microbial consortium. Bars with different letters are significantly different at the 0.05 level among different treatments, while bars with two asterisks are significantly different from bars with one asterisk at the 0.05 level between the concentrations sampled at various tims

These results suggest that the microbial consortium obtained from the spreading cultivation maintained certain degradation capacity of OPs, but it was decreased after spreading cultivation using FW culture medium. Such difference can be attributed to the change in microbial community structure after spreading cultivation. The increase in the solid concentration of FW in the culture increased the amount of substrates for the microorganisms, which consequently increased the biomass of OP degraders. However, it also prompted the growth of non-OPs-utilizers (i.e., *Enterobacteriaceae*), even resulting in the shift of dominant microbe (Fig. 2). Therefore, the microbial consortium obtained from spreading cultivation with FW culture may not maintain its high efficiency for OP degradation, which explained their lower performance of OP degradation in comparison with the degradation by original microbial consortium OP-1.

4. CONCLUSIONS

The microbial consortium OP-1 enriched from the soils of a pesticide production plant could efficiently degrade isocarbophos, dimethoate, and DDVP. Among three types of OPs, isocarbophos was preferentially degraded. Whereas the co-existence of OPs or the supply of extra carbon source (glucose) would affect the biodegradation of OPs. In addition, the microbial consortium obtained from the spreading cultivation using food waste (FW) culture maintained certain degradation capacity of organophosphorus pesticides (OPs). However, the degradation capacity was decreased after spreading cultivation using FW culture medium. The spreading cultivation of microbial inoculum using FW culture should be further optimized.

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