# Effects of sepiolite addition on removing contaminants in anaerobic reactor for treatment landfill leachate

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**Abstract.** The effects of adding sepiolite in the performance of an anaerobic reactor for treating landfill leachate were investigated. Responses of SMP and EPS of the R1 (without sepiolite) and R2 (with sepiolite) reactors were evaluated by EEM and FTIR spectra. Results showed that addition of sepiolite increased the removal of COD removal by 10%. The highest removal rate of ammonia nitrogen was also observed reaching 69.8% in R2 reactor. In the EEM spectra, humic- and fulvic acid-like substances were present in the EPS of the R1 reactor. The peak was located at the excitation/emission wavelength (Ex/Em) of 280/350 nm in the R2 reactor, attributed to tryptophan-like proteins. Meanwhile, dehydrogenase activities were higher than in the R1 reactor, similar with observations on COD removal rate.

## 1. Introduction

The production of leachate from municipal sanitary landfills is harmful to the ecosystem and natural environment due to its chemically reactive products [1]. Popular methods used in treating landfill leachate involve biological mediators, coagulation/flocculation, reverse osmosis, adsorption, advanced oxidation processes (AOPs), and their combinations [1-4]. In particular, anaerobic biological treatment has been widely used due to its unique advantages, especially in treating high chemical oxygen demand (COD) or refractory organic matter contained wastewater [3]. However, The inhibition of high-concentration ammonia poses a great challenge to anaerobic biological treatment for ammonium-rich wastewater. The addition of ammonia-adsorption materials such as activated carbon, resin, biochar, zeolite, sepiolite has been demonstrated to be a promising option for enhancing the efficiency of anaerobic biological reactors [4, 5]. In this study, a sepiolite fixed-bed anaerobic reactor was developed to improve the treatment efficiency of anaerobic reactor for landfill leachate. Soluble microbial products (SMP) and extracellular polymeric substances (EPS) of anaerobic granular sludge was demonstrated by three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy and Fourier transform infrared (FTIR) spectroscopy. In addition, dehydrogenase activity and protease activity of the granules were determined.

## 2. Materials and methods

Two fermenter bottles, each with 2000 mL working volume, were used as the anaerobic bioreactors. Two set-ups were used, one without affixed sepiolite which served as the control (R1) and another with fixed sepiolite (R2). In the contained sepiolite bioreactor, 40 g/L sepiolite which was activated by 0.5 mol/L sulfuric acid was fixed in the porous nylon materials and suspended in the landfill leachate [6, 7]. After treatment 24 hours, the landfill leachates were discharged and then new ones were added to the R1 and R2 reactors. Everyday, the samples were withdrawn from the effluent port on the R1 and R2 reactor to measure COD and ammonium concentration [6]. After the batch experiments, the

anaerobic granular sludge in each reactor was collected to analyze for the SMP and EPS. The pH, COD and ammonium nitrogen were determined following standard methods [8]. According to the reference [9], SMP and EPS were measured. Dehydrogenase activity was determined by the 2-3-5-triphenyltetrazolium chloride spectrophotometry [10]. While protease activity was determined using spectrophotometry based on the recommendations of Guo et al.[11].

