IIETA International Information and Engineering Technology Association
Advancing the World of Information and Engineering

Revue des Composites et des Matériaux Avancés
Vol. 29, No. 3, June, 2019, pp. 165-169
Journal homepage: http://iieta.org/journals/rcma

Microbial Communities on Different Packing Media in Biofilter

Xiaomeng Wang, Xudong Li, Jiangping Qiu, Yinsheng Li*

School of Agriculture and biology, Shanghai Jiao Tong University, Shanghai 200240, China

Corresponding Author Email: yinshengli@sjtu.edu.cn

https://doi.org/10.18280/rcma.290306

Received: 6 April 2019
Accepted: 30 May 2019

Keywords:
multi-layer biofilter (MBF), packing media; water quality, bacteria diversity, community richness

ABSTRACT

Many scholars have explored the treatment effect of biofilters with different packing media. However, there is little report on the microbial communities of the packing media. Therefore, this paper attempts to disclose the differences between two popular packing media of biofilter, namely, volcanic rock and ceramiste, in terms of the attached bacteria. Firstly, two multi-layer biofilters (MBFs) were designed, and respectively packed with volcanic rock and ceramiste. The packing media were collected after the MBFs entered stable operation. The DNA was extracted from the media and pyro-sequenced. After that, the community structure and diversity of the microorganisms were analyzed in details. The main results are as follows: The values of ACE estimator, Chao1 estimator, and Simpson's diversity show that volcanic rock had the higher bacterial diversity. The libraries with volcanic rock and ceramsite respectively consist of 1,810 and 1,352 operational taxonomic units (OTUs). Surprisingly, the Venn diagram of OTUs indicates that the two-packing media only share 13.2 % OTUs. The volcanic rock contained sequences in 16 phyla while ceramiste only covered sequences in 11 phyla. Among all phyla, Tenericutes was unique to ceramiste, while Chloroflexi, GN02, NKB19, Thermi, Chlorobi and TM7 were only observed in volcanic rock. Ceramiste contained more Proteobacteria (67.06 % vs. 64.12 %), Bacteroidetes (25.11 % vs. 22.72 %) andFirmicutes (6.20 % vs. 4.55 %) than volcanic rock. The research findings clarify the research direction and provide the theoretical basis for improving ceramiste technology and developing alternatives for volcanic rock.

1. INTRODUCTION

Volcanic rock and ceramsite are two common materials in biofilters [1, 2]. The former is a natural product which is abundant on Earth, and the latter is a novel artificial material [3]. Both volcanic rock and ceramsite have good bearing capacity and corrosion resistance. In addition, the two materials enjoy high gas permeability, a huge specific surface area, and thus the ability to absorb lots of microorganisms. As a result, volcanic rock and ceramsite are excellent materials for wastewater treatment. Comparatively, volcanic rock has the better treatment effect, while ceramiste is easier to obtain. Many scholars have explored the treatment effect of the two materials, but failing to compare the microorganism communities between them [4-7].

In 2018, Jiang et al. [8] studied the features and composition of nitrobacteria (NOB) and ammonia oxidizing bacteria (AOB) in a partial nitrification-anammox biofilter (PN/AF) system, revealing that the NOB is more stable when dissolved oxygen (DO) is high. Yang et al. [9] investigated the denitrification performance of volcanic rock in aerated biofilters, and found that the AOB can coexist with anammox bacteria. Gao et al. [10] developed and implemented a novel volcanic rock-based filter, discovered the positive correlation between DO and the operating efficiency of the filter, and disclosed the importance of Nitromonas, Microsporumand Candida krusei in the removal of organic matter and ammonia nitrogen. These studies mainly focus on the NOB in volcanic rock medium, paying less attention to the total microbial communities. In addition, there is little report on the ceramsite medium or multi-layer biofilter (MBF).

