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Co-metabolic Biodegradation of Nitrified Old Landfill Leachate with Nitrate-N in Anoxic Sequencing Batch Biofilm Reactors (ASBBRs)

Yancheng Li, Benzhou Gong, Yingmu Wang, Jiale Wang and Jian Zhou†
Key Laboratory of the Three Gorges Reservoir's Eco-Environments, Chongqing University, Chongqing, 400045, P.R. China
†Corresponding author: Jian Zhou

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ABSTRACT

In consideration of the high concentration of ammonia and refractory organics, co-metabolic biotreatment system of old landfill leachate after bio-nitrification was constructed in ASBBRs with nitrate-N as growth substrate. Under the conditions of 0.25 kg COD/(m^3·d) and 0.20 kg NO_3^--N/(m^3·d), the degradation rates of chemical oxygen demand (COD), total organic carbon (TOC), NO_3^--N and colour index, after the operation for 67 d were 67.60%, 53.78%, 45.54% and 53.57%, respectively, while the BOD_5/COD value of old landfill leachate was 0.068. Analyses of molecular weight cut off (MWCO) showed that micro-molecular refractory organics of 5~10 KDa in inflow were effectively degraded, of which parts of organics became more difficult to be biodegraded with smaller molecular weight. Fourier transforms infrared spectroscopy (FTIR) analysis demonstrated that aldehydes, aliphatics, phenols and alcohols, etc. decreased and O-H functional group increased in the effluent. A majority of bacteria in the co-metabolic system were discovered to be globular and short-bar-shaped, with a few disc-shaped bacteria by scanning electric microscopy (SEM) testing. Moreover, benzene and toluene, as particular pollutants in old landfill leachate, were researched in different co-metabolic systems with nitrate-N. The result showed that the dehydrogenase concentration might accelerate the degradation of refractory organics.

INTRODUCTION

Old landfill leachate was featured with high concentration of ammonia and refractory organics (Kulikowska & Klimiuk 2008) complicated pollutants, and great water quality changes (Renou et al. 2008) and so on. Ammonia and refractory organics were normally researched separately in physico-chemical methods, including SNAP technology (Vo & Nguyen 2016), MBR and MBBR (Canziani et al. 2006), partial nitritation and anammox system for ammonia treatment (Nhat et al. 2014), Fenton (Singh et al. 2013), photo-Fenton (Silva et al. 2015), TiO_2 photocatalysis (Hassan et al. 2016) and electrochemical treatment (Cossu et al. 1998) for refractory organics. However, some related studies have discovered that biological co-metabolism was actually an effective, economic and environmentally-friendly way to degrade refractory organics, (Nzila 2013) and it could be used for the degradation of refractory organics together with nitrate-N in a hypoxia condition (Heider et al. 1998). For example, Rabus & Widdel (1995) adopted Strain EbN1 for the co-metabolic degradation of ethyl-benzene with nitrate-N as the growth substrate; Arcangeli & Arvin (1995) adopted a biofilm system to degrade o-xylene under nitrate reducing conditions; Luque-Almagro et al. (2006) adopted *Rhodobacter capsulatus* E1F1 for co-metabolic degradation of 2,4-dinitrophenol in the nitrate environment; and Dou et al. (2008) degraded BTEX under nitrate reducing conditions.

According to the water quality characteristics of nitrified old landfill leachate, a co-metabolic system of nitrate-N and refractory organics was constructed to study the synchronous removal capacities using three-stage ASBBRs. Organic changes during the co-metabolic process were analysed by MWCO and FTIR, and microbial communities' changes were analysed with SEM. In addition, particular pollutants in old landfill leachate were researched in different co-metabolic systems with nitrate-N.