## 3. Results and discussion

### 3.1. Removal of COD and $NH_3$

Changes in the removal rate of the COD and ammonia nitrogen by the R1 and R2 reactors were shown in Fig. 1. In the early stage, since the anaerobic granular sludge needed to adapt to the landfill leachate, the removal rate of COD was not high. On the 6th day, the COD removal rate reached to 77.5% and 85.2%, respectively, in the R1 and R2 reactors. During the experiment, COD removal rate was maintained at around 70% in the R1 reactor (Fig. 1A) This suggested that the addition of sepiolite increased the removal rate of COD. The addition of sepiolite was helpful due to its high adsorption capacity, providing longer time for microorganisms to degrade the particulate organic compounds, and probably increase both methanogens and hydrolytic bacteria in the reactors [12]. It showed that the average removal rate of ammonia nitrogen was 27.8% and 52.2%, respectively, in the R1 and R2 reactors (Fig. 1B). The maximum removal rate of ammonia nitrogen reached to 69.8% in the R2 reactor. Indeed, the removal rate of ammonia nitrogen from landfill leachate increased with the addition of sepiolite. It was illustrated three reaction main mechanisms, namely ion-exchange, adsorption, and struvite crystallization [13]. Sepiolite exchanged ammonium nitrogen from the medium for one of its own cations, mainly $Ca^{2+}$ and $Mg^{2+}$ [14]. Meanwhile, sepiolite is a magnesium silicon mineral, $Mg^{2+}$ dissolution in the solution could be used as the source of magnesium in struvite crystallization, then struvite crystallization contributed to the removal of ammonia [13]. In addition, the pH of the reactors with and without sepiolite were similar, reaching values close to 7.5. Struvite crystallization could proceed on a wide pH range of 7-11 [12, 13]. Therefore, the ammonia nitrogen was removed in conditions with a pH of 7.5, as seen in the R2 reactor.
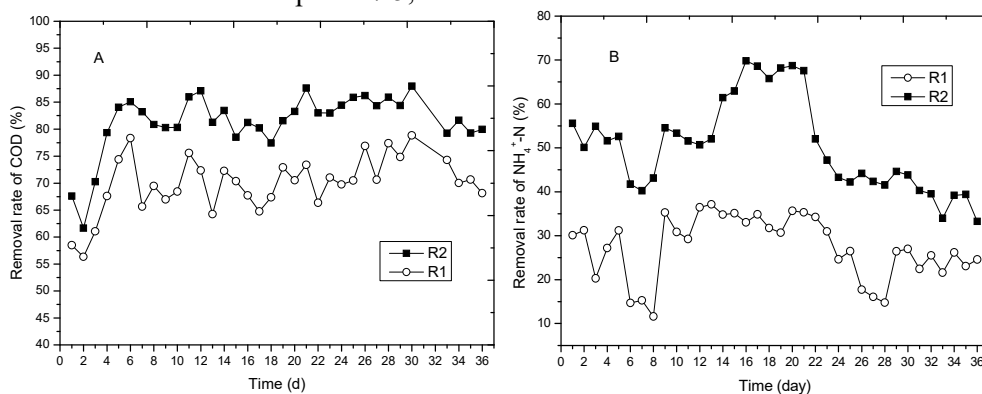


Figure 1. Removal rate of COD and $NH_4^+$-N.

### 3.2. SMP and EPS

SMPs are components of wastewater effluents that originated from substrate metabolism and biomass decay, and the presence of SMP influences the efficiency of post-treatment reactions [9]. The main components of the SMP were analyzed by the FTIR. Results showed that the predominant spectral bands were as follows in the R1 reactor: 3340 cm^{-1} (amino group), 2950 cm^{-1} (fatty acids), 2530 cm^{-1} (CH-SH stretching), 1480 cm^{-1} (methyl groups), 615 cm^{-1} (unsaturated bonds) [9]. With the addition of the sepiolite, the peaks at 2530 cm^{-1} and 1480 cm^{-1}weakened in the R2 reactor. Especially, the peak at 1480 cm^{-1} was a result of the combined effects of C-H stretching vibrations of benzene ring. These

also demonstrated that the removal efficiency of macromolecular organic pollutants was improved by the adsorption of sepiolite.
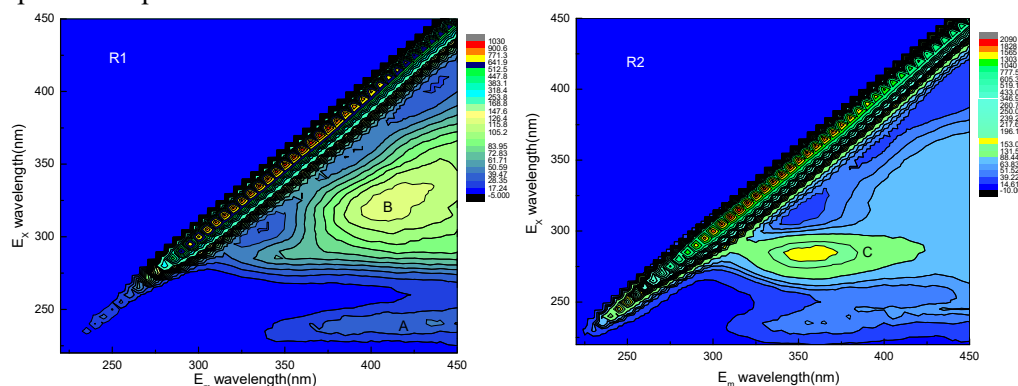