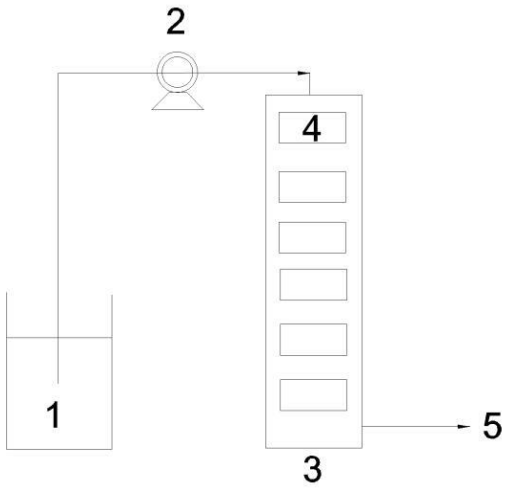
With good conservation and variability, the 16S rRNA is an important tool to analyze microbial diversity. For instance, Yasuda et al. [11] relied on this tool to explore the community structure and total bacterial count of denitrifying bacteria (DNB) in ammonia-nitrogen biofilters. Similarly, the high-throughput sequencing (HTS), a rapidly evolving method, has been widely applied to analyze the diversity and composition of environmental microorganisms [12]. Brinkman et al. [13] adopted the HTS to monitor the seasonal changes of the virus in wastewater. Martínez et al. [14] examined the abundance variation of archaea in sludge by the HTS. All these studies have shown that the HTS is extremely efficient and convenient in determining the properties of environmental microorganisms [15, 16].

In this paper, two pilot scale MBFs are designed, respectively packed with volcanic rock and ceramiste. Under steady operation, the two MBFs were compared in terms of operating state, microbial features, dominant population and microbial diversity. The research findings clarify the research direction and provide the theoretical basis for improving ceramiste technology and developing alternatives for volcanic rock.

2. MATERIALS AND METHODS

2.1 Pilot scale MBFs

Two pilot scale MBFs were fabricated with stainless steel (L×W×H: 42cm×31cm×2,200cm). Each MBF consists of six equal-height chambers. The inside dimension ofeach chamber is 250mm×200mm×220mm. The total volume of the packing is 66L. For natural ventilation, both the shell and the inner layers are highly porous. One of the MBF was packed with volcanic rock and the other with ceramiste. The particle size of both materials is about 1cm. The structure of each pilot scale MBF is shown in Figure 1.



Note: 1. Wastewater tank; 2. Peristaltic pump; 3. Main body of trickling filter; 4. Chamber; 5. Perforated rubber hose

Figure 1. Sketch map of a pilot scale MBF

The wastewater was prepared manually by mixing 281.25mg glucose, 57.25 mg NH_4Cl, 13.25 mg KH_2PO_4, 75 mg $NaHCO_3$, 25 mg peptone, 10mg yeast extract, 16.5 mg $MgSO_4 \cdot 7\ H_2O$, 1.5 mg $MnSO_4 \cdot 7\ H_2O$, and 0.1 mg $FeSO_4$ with each liter of tap water. The water quality indices of the wastewater are as follows: chemical oxygen demand (COD), 300 $mg \cdot L^{-1}$; total nitrogen (TN), 18 $mg \cdot L^{-1}$; ammonia nitrogen (NH_4-N), 15 $mg \cdot L^{-1}$; total phosphorus (TP), 3$mg \cdot L^{-1}$ (C/N/P=100/6/1). The wastewater was transferred to the surface of the packing medium via a perforated rubber hose at the constant flow of 0.0885 $m^3 \cdot d^{-1}$.

2.2 Water quality analysis

To keep the water quality consistent, the wastewater was prepared once every two days. The effluent was collected by a plate beneath the MBF. The COD, NH_4-N and TN were analyzed by the methods specified in Chinese national standards [17-19].

2.3 Sample collection and processing

At 60d, the biofilm samples were collected from every layer of the two MBF. The samples from the same MBF were mixed together. The mixture was subjected to 5min ultrasonic treatment, and then flushed with deionized water. After that, the sample was relocated to a 50mL tube, and centrifuged at 8,000×g for 10min. The sediments were stored at -80℃ until DNA extraction.