MATERIALS AND METHODS

Chemicals: Old landfill leachate was acquired from a landfill of Chongqing, and used as test water after ammonia was transformed into nitrate-N in one aerobic reactor. Test water quality was as shown in Table 1 and the BOD_5/COD value was 0.068. Experimental chemicals were analytical or reagent grades. Activated seeding sludge came from a municipal wastewater treatment plant. To meet the nutritional demands of microorganisms, the following microelements were added (mg/L): KH_2PO_4, 32.13; NaCl, 0.25; $CoCl_2·6H_2O$, 0.41; $MgSO_4·7H_2O$, 12.32; $CaCl_2$, 0.55; $ZnSO_4·7H_2O$, 0.43;

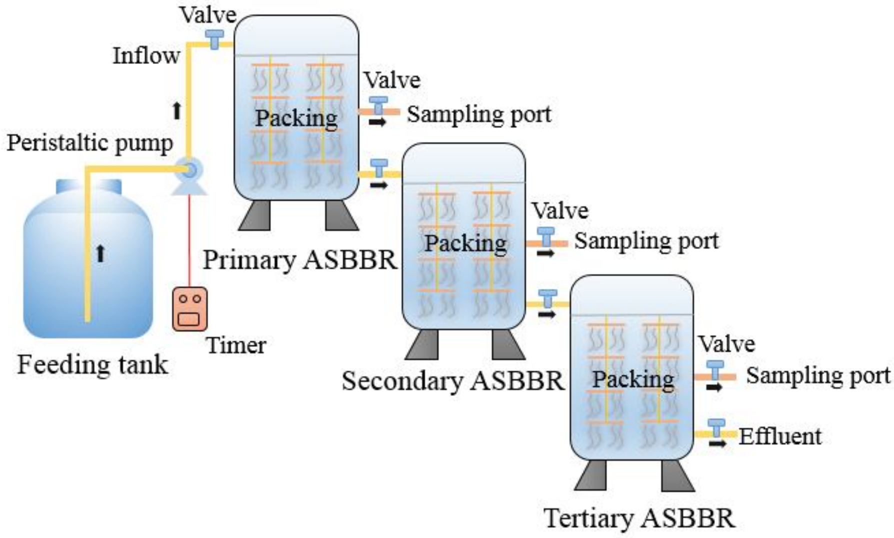

Fig. 1: Experiment equipment diagram of three-stage ASBBRs.

$FeSO_4·7H_2O$, 2.50; $Na_2MoO_4·2H_2O$, 1.26 and $MnSO_4$, 0.75.

Bioreactor: Three-stage ASBBRs (Fig. 1) was employed in the experiment. Single-stage reactor was 5 L of working volumes (30 cm high, 15 cm wide), of which 40% were filled with combination packing fillings (consisting of hanging 6 g ~ 7 g white semi-flexible filiform materials on each disc). Equipment temperature was adjusted by constant incubator.

Experimental procedure: Firstly, ASBBRs were seeded by the sludge (10 g/L TSS), and then operated in a cycle of “inflow 0.5 h -reaction 22.5 h-sedimentation 0.5 h-effluent 0.5 h”. Hydraulic retention time (HRT) of a single-stage reactor lasted for 22.5 h, and exchange ratio of volume was close to 1/5. The landfill leachate into ASBBRs from the primary one to the secondary and then the tertiary one in sequence. The temperature was maintained at 30°C ± 0.5°C, with no control to pH value. COD load was 0.25 kg COD/(m^3·d) and nitrate-N load was 0.20 kg NO_3^--N/(m^3·d).

Secondly, benzene and toluene, particular pollutants in old landfill leachate (Kjeldsen et al. 2002, Kjeldsen & Christophersen 2001, Kulikowska & Klimiuk 2008) were selected for the co-metabolic degradation research through contrast tests. Four reactors were employed with benzene into No. 1 and No. 2 reactors, toluene in No. 3 and No. 4 reactors, besides, No. 2 and No. 4 were added nitrate as the growth substrate. Organic load was 0.12 kg TOC /(m^3·d), and nitrate-N load was 0.1 kg NO_3^--N /(m^3·d) in the four reactors.

Wastewater quality analysis: Potassium dichromate digestion method was employed for COD measurement (DRB200 and DR2800, Hach, USA). NO_3^--N concentration was measured using an ultraviolet (UV-visible spectrophotometer DR5000, Hach, USA), and colour index was tested by colorimeter (LICO 500, Hach, USA), and TOC was analysed by UV-light catalytic oxidation (ElementarLiqui TOC, Germany). Same detection method of TTC-dehydrogenase activity was used as Xie et al. (2014) did.

