

Molecular Biology Technology in Environmental Biology under the Background of Artificial Intelligence

Cioara Tudor*

Charles Sturt University, Australia

**corresponding author*

Keywords: Artificial Intelligence, Molecular Biology Technology, Environmental Biology, Activated Sludge Denitrification

Abstract: The accelerating development of artificial intelligence not only promotes a qualitative leap in my country's science and technology, but also further stimulates social and economic development. The application of molecular biology technology (MBT) in environmental biology can improve the current ecological environment, so as to better meet the needs of modern people's life. In this paper, taking activated sludge denitrification as an example, the sludge DNA was first extracted, and then the sludge DNA was amplified by PCR using fluorescence quantitative PCR technology, and then the IFAS system was used to denitrify the sewage, BOD5 inlet and outlet water quality changes, in order to evaluate the water quality standards. The experimental results show that after denitrification, the water quality can basically meet the discharge standard of Class B, and the removal rate of organic matter in sewage can also reach about 90%, which verifies that MBT can effectively degrade and remediate complex pollutants in the environment.

1. Introduction

Microbial resources are extremely rich. How to effectively use these resources to promote the development of science and technology, thereby bringing economic and social benefits, especially how to apply environmental microbes to the control of environmental pollution is worthy of attention. The study of environmental microbial diversity can understand the micro-ecological structure, and summarize the distribution characteristics of microorganisms in different habitats in terms of species and quantity, so as to further control and optimize the microbial community structure, it is of great significance to correctly handle the relationship between microorganisms and the increasingly harsh human living environment [1].

Today, molecular biology techniques are widely used in environmental biology. For example, in the study of microbial flora, a scholar used fluorescence quantitative PCR technology to measure

the AOB and NOB flora in the biofilm and suspended sludge phase. From the perspective of the number of microbial flora, it is revealed that the IFAS system is superior to the traditional activated sludge method in the process of sewage denitrification [2]; a scholar used high-throughput pyrosequencing technology for the IFAS process to reveal that the two phases of suspension and adhesion dominate. A certain scholar used FISH technology to quantitatively identify and distribute the nitrifying bacteria in IFAS, and concluded that the attached biofilm in the system is the main place for the nitrification reaction [4]. Some scholars combined high-throughput sequencing technology to study the utilization of acetate by mixed microbial communities during the adaptation period under different growth conditions. This study shows that the microbial community structure and its change process are affected by the culture process. The sludge with low sludge age has a distinctive community structure, and the kinetic changes of its growth process and the storage capacity of biopolymers are also affected by the bacterial community structure [5]. In terms of plant karyotype research, traditional karyotype analysis can no longer meet the rapid development of molecular cytology and biology. However, fluorescence in situ hybridization (FISH) technology solves this problem, and the medicinal Plant karyotype analysis is of great significance to cytogenetic research. A scholar uses FISH technology to label multiple different probes with different fluorescein and perform fluorescence in situ hybridization, and identify chromosomes according to the distribution sites of probes on different chromosomes, so as to solve the problem of identifying chromosomes only by chromosome length and centromere position. Chromosome uncertainty, and can identify chromosome information according to the number and location of signal points [6]. Although the application of MBT in environmental biology involves many fields, in general, it is for biological genetics and environmental governance, so as to make the ecology more stable.

This paper first introduces the application of MBT in environmental microorganisms, and then proposes the experimental content and experimental methods of this paper, aiming to analyze the microbial structure in sludge through modern technologies such as MBT, and then denitrify sewage, In order to achieve the sewage discharge standard, the research of this paper shows the excellent effect of MBT in environmental pollution control.

2. Molecular Biology Techniques and Their Applications

With the development of molecular biology theory, experimental technology has also advanced by leaps and bounds. The continuous innovation of technology provides new research methods for theoretical research, and new technologies gradually replace traditional analysis methods with many limitations. Since the concept of molecular ecology was proposed, environmental microbial analysis based on modern molecular biology techniques has also developed rapidly. Microorganisms have characteristics that are different from other organisms. In a changing environment, it is easy to mutate and adapt to the new environment. According to its characteristics, researches at different levels are also carried out correspondingly, such as metabolic pathways and the relationship between flora and so on [7-8]. The research on the structural characteristics and functional characteristics of environmental microorganisms is the most important research in the field of environmental microecology.