2.4 DNA extraction and polymerase chain reactions (PCRs)

The total genomic DNA was extracted by E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, US).To set up the clone library, the primer sets 341F (5'-CCTACGGGAGGCAGCAG-3') and 907R (5'- CCGTCAATTCMTTTRAGT-3') were selected to amplify the hypervariable V3~V5 region(about 560bp) of bacterial 16S rRNA gene. To identify each sample in the mixed reaction, the fused forward primer contains a10-base long barcode, inserted between the 454 adapter and the 341F primer.

The PCRs were carried out in a 25 μL volume containing 2.5 μL 10×buffer, 2 μL dNTP, 1 μL of each primer (10 μM),2 μL of DNA(20 ng/μL) and 0.125 μL pyrobestpolymerase(5 U/μL, Takara, China).The thermocycling was carried out at 94 ℃for 4min, followed by 27 cycles at 95 ℃ for 30s, 55 ℃ for 45s, 72 ℃ for 1min and a final extension at 72 ℃ for 7 min. The number of PCR cycles was reduced for the accuracy and reliability of the subsequent analysis. The PCR products were obtained with a 0.8 % agarose gel, with the amplicon size of 500bp.

2.5 Pyrosequencing

The PCR products were purified withAMPureXP beads, quantified byQuant-iTPicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, US), and finallypyro-sequenced on 454 Gs Flx Titanium Platform (Roche, US).

2.6 Sequence analysis

The genetic sequences were analyzed on QIIME, an open-source bioinformatics pipeline for performing microbiome analysis from raw DNA sequencing data. Any sequences failing to meet the following requirements were considered ineffective and removed: the length falls within 200~1,000bp, fewer than 6 consecutive bases are identical, no ambiguous base is included, and the quality score is above 25. Next, the sequences with greater-than-97 % similarities were clustered into one operational taxonomic unit (OTU), using the QIIME program. Community richness and diversity indice, such as Chao1 estimator, ACE estimator and Simpson's diversity, and rarefaction curves were obtained with mothur. The species in each OTU were identified through blasting with the genes in Greengene database. The Venn diagram of each OTU was drawn by Venny 2.1.0.

3. RESULTS AND DISCUSSION

3.1 MBF performance

Table 1 lists the water quality indices of the two MBFs under stable operation. As shown in the Table, the pH value only change slightly through the monitoring. The influent is basically pH neutral (6.827), while the effluents of both packing media is faintly alkaline (7.897 for volcanic rock and 7.692 for ceramiste). The DO of both MBFs is on the rise, signifying the increase in the content of dissolved oxygen. The effluent of volcanic rock contains more dissolved oxygen than that of ceramiste.

The removal rates of COD, NH_3-N and TN of the two MBFs are shown in Figure 2. It can be seen that, the COD of volcanic rock (76.8 %) was higher than that of ceramist (60.8 %).

Table 1. Water quality indices (temperature: 23.45±0.49 °C)

	pH	DO	COD (mg/L)	NH_3-N(mg/L)	TN (mg/L)
Influent	6.827	1.31	360	13.21	12.53
Effluent (volcanic rock)	7.897	4.14	77	2.79	2.72
Effluent (ceramsite)	7.692	3.78	141	2.18	7.91

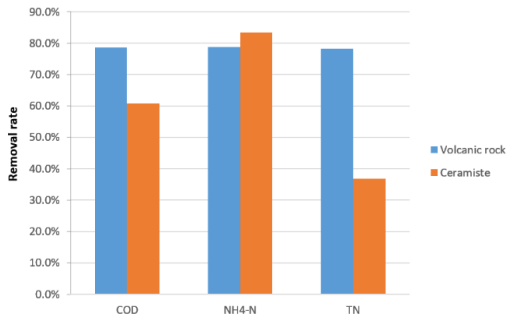


Figure 2. The removal rates of COD, NH_3-N and TN

In terms of NH_3-N removal rate, ceramiste slightly outperformed volcanic rock (83.5 % vs. 78.8 %). The high NH_3-N content in volcanic rock is the result of weak nitrification, which is an aerobic reaction. The more intense the reaction, the lower the DO of the effluent. Thus, it is speculated that volcanic rock has more but smaller pores than ceramiste, which favors anoxic reaction. The inverse is also true. This explains the relatively weak nitrification in volcanic rock.

