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Promoting degradation of 2,4-dichlorophenoxyacetic acid with fermentative effluents from hydrogen-producing reactor

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HIGHLIGHTS

- Addition of the effluents to an enrichment culture promoted 2,4-D degradation.
- The effluent additions altered the bacterial community composition of the enrichment.
- VFAs in effluents acted as beneficial substrate for co-metabolic degradation of 2,4-D.

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ABSTRACT

This research aims to identifying the potential effect of using a hydrogen-producing reactor's effluent as an enrichment amendment for enhancing the degradation rates of 2,4-dichlorophenoxyacetic acid (2,4-D) during the bioremediation of contaminated paddy soils. The results showed that addition of the effluents to 2,4-D- degrading enrichment culture enhanced (up to 1.3-fold) the degradation rate constant of 2,4-D. The enhancement effect most probably resulted from the co-metabolic degradation of 2,4-D facilitated by volatile fatty acids (e.g., acetate, propionate, and butyrate) in the effluents which served as the beneficial substrates. Results from DNA sequencing analysis showed that the effluent additions shifted the bacterial community composition in the enrichment culture. *Dechloromonas* and *Clostridium* were two dominant bacterial genera involved in 2,4-D degradation. The findings will make a substantial contribution to remediation of soils contaminated with 2,4-D.

1. Introduction

2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most commonly used herbicides worldwide (Deml and Dettner, 2001). It is usually found in low concentrations in the environment (Boyle et al., 1999; Gold et al., 1988), but its presence may lead to unpredictable toxicological effects on organisms (Odukkathil and Vasudevan, 2013; Willemsen and Hailey, 2001). Thus, it is imperative to remove 2,4-D from the environment.

Data has demonstrated that 2,4-D degradation was impacted by the availability of organic substances (Greer and Shelton, 1992; Robles-González et al., 2006). However, the indigenous organic substances in soils may have adverse effect on 2,4-D degradation, as an absorption of 2,4-D to the organic substances rendered 2,4-D less accessible to microbes for decomposition (Greer and Shelton, 1992; Robles-González et al., 2006). Hence, in addressing this question, others have focused on the exogenous addition of organic substances that may enhance 2,4-D degradation. These included glucose (Wang et al., 2009), sucrose (Robles-González et al., 2006), acetate (Boyle et al., 1999). For example, Robles-González et al. (2006) showed that supplementation of sucrose to an agricultural soil enhanced (up to 30%) 2,4-D degradation rate. Boyle et al. (1999) demonstrated that addition of acetate and H_2 to the sediment enrichment culture resulted in a 2-fold increase in 2,4-D dehalogenation rate. These findings revealed the potential effect of using the exogenous organic substances as an amendment to promote 2,4-D degradation. However, the likely prohibitive cost of preparing them may hamper their practical application in remediation of soils contaminated with 2,4-D. Therefore, it is necessary to develop a cost-effective strategy for the cleanup of contaminated soils.

Effluents are the by-products produced via biological hydrogen production from cheap organic wastes (e.g. food wastes), which

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contained considerable amounts of organic substances (e.g., volatile fatty acids, VFAs) (Wong et al., 2014; Dahiya et al., 2015; Han et al., 2015; Yang et al., 2016a). It is noteworthy that 2,4-D degradation was accompanied with the consumption of VFAs in a sequencing batch reactor treating 2,4-D, as reported by Celis et al. (2008). Therefore, based on the aforementioned findings, it was hypothesized that the effluent additions might facilitate 2,4-D degradation.

Here, the effluents were firstly produced via biological hydrogen production from potato slurry. The potential effect of using the effluents as an enrichment amendment for enhancing 2,4-D degradation was subsequently investigated. The strategy developed in this work plays dual roles in remediation of soils contaminated with 2,4-D and high-value utilization of effluents.

2. Materials and methods

2.1. 2,4-D, enrichment culture and effluents

Analytical grade 2,4-D (purity, 97%) was supplied by Aladdin (Shanghai, China). An anaerobic 2,4-D-degrading culture from paddy soil enriched with 2,4-D and acetate has been successfully established (Yang et al., 2017), and was used as inoculum for 2,4-D degradation. Briefly, the enrichment culture was grown by batch cultivation in fresh medium containing acetate (10 mM) and 2,4-D (25 mg/L) at 30 °C, and was harvested when 2,4-D was completely degraded. The enrichment culture was centrifuged and resuspended in 10 mM phosphate buffer medium (pH 7.0) prior to use. 63% of total solids (TS) in the enrichment culture were volatile solids (VS). DNA-sequencing analysis showed that *Dechloromonas* (23.3%) and *Pseudomonas* (37.7%) were the dominant genera, and that *Clostridium* (0.1%) was the minor genus.