The ultra-filter system (SCM-300, Shanghai, China) was used for MWCO analysis of inflow and effluent, with filter membranes of 0.45 μm, 0.22 μm, 100 KDa, 50 KDa, 30 KDa, 10 KDa, 5 KDa, 1 KDa and 500 Da, respectively. Firstly, filter membranes were soaked in ultrapure water for 24 h, and then placed in the ultra-filter cup after rinse. The ultra-filter cup was cleaned by ultrapure water for 3~5 times, and then by water samples for 3~5 times. Stirring rate of magnetic stirrer was controlled to be 100~120 rpm, and constant pressure of nitrogen cylinder was 0.2 Mpa. COD and TOC of effluent filtered by different membranes were tested.

Fourier transforms infrared spectroscopy (FTIR) analysis was used for wastewater composition. Firstly, inflow and effluent were collected for FTIR study; secondly, sample of 100 mL was frozen to solid state and powdered by vacuum freeze drier; and thirdly, the powder was grounded by pestle and mortar with KBr in infrared light, and pressed into slice under (5~10)×10^7Pa. The spectra were collected in FTIR system (IR Prestige-21, Japan) with diffuse reflectance accessory in a range of 400-4000 cm^{-1}. All spectra were plotted using the same scale on the transmittance axis.

Microorganism was analysed by scanning electron microscope (SEM). Sludge was firstly scraped from combina-