The application of molecular biology in environmental microbiology research has the following aspects:

(1) Monitoring of environmental microorganisms

Traditional culture methods limit the detection possibilities of most microorganisms. This greatly

limits the development of environmental microbial research. However, modern molecular biology can avoid the problem that some live bacteria cannot be cultivated, and can directly analyze at the molecular level. PCR technology and related derivative technologies can amplify microbial genes with low content in complex environments, and can even detect specific genes of a specific flora [9-10]. For example, a scholar used PCR and RT-PCR techniques to detect pathogenic bacteria in organic wastes such as compost [11].

(2) Application of genetically engineered bacteria in environmental pollution control

Nature provides human beings with microorganisms with diverse functions. On the other hand, human beings modify microorganisms based on the characteristics of microorganisms to meet the needs, especially the requirements of environmental pollution control. For example, by increasing the expression of intracellular enzymes, the ability of the original strain to degrade specific pollutants can be improved, or the ability of specific strains to degrade pollutants can be given [12]. A scholar expresses organophosphorus hydrolase on the cell surface, so that the cell can effectively degrade phosphorus, sulfur and Paraoxon brought into the soil by agricultural pesticides while keeping the cell intact. The organophosphorus hydrolase obtained by this method is higher than expression in normal cells is several-fold higher [13].

3. Research Experiments

3.1. Experiment Content

This paper uses MBT to analyze the colonies in activated sludge, uses fluorescence quantitative PCR modern MBT to analyze the microbial community in sewage, and uses the IFAS system to denitrify the sewage to monitor the water quality indicators after denitrification.

IFAS system: Because the temperature changes greatly throughout the year, especially in winter when the temperature is low, the water temperature also decreases. When the water temperature is lower than 12 °C, the microbial activity involved in nitrification and denitrification will also decrease, thus affecting the process. The denitrification efficiency of the IFAS process is often faced with similar problems. Therefore, periodic and continuous monitoring of the microbial community structure and diversity in the fixed membrane and activated sludge of the IFAS system, as well as the composition and quantity of important microorganisms involved in the biological denitrification process, will help the IFAS system to solve the problem of denitrification in different seasons. The promotion, maintenance, improvement and perfection of nitrogen efficiency provide important theoretical basis [14-15].

3.2. Experimental Method

(1) Extraction of total DNA from sample colonies

Establishing an efficient and convenient DNA extraction method is one of the key technologies to be solved. In the experiment, the molecular biology method was used to analyze the bacterial community structure in activated sludge, and the extraction effect of total bacterial DNA in the sample directly affected the accuracy of subsequent PCR-DGGE analysis [16]. Quantitative detection adopts fluorescence quantitative PCR technology, and selects the NSR gene of *Nitrospira* as the target gene and the bacterial gene as the internal reference gene for fluorescence quantitative PCR amplification.

Sludge total DNA extraction method:

The sludge kit was selected, and the sludge was extracted according to the kit instructions. The

extracted DNA was electrophoresed for 30 min at a voltage of 85V, and then imaged and photographed in a gel imager. Qualified DNA solutions should be stored in a -20 °C refrigerator for later use, and appropriately aliquoted [18]. Quantity One software was used to analyze the acquired DGGE images to obtain information such as the number of bands in each lane, band peak area, etc. The similarity index Cs was calculated through the software's built-in function, and cluster analysis was performed [19].

$$H = -\sum_{i=1}^S P_i \log P_i \tag{1}$$

$$P_i = \frac{n_i}{N} \tag{2}$$

where P is the band intensity, n_i is the peak area, and N is the total area of all peaks.

(2) PCR amplification

PCR amplification used a 25ul reaction system. The PCR template selects samples diluted to 20ng/ul, and the amplification conditions are: (1) Denaturation: 94 °C, 5min; (2) Annealing: 94 °C, 30s; 58 °C, 30s; 72 °C, 30s; (3) Extension: 72 °C, 5min; (4) Storage, 10 °C. The number of cycles in the annealing process needs to be pre-tested before the test to ensure the accuracy and reliability of information analysis in the subsequent high-throughput sequencing of the sample, because the PCR process should use as few cycles (25-30 cycles) as possible to reduce DNA Replication errors, in addition, the cycle number of the same batch of sequencing samples should also be kept similar [20].