Figure 2. EEM spectra of EPS.

EPS usually stabilize anaerobic granular sludge structure, and it may play significant roles for influencing the performance of anaerobic granular sludge system [9]. As shown in Fig. 2A, two major peaks (Peak A and Peak B) were identified at the excitation/emission (Ex/Em) wavelengths of 230/430 nm and 320/410 nm, respectively. These peaks were assigned to fulvic acid-like substances (Peak A) and humic acid-like substances (Peak B), respectively [15]. It was demonstrated that the anaerobic sludge undergone a certain degree of decomposition in the R1 reactor. In contrast, the peak was located at the excitation/emission wavelength (Ex/Em) of 280/350 nm (Peak C) in the R2 reactor (Fig. 2B), which was attributed to the tryptophan-like proteins. The comparison of the EEM spectra of sludge in R1 and R2 reactors revealed that the location of the fluorescence peaks in the EEM spectra changed significantly with the sepiolite, which has a large calcium and magnesium ion. These ions are necessary trace metals for the growth of the anaerobic microorganisms, especially for anaerobic granular sludge formation. The performance of the sludge was good, and with sepiolite, was advantageous for the long-term and stable operation of an anaerobic reactor. Although addition of sepiolite in anaerobic reactor increases some operation costs, it can improve the efficiency of anaerobic reactor and the performance of the anaerobic sludge. Furthermore, sepiolite as a mineral is not expensive, making it an effective alterative for treating wastewater.
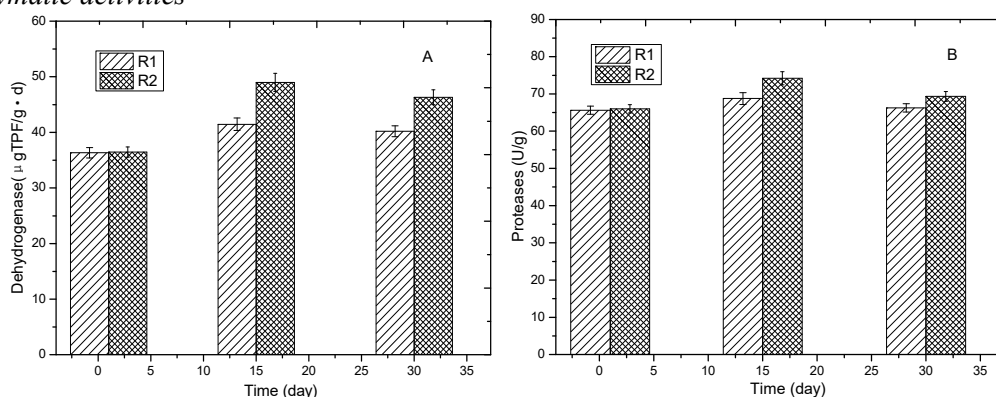
*3.3. Enzymatic activities*



Figure 3. Dehydrogenase and proteases.