By contrast, the effluent of volcanic rock had a TN removal rate (78.3 %) much higher than that of ceramiste (36.8 %). This means volcanic rock has a stronger denitrification effect than ceramiste. Denitrification is an anaerobic reaction. The result further validates the previous speculation.

3.2 Bacteria diversity

The data in Table 2 show that11,844 and 10,904 sequences with high-quality 16S rRNA genes were recovered from volcanic rock and ceramsite, respectively, and subjected to community analysis. The libraries with volcanic rock and ceramsite respectively consist of 1,810 and 1,352 OTUs.

Table 2. Community richness and diversity indices

Sample number	Number of effective sequences	Number of high-quality sequences	OTUs	ACE estimator	Chao1 estimator	Simpson's diversity	Coverage
Volcanic rock	12,576	11,844	1,810	3,082.413	3,008.355	0.0206	0.909
Ceramsite	11,431	10,904	1,352	3,125.282	2,287.846	0.0715	0.929

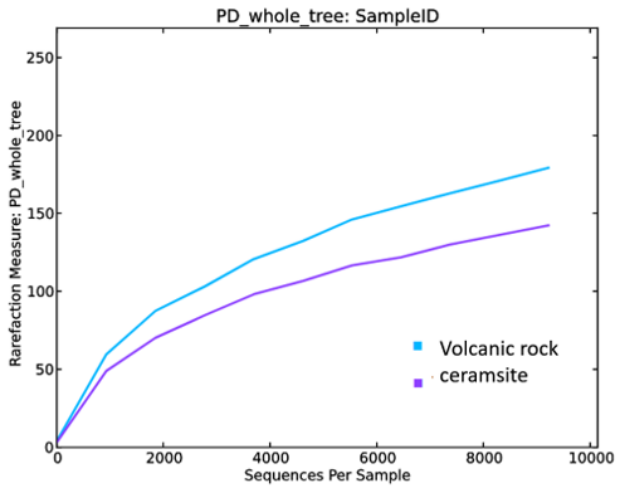


Figure 3. Rarefaction curves of OTUs in volcanic rock and ceramiste

To compare the the community richness, the rarefaction curves are plotted based on the OTUs at 3 % cutoff (Figure 3). Obviously, neither curve approached a plateau, indicating the need for more OTUs in each sample. However, the coverage of both samples reached 90 %. This means pyrosequencingoutputenough OTUs to provide the genetic information needed for identification of scarce species [20].

It can also be seen from Table 2 that volcanic rock contained richer species than ceramsite. In addition, the values of ACE estimator, Chao1 estimator, and Simpson's diversity show that volcanic rock had the higher bacterial diversity.

The comparison of bacterial diversity reflects the features of the packing media. The volanic rock has more but smaller pores than ceramiste, which reduce the impact of water flow. Thus, microorganisms are more likely to stay on volanic rock for a long time than ceramiste, pushing up the total bacterial count and diveristy on this medium. This agrees well with the number of OTUs in the two-packing media.

The two samples are ranked by OTU abundance. Then, the distribution curves of OTU abundance are drawn and recorded as Figure 4. For each curve, the horizontal length reflects community richness, and the shape describes the species evenness. The curve of ceramiste is flatter than that of volcanic rock, revealling that the ceramiste medium is more evenly distriubted than volcanic rock medium.