4. Analysis of Water Quality Index Test Results

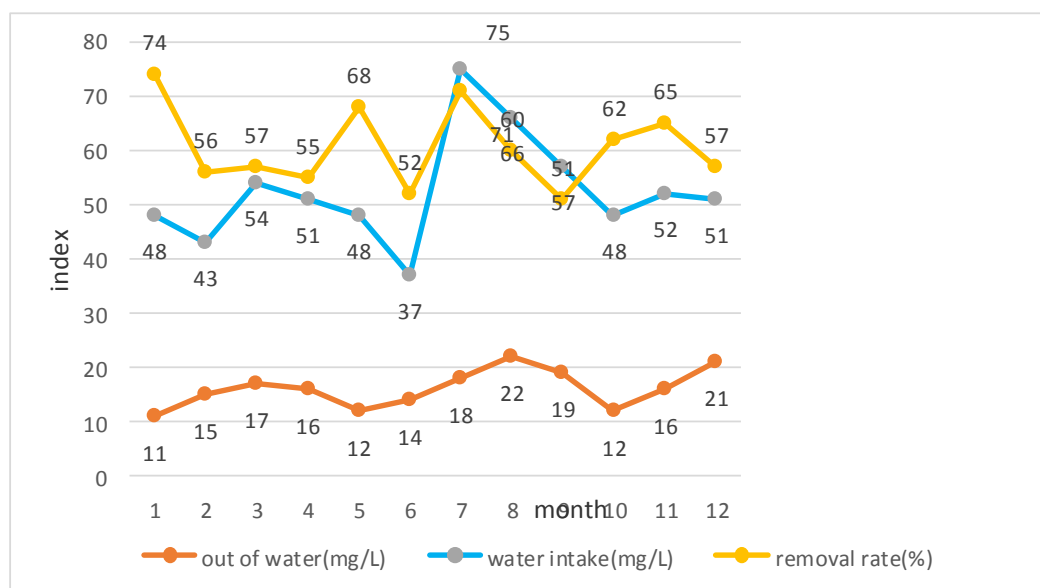


Figure 1. Changes in total nitrogen

Figure 1 shows the change of total nitrogen. It can be seen that the water quality of total nitrogen in the system fluctuates between 40-80mg/L, but the water quality of the effluent is maintained at about 20mg/L, except that the water quality of the effluent in some months meets the second grade.

In addition to the standard, the quality of the effluent in the rest of the months is about Grade B or better than Grade B. The removal rate fluctuated between 55% and 80%, and was mainly maintained at around 60%. Except for a few months, it generally showed a trend of rising in summer, falling in autumn, stable in winter, and rising again in spring.

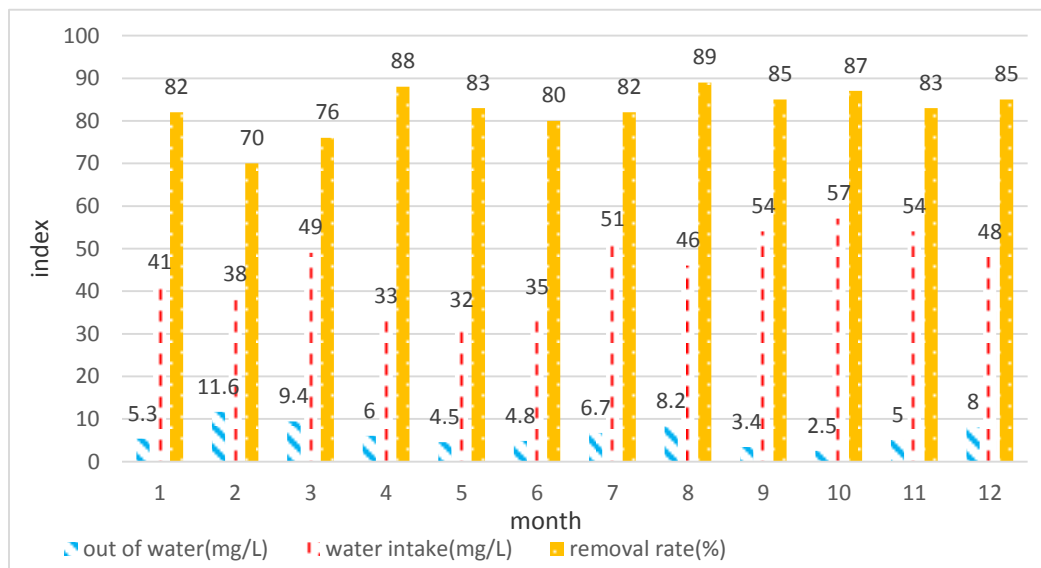


Figure 2. Changes in ammonia nitrogen

Figure 2 shows the changes of ammonia nitrogen. When the influent water quality of ammonia nitrogen in the system fluctuates between 30-60 mg/L, the effluent water quality fluctuates between 2.5-9.5 mg/l. Except for February and March, other months can meet the requirements Level 1 B emission standard, and even up to Level 1 A emission standard in some months. Except for February and March when the removal rate was less than 80%, the other months were all over 80%, and basically fluctuated around 90%, with a small change trend.