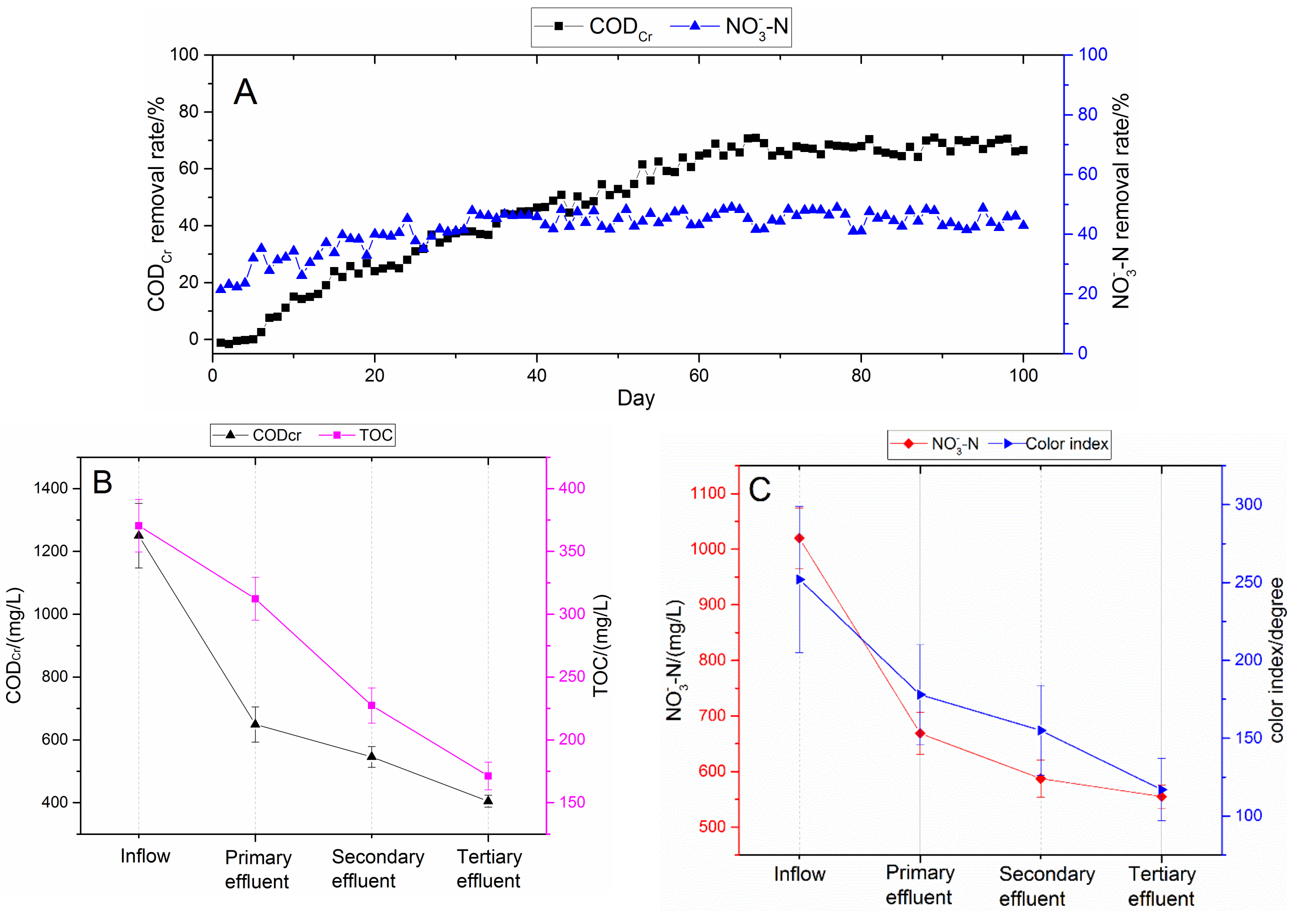

Fig. 2: (A): The gradual degradation curves of COD and NO_3^--N in the construction process of cometabolic system; (B): Degradation properties of COD and TOC at primary, secondary and tertiary stage; (C): Degradation properties of NO_3^--N and color index atprimary, secondary and tertiary stage.

tion packing fillings in reactors and fixed in stationary liquid, then scanned and analysed by SEM (S-3400 N, Denmark), to observe the microstructure of microorganism by 8,000 times of magnification.

RESULTS AND DISCUSSION

The start-up and properties of co-metabolic biotreatment system: A co-metabolic system of old landfill leachate with nitrate-N was constructed in ASBBRs under 0.25 kg COD/(m^3·d) and 0.20 kg NO_3^--N/(m^3·d). Fig. 1 (A) indicated that the degradation rate of COD was close to zero in the first 5 days after seeding sludge; then it rose slowly since the 6th day and reached 70.88% on 67th day, and synchronously, degradation rate of NO_3^--N was 41.62%. It could be known from Fig. 2 (B)~Fig. 2 (C) that, after the system was constructed, the average total degradation rate of COD, TOC, colour index and NO_3^--N was 67.60%, 53.78%, 53.57% and 45.54%, respectively. COD sharing rates at primary, secondary and tertiary stage were 48.08%, 8.24%, 11.28%; TOC were 15.71%, 22.92%, 15.15%, and NO_3^--N were 34.42%, 7.97%, 3.15%, respectively, showing that synchronous removals of both refractory organics and nitrate-N at each stage were achieved, and the removal rate of old landfill leachate increased with the increase of the reactor stage.

Degradation properties analyses of particular pollutants: Degradation results of benzene and toluene are shown in Fig. 3, and it is discovered by comparison that, benzene and toluene were degraded with nitrate-N as growth substrate in cometabolic systems, which indicated that nitrate-N obviously promoted the removal of refractory organics. TOC degradation rate in the reactor with benzene and nitrate-N was 63.65%, and NO_3^--N was 85.12%, while compared with the control group without nitrate-N, TOC degradation rate increased by 46.66% and sludge dehydrogenase concentration increased by 1.33μg TF/g MLSS·h. TOC degradation rate in the reactor with toluene and nitrate-N was 68.19%, and NO_3^--N was 74.91%, while compared with the control group without nitrate-N, TOC degradation rate increased