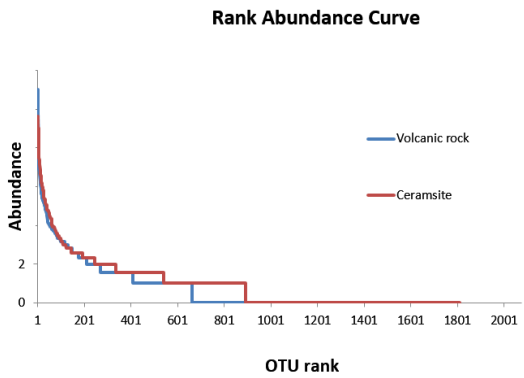


Figure 4. Distribution curves of OTU abundance

As shown in Figure 5, the two-packing media only share 13.2 % OTUs, reflecting a huge difference in species between volcanic rock and ceramiste. The difference may be attributable to the origin of the two materials; volcanic rock is

a natural packing material, while ceramist is artificially prpeared. As a natural material, volcanic rock may originally contain some microorganisms [21-24]. Thus, the microorganims in ceramist mainly come from the local environment, while only a part of those in volcanic rock has the same origin.

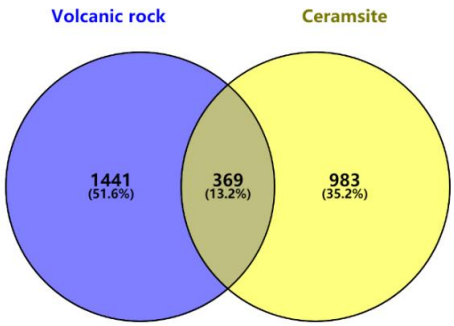


Figure 5. The Venn diagram of OTUs

3.4 Bacteria composition

As shown in Figure 6, the volcanic rock contains sequences in 16 phyla while ceramiste only coveres sequences in 11 phyla. This is an indirect evidence to the relatively high concentration of microbial species in ceramiste. Among all phyla, Tenericutes was unique to ceramiste, while Chloroflexi, GN02, NKB19, Thermi, Chlorobi and TM7 were only observed in volcanic rock. The top five phyla in the packing media were Proteobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes and Actinobacteria in turn. Ceramiste contained more Proteobacteria (67.06 % vs. 64.12 %), Bacteroidetes (25.11 % vs. 22.72 %) andFirmicutes (6.20 % vs. 4.55 %) than volcanic rock, but fewer Gemmatimonadetes (1.18 % vs. 3.87 %) and way fewer Actinobacteria.

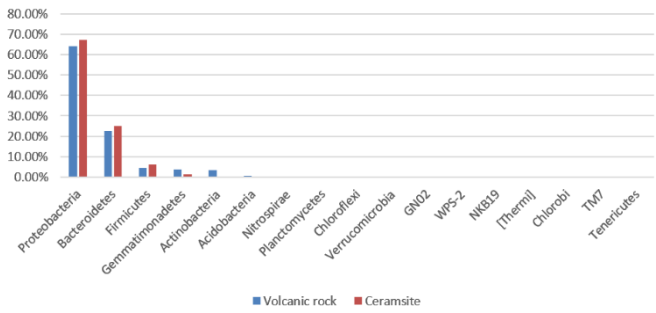


Figure 6. Proportion of sequences in each phylum

4. CONCLUSIONS

This paper pinoneers the comparison of microbial communities between pilot scale MBFs packed with volcanic rock and ceramiste, respectively. The analysis results show that, compared with ceramiste, volcanic rock can reduce COD and TN effecively, and ensure a high DO in the effluent. However, ceramiste has a slight edge in the removal of NH_3-N. A possible reason for these results lies in the uniform shape of and wide gap between ceramiste particles. In the biofilter, ceramist medium contains more oxygen than volcanic rock medium, and thus acquires strong nitrification effect and weak denitrification effect.