The effluents were obtained from the hydrogen-producing reactor treating the potato slurry, as described previously (Yang et al., 2016a). Briefly, a 600 mL continuously stirred tank reactors with a working volume of 400 mL was operated in a batch mode at 37 °C. The reactor was operated with the sludge pretreated at 120 °C for 30 min (1 g-VS/L) as inoculum and fresh potato slurry (10 g-VS/L) as fermentative substrate under anaerobic conditions. Hydrogen production reached a plateau after 96 h of fermentation, and a hydrogen yield of 42 mL/g-VS was obtained. The effluents were subsequently harvested and stored at 4 °C. The organic substances in the effluents were composed of 9.1 mM glucose as well as 19.1 mM of VFAs and ethanol. The latter consisted of acetate (70.1%), propionate (8.8%), butyrate (4.9%), and ethanol (16.2%). 80% of TS in the effluents was VS.

2.2. 2,4-D degradation

Fig. 1 shows the schematic representation of the batch experiment. Three batch experiments were carried out at 30 °C in 60 mL of bottles with a working volume of 30 mL, where 0.3 g-VS/L of the enrichment culture was used. Each test was conducted in duplicate under anaerobic conditions. Medium (pH 7.0) was prepared as described previously (Yang et al., 2015), which contained 25 mg/L of 2,4-D (final concentration).

In experiment 1, the enrichment culture was detected using acetate (10 mM) or effluents (0.4 g-VS/L) as an enrichment amendment; In experiment 2, the enrichment culture was tested using the following feedstock (0.4 g-VS/L): (1) the non-sterilized effluents (named as NSTE); (2) the sterilized effluents (named as STE), where the effluents were pretreated at 121 °C for 4 h to inactivate the microbial activity in an autoclave; and (3) control, where only the effluents and 2,4-D were used. In experiment 3, the enrichment culture was tested using the following feedstock (10 mM): (1) ethanol; (2) acetate; (3) propionate; (4) butyrate; and (5) glucose.

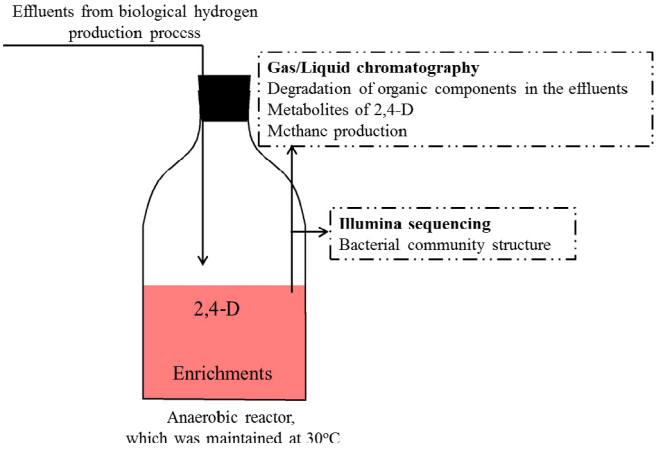

Fig. 1. Schematic diagram of the batch experiment.

2.3. Analytical methods

Methane, TS, VS, ethanol, VFAs and 2,4-D were determined as described previously (Yang et al., 2015, 2017). The glucose was detected using the phenol-sulfuric acid method (Dubois et al., 1956).

The degradation kinetics of 2,4-D were determined using a pseudo-first-order reaction:

$$lnCt = -kt + lnC0$$

where C0 represents the initial concentration of 2,4-D, Ct is the concentration of 2,4-D at time t, k is the degradation rate constant (d^{-1}), and t is the cultivation time (d).

For the analysis of bacterial community structures, DNA extraction, PCR amplification and library construction were carried out according to a reported protocol (Fu et al., 2016). Illumina sequencing was conducted at GENEWIZ (Suzhou, China). Sequence analysis and taxonomic classifications followed the protocol as reported previously (Yang et al., 2015).

3. Results and discussion

3.1. Methane production and 2,4-D degradation

The cumulative methane production by the enrichment culture was shown in Figs. 2a and 3. The methane yield in the effluent-supplemented enrichment culture ranged from 203.6 to 238.9 mL/g-VS, which was lower than the previous reported value of 346.5 mL/g-VS (Yang et al., 2016b). In addition, the methane yields from per gram of acetate, ethanol, propionate, butyrate and glucose were 12.5–203.6, 86.6, 26.6, 166.6 and 6 mL, respectively. These values were lower than the corresponding theoretical values (373 mL/g-acetate, 730 mL/g-ethanol, 530 mL/g-propionate, 509 mL/g-butyrate and 373 mL/g-glucose). These results indicated that only parts of organic substances in the effluents were consumed by methanogens. Previous reports showed that 2,4-D degradation was independent of methane production, while methanogens could provide an easy access to substrate oxidation (Boyle et al., 1999; Warner et al., 2002; Yang et al., 2017). Therefore, the methanogens in the enrichment culture might offer an easy access to oxidation of organic substances in the effluents, thereby providing