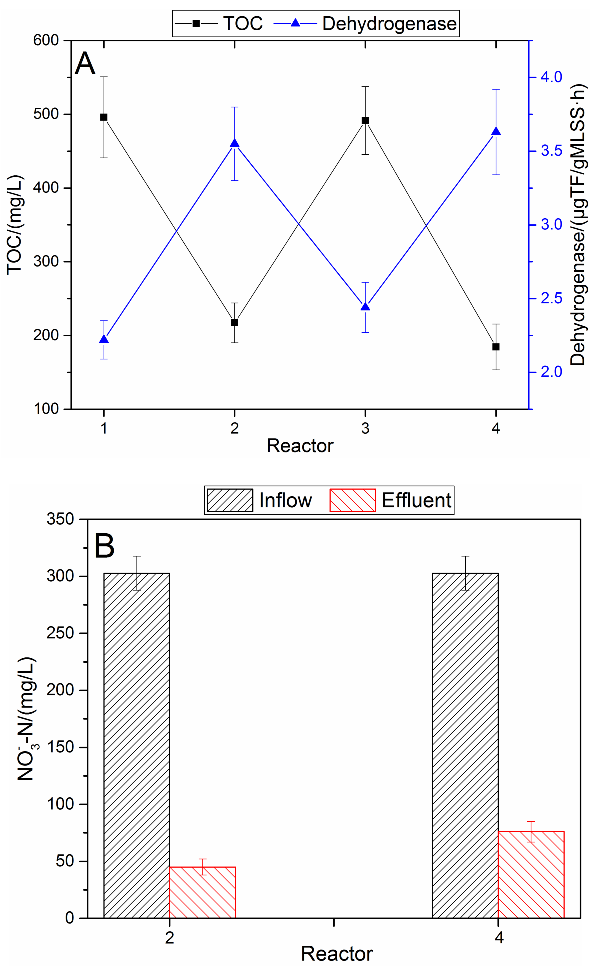

Fig. 3: (A): TOC and dehydrogenase concentration in the effluent of four reactors; (B): Nitrate-N concentration of inflow and effluent in cometabolic reactors with nitrate-N (Reactor 1: benzene system; Reactor 2: cometabolic system of benzene and nitrate-N; Reactor 3, toluene system; Reactor 4, benzene and nitrate-N of cometabolic system).

Table 1: Test water quality.

No.	Index	Test Water
1	COD (mg/L)	1250±103
2	BOD_5 (mg/L)	85±23
3	TOC(mg/L)	370.4±38
4	TN (mg/L)	483±52
5	NH_4^+-N (mg/L)	24.3±4.4
6	NO_2^--N (mg/L)	1.7±0.1
7	NO_3^--N (mg/L)	446±54
8	PO_4^{3-} (mg/L)	4.2±0.9
9	pH	6.8~7.2
10	Alkalinity ($CaCO_3$mg/L)	1800±212

by 52.97% and sludge dehydrogenase concentration increased by 1.19 μg TF/g MLSS·h. Dehydrogenase concentration in co-metabolic systems with nitrate-N as the growth substrate was higher than control groups, so nitrate-N might improve the biological activity, increase dehydrogenase concentration (Franta & Wilderer 1997) and accelerate the degradation of benzene and toluene.

Molecular weight change law in the degradation process of refractory organics: It was known from molecular weight cut-off experiment (Fig. 4) that inflow of ASBBRs could not pass ultrafiltration membrane of 5 KDa, with uneven molecular weight distribution, indicating dominant organics of the inflow exceeded 5 KDa (Wu et al. 2004). However, organics in effluent at the different level of the molecular weight were relatively well-distributed. Organics between 5 KDa and 10 KDa in the effluent decreased by 59.94% compared with the inflow, while organics between 10 KDa and 0.22 μm increased by 21.88% and organics less than 1 KDa occupied 32.34%. Therefore, the results showed that low molecular weight organics between 5~10 KDa of inflow was effectively degraded in the co-metabolic system, and parts of organics were transformed into even lower molecular weight organics (Pi et al. 2009), that were harder to

Table 2: Vibration descriptions of the functional groups by FTIR.