Table 1. Variation of nitrite and nitrate effluent

	1	2	3	4	5	6	7	8	9	10	11	12
Nitrous nitrogen	0.36	0.43	0.47	0.51	0.56	0.62	0.46	0.75	0.56	0.42	0.40	0.38
Nitrate	1.54	1.37	1.24	1.21	1.18	0.55	0.58	1.07	0.84	0.96	1.15	1.16

It can be seen from Table 1 that the water quality of nitrate-nitrogen effluent mainly fluctuates between 0.5-1.6 mg/L, and the nitrous-nitrogen content of effluent in winter is higher than that in summer. The effluent quality of nitrous nitrogen fluctuates between 0.35-0.8 mg/L, and the effluent concentration is low, which is mainly related to its easy oxidation and unstable properties, and its changing trend is opposite to that of nitrous nitrogen.

Table 2 shows the changes of COD_{Cr}. The influent water quality of the system COD_{Cr} varies widely, fluctuating between 260-500 mg/L; the removal rate is basically stable and maintained between 80%-95%, and is around 90% most of the time, and the removal efficiency is high; The quality of the effluent is relatively stable, with the exception of more than 60mg/L in February and April, which is between 30-55mg/L, which is far better than the first-class B emission standard, and can even meet the first-class A standard.

Table 2. Changes in COD_{cr}

	1	2	3	4	5	6	7	8	9	10	11	12
Water intake(mg/L)	473	345	426	412	376	264	325	420	388	415	457	463
Out of water(mg/L)	48	63	55	76	52	36	30	45	51	55	54	52
Removal rate(%)	86	78	82	79	81	83	92	87	85	94	95	93

Table 3. Changes in BOD₅

	1	2	3	4	5	6	7	8	9	10	11	12
Water intake(mg/L)	287	195	236	211	205	184	262	227	215	220	263	239
Out of water(mg/L)	12	16	17	12	15	16	12	18	17	14	12	16
Removal rate(%)	92	94	93	95	97	94	92	96	95	94	91	92

Table 3 shows the variation of BOD₅. When the variation range of the influent water quality of the system BOD₅ fluctuates between 180-300 mg/L, the removal rate can be maintained at about 95%, the removal efficiency is good, and the fluctuation of the effluent water quality is also small, stably maintained at about 10mg/L-20mg/L, far better than the first-class B emission standard, and basically close to the first-class A standard.

According to the above analysis results, after the use of MBT and IFAS system to denitrify the sewage, the total nitrogen and ammonia nitrogen in the effluent can basically reach the first-class B emission standard, and even reach the first-class A standard in some months, even in winter when the temperature is low., also showed a good effect of nitrogen removal, in line with the characteristics of the IFAS system, verifying that the IFAS system has a good denitrification effect. The IFAS system has a very strong ability to remove organic matter. In a year, the COD_{cr} removal rate is about 90% most of the time, and the BOD₅ removal rate is about 95%, which proves that the IFAS system is very effective in the removal of organic matter.

5. Conclusion

At present, the problem of environmental pollution is serious, and the use of MBT to solve environmental problems brought about by human development is of great practical significance. However, the traditional analytical methods have great limitations and cannot obtain detailed environmental microbial information. Therefore, in this paper, PCR-DGGE technology was selected to extract sludge DNA to analyze sludge microbial community structure, and to provide a basis for sewage denitrification in IFAS system. The experimental results show that the sewage after denitrification can meet the discharge standard and alleviate the environmental problems caused by the sewage.

Funding

This article is not supported by any foundation.

Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

References

- [1] Colombo N, Sessa C, Bois A D, et al. ESMO–ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Annals of Oncology*, 2019, 30(5):672-705.
- [2] Mccord R P, Taipale M, Teichmann S, et al. Voices on technology: The molecular biologists' ever-expanding toy box. *Molecular Cell*, 2020, 82(2):221-226.
- [3] Abdallah B B, Andreu I, Chatti A, et al. Size Fractionation of Titania Nanoparticles in Wild *Dittrichia viscosa* Grown in a Native Environment. *Environmental Science and Technology*, 2020, 54(14):8649-8657. <https://doi.org/10.1021/acs.est.9b07267>
- [4] P Spégel, Mulder H . Metabolomics Analysis of Nutrient Metabolism in β -Cells. *Journal of Molecular Biology*, 2020, 432(5):1429-1445. <https://doi.org/10.1016/j.jmb.2019.07.020>
- [5] Santos, R. O, Rehage, et al. Combining data sources to elucidate spatial patterns in recreational catch and effort: fisheries-dependent data and local ecological knowledge applied to the South Florida bonefish fishery. *Environmental Biology of Fishes*, 2019, 102(2):299-317.
- [6] Rahman M J, Amin S, Nahiduzzaman M, et al. Influence of seasons, habitat sanctuaries, gears and environmental variables on the catches of hilsa shad (*Tenualosa ilisha*) in Bangladesh waters. *Journal of environmental biology*, 2018, 39(5SPEC.):767-776. [https://doi.org/10.22438/jeb/39/5\(SI\)/21](https://doi.org/10.22438/jeb/39/5(SI)/21)
- [7] Tabti D, Laouar M, Rajendran K, et al. Identification of desirable mutants in quantitative traits of lentil at early (M_2) generation. *Journal of environmental biology*, 2018, 39(2):137-142. <https://doi.org/10.22438/jeb/39/2/MRN-476>
- [8] Thrall J H, Li X, Li Q, et al. Artificial Intelligence and Machine Learning in Radiology: Opportunities, Challenges, Pitfalls, and Criteria for Success. *Journal of the American College of Radiology*, 2018, 15(3):504-508. <https://doi.org/10.1016/j.jacr.2017.12.026>
- [9] Bin Y U, Kumbier K . Artificial intelligence and statistics. *Frontiers of Information Technology & Electronic Engineering*, 2018, 19(01):6-9. <https://doi.org/10.1631/FITEE.1700813>
- [10] Koch, Marta. Artificial Intelligence Is Becoming Natural.. *Cell*, 2018, 173(3):531-533. <https://doi.org/10.1016/j.cell.2018.04.007>
- [11] Syam N, Sharma A . Waiting for a sales renaissance in the fourth industrial revolution: Machine learning and artificial intelligence in sales research and practice. *Industrial Marketing Management*, 2018, 69(FEB.):135-146.
- [12] Selvarajoo K, Piras V, Chiow A . Long Range Order and Short Range Disorder in *Saccharomyces cerevisiae* Biofilm. *Engineering Biology*, 2019, 3(1):12-19. <https://doi.org/10.1049/enb.2018.5008>
- [13] Roger M, Brown F, Gabrielli W, et al. Efficient Hydrogen-Dependent Carbon Dioxide Reduction by *Escherichia coli*. *Current Biology: CB*, 2018, 28(1):140-145.e2. <https://doi.org/10.1016/j.cub.2017.11.050>

- [14] Pullaguri N, Nema S, Bhargava Y, et al. Triclosan alters adult zebrafish behavior and targets acetylcholinesterase activity and expression. *Environmental toxicology and pharmacology*, 2020, 75(Apr.):103311.1-103311.7. <https://doi.org/10.1016/j.etap.2019.103311>
- [15] Fasciglione G, Goi M G, Yommi A, et al. Revaluation of waste from fishing industry through generation of chitosan coatings to improve quality and extend shelf-life of minimally processed lettuce. *Postharvest Biology and Technology*, 2020, 170(12):1-9.
- [16] Reyna-Figueroa J, MF Rodríguez-Sánchez, Matsumoto P, et al. Decrease in the Hospital Stay of Neonates with Suspected Nosocomial Sepsis with the Use of a Molecular Biology Technique. *Journal of Biosciences and Medicines*, 2019, 07(3):44-51. <https://doi.org/10.4236/jbm.2019.73005>
- [17] Bucci E M . Xylella fastidiosa, a new plant pathogen that threatens global farming: Ecology, molecular biology, search for remedies. *Biochem Biophys Res Commun*, 2018, 502(2):173-182. <https://doi.org/10.1016/j.bbrc.2018.05.073>
- [18] Kaul D . An Overview of Coronaviruses including the SARS-2 Coronavirus – Molecular Biology, Epidemiology and Clinical Implications. *Current Medicine Research and Practice*, 2020, 10(2):54-64. <https://doi.org/10.1016/j.cmrp.2020.04.001>
- [19] Ratner L . Molecular biology of human T cell leukemia virus. *Seminars in Diagnostic Pathology*, 2020, 37(2):104-109. <https://doi.org/10.1053/j.semdp.2019.04.003>
- [20] Precious S V, Rosser A E, Dunnett S B . [Methods in Molecular Biology] Huntington's Disease Volume 1780 || Translating Antisense Technology into a Treatment for Huntington's Disease. 2018, 10.1007/978-1-4939-7825-0(Chapter 23):497-523. <https://doi.org/10.1007/978-1-4939-7825-0>