On the first day, the dehydrogenase activity was similar on the two reactors, with 36.33 µgTPF/g and 36.45 µgTPF/g, respectively, in the R1 and R2 (Fig. 3A). After the 15th and 30th days, however, dehydrogenase activities increased to 48.99 µgTPF/g and 46.31 µgTPF/g, respectively, in the R2 reactor. Meanwhile, dehydrogenase activities were higher than in the R1 reactor, similar with observations on COD removal rate. Sepiolite contains a lot of calcium and magnesium ions, which are necessary trace elements for microbial growth. In addition, metals ions like magnesium, are also

involved in the synthesis of the enzyme [16]. Protease is mainly involved in the decomposition of organic nitrogen into inorganic nitrogen that could then be used by the microbes. As shown in Fig. 3B, at 1, 15, and 30 days, the activities of protease were 65.63 U/g, 68.77 U/g, and 66.26 U/g, respectively in the R1 reactor with no significant variations throughout the experiment. However, the activities of protease increased from 66.02 U/g to 74.18 U/g at day 15 in the R2 reactor. Therefore, the removal rate of ammonia nitrogen was good. At day 30, protease activity decreased to 69.35 U/g in the R2 reactor. This indicted that the removal of ammonia nitrogen was a result of the combined actions of physicochemical and biological reactions.

## 4. Conclusions

This study demonstrated that the addition of sepiolite to the anaerobic reactors could improve its performance in treating landfill leachate. In particular, the COD removal rate increased by 10%, and the ammonia nitrogen removal rate increased by 20% compared to the anaerobic reactor without sepiolite. Further, the addition of sepiolite was demonstrated to be an effective approach to enhance the removal of macromolecular organic pollutants and could stabilize the performance of the anaerobic granular sludge.

## Acknowledgements

## References

[1] Contrera RC, Silva KCC, Morita DM, Rodrigues JAD, Zaiat M and Schalch V, 2014. *J. Environ. Manage*. **145,** 385-393
[2] Wang LQ, Yang Q, Wang DB, Li XM, Zeng GM, Li ZJ, Deng YC, Liu J and Yi KX, 2016. *J. Hazard. Mater*. **318,** 460–467
[3] Meng LW, Li XK, Wang K, Ma KL and Zhang J, 2015. *J. Chem. Eng.* **274,** 94–101
[4] S. Balci, 2014. *J. Water Res.* **38,** 1129–1138
[5] Vu TM, Trinh VT, Doan DP, Van HT, Nguyene TV, Vigneswaran S and Ngo HH, 2017. *J. Sci. Total Environ.* **579,** 612–619
[6] Zheng HY, Li DW, Stanislaus MS, Zhang N, Zhu Q, Hu XH and Yang YN, 2015. *J. Chem. Eng.* **280,** 106–114
[7] Wang QH, Yang YN, Yu C, Huang H, Kim MJ, Feng CP and Zhang ZY, 2011. *J. Bioresource Technol.* **102,** 7064-7068
[8] Eaton AD, Clesceri LS, Rice EW and Greenberg AE, 2005. *American Public Health Association.* **220**, 518-519
[9] Li K, Wei D, Yan T, Du B and Wei Q, 2016. *J. Environ. Manage.* **183,** 594-600
[10] Liu B, Li YX, Zhang XL, Wang J and Gao M, 2014. *J. Soil Biol. Biochem.* **74,** 148-155
[11] Guo XL, Gu J, Gao H, Qin QJ, Chen ZX, Shao L, Chen L, Li HL, Zhang WJ, Chen SN and Liu J, 2012. *J. Bioresour. Technol.* **108,** 140-148
[12] Dutta K, Tsai CY, Chen WH and Lin JG, 2014. *J. Inte. Biodeter. Biodegr.* **95,** 84-88
[13] Huang HM, Xiao D, Pang R, Han CC and Ding L, 2014. *J. Chem. Eng.* **256,** 431–438
[14] Montalvo S, Martin JS, Huilĩnir C, Guerrero L and Borja R, 2014. *J. Process Biochem.* **49,** 2220–2227
[15] Sun J, Guo L, Li QQ, Zhao YG, Gao MC, She ZL and Wang GC, 2016. *J. Bioresource Technol.* **219,** 614–623
[16] Su CY, Li WG, Chen ML, Huang Z and Wu L, 2016. *J. Bioresource Technol*. **200,** 1065-1072