No.	Absorption peaks /cm^{-1}	Vibration descriptions of the functional groups
1	3444	O-H stretching vibration
2	2402, 2331	P-H stretching vibration
3	2083	Aromatic isothiocyanates -N=C=S asymmetrical stretching vibration
4	1760	Aliphatic carboxylic monomer C=O stretching vibration
5	1675, 1641	Aldehyde C=O stretching vibration
6	1513	Aromatic ring C=C stretching vibration or aromatics C=N stretching vibration
7	1402, 1385	NH_4^+ asymmetrical deformation vibration
8	1193	Phenols C-OH stretching vibration
9	1100	Aliphatics CF stretching vibration
10	997	SO_4^{2-} symmetrical stretching vibration
11	838	Benzene ring =CH in-plane bending vibration
12	620	Alcohol COH in-plane bending vibration
13	513, 497	P-Cl stretching vibration

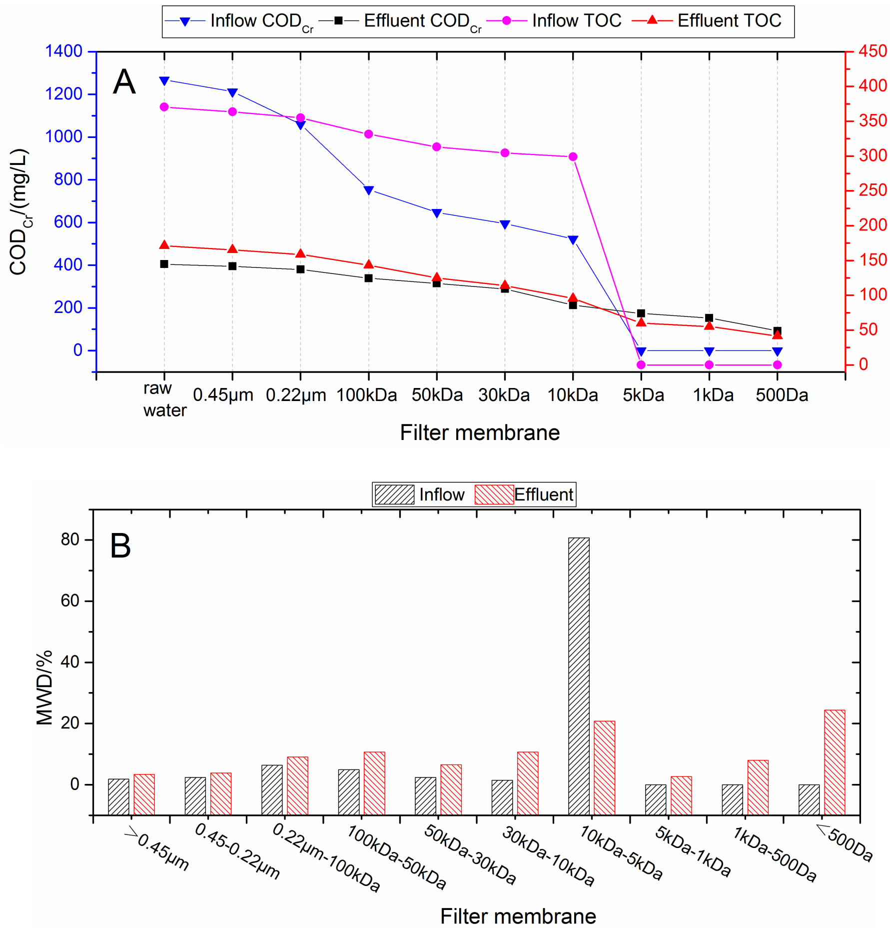

Fig. 4: (A): COD and TOC concentration changes of inflow and effluent by MWCO analysis; (B): Molecular weight distribution characteristics (represented by TOC).

degrade. Thus, organics with the molecular weight less than 1 KDa increased.

Functional group changes analyses during the degradation process of refractory organics: FTIR analyses results (Fig. 5 and Table 2) showed that old landfill leachate might contain aromatics, phenols, aldehydes, alcohols and aliphatics and so on (Huo et al. 2008, Lenz et al. 2016) which were effectively degraded after co-metabolic treatment. Absorption peak at 3444 cm^{-1} was obviously enhanced while absorption peaks at 1641 cm^{-1}, 1110 cm^{-1} and 620 cm^{-1} gradually were weakened from primary stage to tertiary stage. It showed reductions of aldehydes, aliphatics, phenols and alcohols, as well as an increase of O-H functional group.