In terms of microbial features, volcanic rock contains more diverse microogranisms than ceremiaste. This is because the multiple pores on and inside volcanic rock reduce the shear force of water flow, allowing more kinds of microorganisms to remain on its surface. Phyla analysis shows that the two packing media have similar dominant populations, but vary greatly in the proportion of each phylum. The above results show that ceramiste technique can be improved by optimizing the particle shape and increasing the particle porosity.

REFERENCES

[1] Sekomo, C.B., Rousseau, D.P.L., Lens, P.N.L. (2012). Use of gisenyi volcanic rock for adsorptive removal of cd(ii), cu(ii), pb(ii), and zn(ii) from wastewater. Water Air and Soil Pollution, 223(2): 533-547. https://doi.org/10.1007/s11270-011-0880-z

[2] Kele, B. (2010). 'Volcanic rock filters in decentralised wastewater treatment' in institute for resource industries and sustainability 2010, 2nd annual conference of the iris postgraduate students: 'resourcing for the future'. Conference Program, 1st December, Rockhampton, Qld, 9-9. http://hdl.cqu.edu.au/10018/57127

[3] Gomes, J., Frasson, D., Quinta-Ferreira, R., Matos, A., Martins, R. (2019). Removal of enteric pathogens from real wastewater using single and catalytic ozonation. Water, 11(1): 1-12. https://doi.org/10.3390/w11010127

[4] Chao, C., Zhao, Y., Song, Q., Min, J., Wang, Z., Ma, H., (2019). Volcanic rock based ceramsite adsorbent for highly selective fluoride removal: function optimization and mechanism. Journal of Chemical Technology and Biotechnology, 94(7): 2263-2273. https://doi.org/10.1002/jctb.6014

[5] Sun, M.T., Yang, Z.M., Fu, S.F., Fan, X.L., Guo, R.B. (2018). Improved methane removal in exhaust gas from biogas upgrading process using immobilized methane-oxidizing bacteria. Bioresource Technology, 256: 201-207. https://doi.org/10.1016/j.biortech.2018.02.020

[6] Agegnehu, A., Brook, L., Nigus, G., Tadele, A.M., Teferi, D.M. (2018). Removal of chromium (vi) from aqueous solution using vesicular basalt: A potential low-cost wastewater treatment system. Heliyon, 4(7): e00682. https://doi.org/10.1016/j.heliyon.2018.e00682

[7] Sumiyati, S., Purwanto, S. (2018). Decreasing of BOD concentration on artificial domestic wastewater using anaerob biofilter reactor technology. E3s Web of Conferences, 31: 03016. https://doi.org/10.1051/e3sconf/20183103016

[8] Jiang, H.C., Liu, G.H., Ma, Y.M., Xu, X.L., Chen, J.H., Yang, Y.Q., Liu, X.H., Wang, H.C. (2018). A pilot-scale study on start-up and stable operation of mainstream partial nitrification-anammox biofilter process based on online ph-do linkage control. Chemical Engineering Journal, 350: 1035-1042. https://doi.org/10.1016/j.cej.2018.06.007

[9] Yang, Q., Zhou, T., Liu, X., Li, H., Peng, Y. (2017). Implementation of integrated autotrophic nitrogen removal system at normal temperature by returned sludge. Ciesc Journal. https://doi.org/10.11949/j.issn.0438-1157.20161699

[10] Gao, C., Liu, L., Yang, F. (2017). Development of a

novel proton exchange membrane-free integrated MFC system with electric membrane bioreactor and air contact oxidation bed for efficient and energy-saving wastewater treatment. Bioresource Technology, 238: 472-483. https://doi.org/10.1016/j.biortech.2017.04.086

[11] Yasuda, T., Waki, M., Fukumoto, Y., Hanajima, D., Kuroda, K., Suzuki, K., Matsumoto, T., Uenishi, H. (2017). Community structure of denitrifying and total bacteria during nitrogen accumulation in an ammonia-loaded biofilter. Journal of Applied Microbiology, 123(6): 1498-1511. https://doi.org/ 10.1111/jam.13603

[12] Bruno, A., Sandionigi, A., Galimberti, A., Siani, E., Labra, M., Cocuzza, C., Ferri, E., Casiraghi, M. (2017). One step forwards for the routine use of high-throughput dna sequencing in environmental monitoring. an efficient and standardizable method to maximize the detection of environmental bacteria. Microbiology Open, 6(1): e00421. https://doi.org/10.1002/mbo3.421

[13] Brinkman, N.E., Fout, G.S., Keely, S.P., Lapara, T.M. (2017). Retrospective surveillance of wastewater to examine seasonal dynamics of enterovirus infections. mSphere, 2(3): e00099-17. https://doi.org/10.1128/msphere.00099-17

[14] Martínez, E., Sotres, A., Arenas, C., Blanco, D., Martínez, O., Gómez, X. (2019). Improving anaerobic digestion of sewage sludge by hydrogen addition: analysis of microbial populations and process performance. Energies, 12(7): 1-15. https://doi.org/10.3390/en12071228

[15] Oshiki, M., Miura, T., Kazama, S., Segawa, T., Ishii, S., and Hatamoto, M., Yamaguchi, T., Kubota, K., Iguchi, A., Tagawa, T., Okubo, T., Uemura, S., Harada, H., Kobayashi, N., Araki, N., Sano, D. (2018). Microfluidic pcr amplification and miseq amplicon sequencing techniques for high-throughput detection and genotyping of human pathogenic rna viruses in human feces, sewage, and oysters. Front Microbiol, 9: 830-830. https://doi.org/10.3389/fmicb.2018.00830

[16] Selvarajan, R., Sibanda, T., Venkatachalam, S., Kamika, I., Nel, W.A.J. (2018). Industrial wastewaters harbor a unique diversity of bacterial communities revealed by high-throughput amplicon analysis. Annals of Microbiology, 68(7): 445-458. https://doi.org/10.1007/s13213-018-1349-8

[17] HJ535. (2009). Water quality-Determination of ammonia nitrogen-Nessler's reagent spectrophotometry.

[18] HJ636 (2012). Water quality-Determination of total nitrogen-Alkaline potassium persulfate digestion UV spectrophotometric method.

[19] HJ/T399. (2007). Water quality-Determination of the chemical oxygen demand-Fast digestion-Spectrophotometric method.

[20] Zhang, X., Yue, S., Zhong, H., Hua, W., Chen, R., Cao, Y. (2011): A diverse bacterial community in an anoxic quinoline-degrading bioreactor determined by using pyrosequencing and clone library analysis. Applied Microbiology and Biotechnology, 91(2): 425-434. https://doi.org/10.1007/s00253-011-3296-1

[21] Haldeman, D.L., Amy, P.S., White, D.C., Ringelberg, D.B. (1994). Changes in bacteria recoverable from subsurface volcanic rock samples during storage at 4 degrees c. Appl Environ Microbiol, 60(8): 2697-2703. https://doi.org/10.1002/bit.260440418

[22] Kelly, L.C., Cockell, C.S., Herrera-Belaroussi, A., Piceno, Y., Andersen, G., Desantis, T., Brodie, E. (2011). Bacterial diversity of terrestrial crystalline volcanic rocks, iceland. Microbial Ecology, 62(1): 69-79. https://doi.org/10.1007/s00248-011-9864-1

[23] Lee, D.W., Park, M.Y., Kim, J.J., Kim, B.S. (2017). Pseudokineococcus basanitobsidens sp. nov. isolated from volcanic rock. International Journal of Systematic and Evolutionary Microbiology, 67(10): 3824-3828 https://doi.org/10.1099/ijsem.0.002206

[24] Lavoie, K.H., Winter, A.S., Read, K.J.H., Hughes, E.M., Spilde, M.N., Northup, D.E. (2017). Comparison of bacterial communities from lava cave microbial mats to overlying surface soils from lava beds national monument, USA. PLOS ONE, 12(2): e0169339. https://doi.org/10.1371/journal.pone.0169339