Bacteria microstructure changes of co-metabolic system: SEM examination results (Fig. 6) showed that bacteria at primary stage existed mainly in a form of zoogloea, and most of the bacteria were globular and short-bar-shaped, with a few disc-shaped bacteria. The short-bar-shaped bacteria were mostly about 1 μm long, and diameter of the globular bacterium was about 0.2 μm. In addition, zoogloeas were out-of-shape, and had comparatively tighter structures, larger quantities and lighter colour. It could be concluded that zoogloeas at primary stage might possess more exuberant vitality and stronger capacity of absorbing and degrading organics. Whereas, zoogloea at secondary and tertiary stages became loose, with gradually decreasing short-bar-shaped bacteria and increasing spores. Volumes of partial globular bacteria also increased gradually and most of them dissociated in the flocs. Lower operating load, a lack of nutrients and energy, and hostile environment might cause zoogloea to become aging until loose. Partial bacteria aggregated the cytoplasm and nucleoplasm to form globular and ellipsoidal spores.

CONCLUSIONS

Synchronous degradation of refractory organics and nitrate-

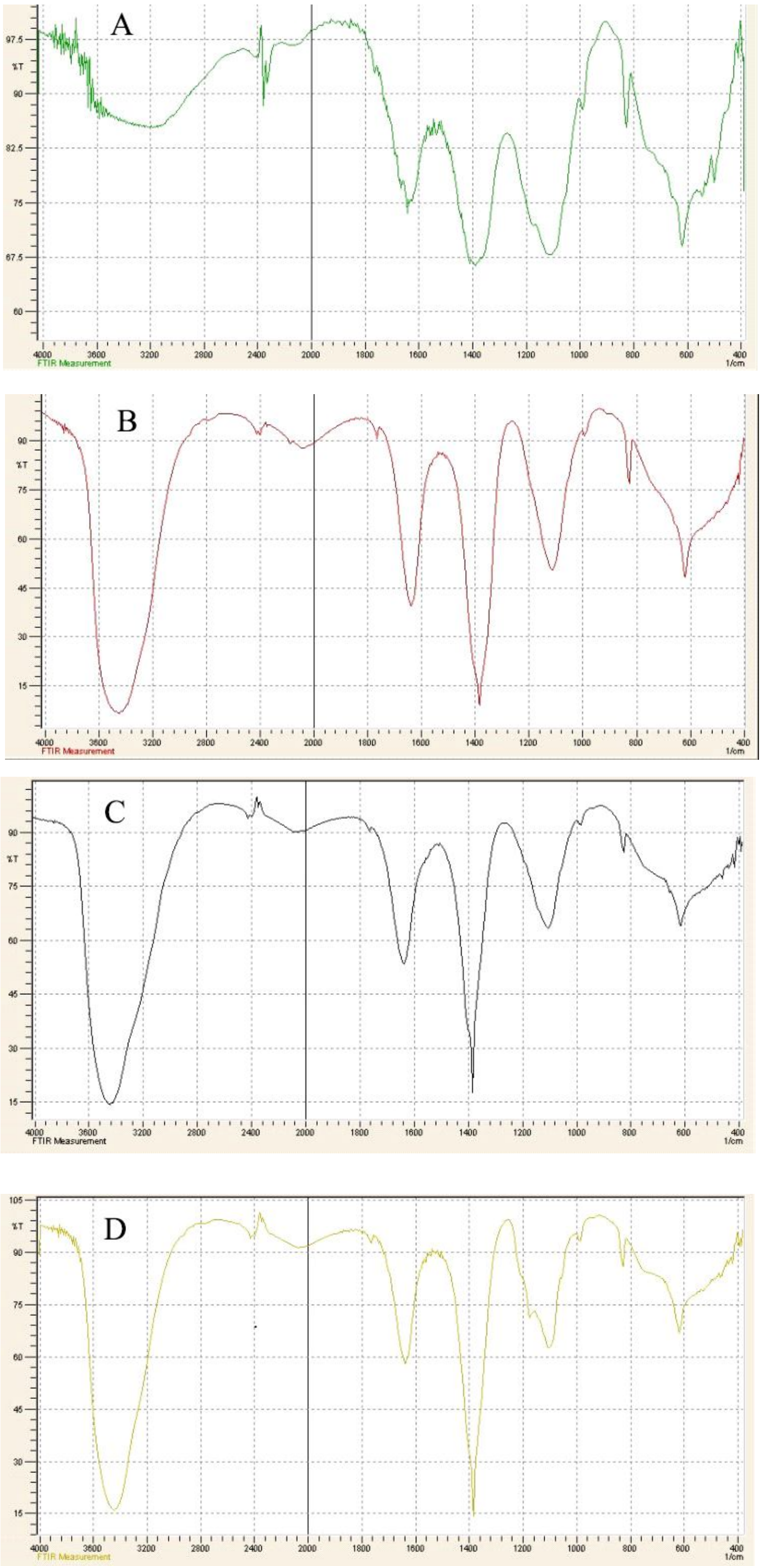

Fig. 5: FTIR analyses (A: effluent; B: primary effluent; C: secondary effluent; D: tertiary effluent).

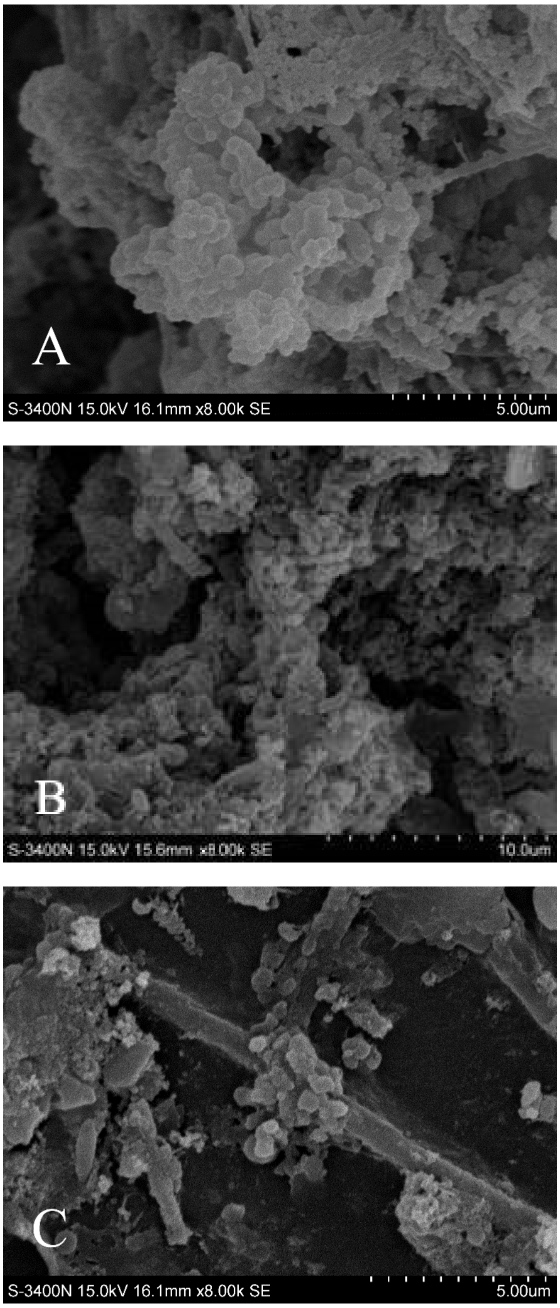

Fig. 6: Bacteria microstructures of reactors (A: the primary stage; B: the secondary stage; C: the tertiary stage).

N was realized in co-metabolic biotreatment system of old landfill leachate. MWCO and FTIR analyses demonstrated that refractory organics in the old landfill leachate were effectively degraded. SEM results showed that there were a large number of globular and short-bar-shaped bacteria, as well as a few disc-shaped bacteria. And benzene and toluene, as particular pollutants, were degraded in different co-metabolic systems with nitrate-N. Co-metabolism increased the concentration of dehydrogenase and might effectively improve the bioactivity. Therefore, this study provided a new suggestion for the biotreatment of old landfill leachate.

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