

Based on MBR Microbial Community Structure Analysis of Shortcut Nitrification Initiation in Different Kinds of Mud

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Abstract: For clear membrane bioreactor (MBR) Microbial Community structure changes before and after the short-cut Nitrification with different sludge inoculation. MBR Nitrifying sludge (R1.), Anaerobic nitrification sludge (R2.) And 1.:1. Mixed inoculation of anaerobic and Denitrifying sludge (R3.) Sludge source for achieving rapid short-cut nitrification. The results show that the combination of Intermittent Aeration and reduced hydraulic retention time (HRT), R1. R2. With R3. Reactor time-consuming 46 d, 8 d and 30 d. The shortcut nitrification was successfully launched, R2. Shortest startup cycle of Reactor. During the stable operation period, R1. R2. And R3. The average nitrite accumulation rate was 92%, 93% and 94%, R3. The reactor showed more stable short-cut Nitrification performance. Ace, Chao, Shannon and Simpson Index results show that after stable operation, R1. And R2. The microbial abundance and diversity of the reactor were significantly lower than that of the inoculated sludge, R3. The abundance of reactor species decreased slightly while the diversity level changed little. After the shortcut nitrification was successfully started, 3. The main bacteria in the reactor were Proteus phyla (Proteobacteria) and bacteriocin (Bacteroidetes) and the abundance of the Main Nitrogen Removal Function bacteria is higher than that of the inoculated sludge. Beta-Proteus 3. The dominant bacteria in the short-cut Nitrification system of each reactor accounted 59.6%, 63.6% and 69.3%. R1. R2. And R3. Reactor in the advantage bacteria of are nitrosation single (Nitrosomonas) Of proportion respectively up 12.8%, Natural 20.2% and 19.7%. Compared R1 Reactor, R2 and R3 Reactor inoculation sludge in there is a certain proportion of nitrification bacteria more conducive to system short-range nitrification of implementation.

Keywords: short-range nitrification; Membrane Biological Reactor; inoculation sludge; start; microbial community structure

Many scholars for this of the difficult problems of a large number of research results show that the high temperature, High FA, Low DO, Intermittent Aeration and real time control Mud source start short-range nitrification of study few reports. And most research are from Start Time, Nitrification rate and macro-angle analysis short-range nitrification start performance of quality for start before and after microbial community structure of Change Characteristics Research is less, so Will macro- and micro-combined with up the different mud source conditions under short-range nitrification start performance difference is very necessary. High Flux sequencing technology as a New Microbial Population Identification Technology Of has analysis results accurate, High-speed, High sensitivity and high automatic [9,10] Of and Characteristics In environment microbial identification field application widely. At the same time, the technology also more and more be applied to short-range nitrification process [11] The microbial community structure detection in help short-range nitrification

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process of further regulation and implementation. Membrane Biological Reactor (MBR) Compared with traditional biological reactor has covers an area of small, Volume load high and left [12] More than sludge of low advantage and membrane filtration water of operation style can achieve system sludge of efficient interception to enrichment growth slow AOB Reduce Short-range nitrification of Start Time. This study Selection MBR Reactor respectively inoculation nitrification Sludge, Anaerobic nitrification Sludge 1:1 Mixed inoculation anaerobic nitrification sludge and anti-nitrification sludge the different inoculation sludge of short-range nitrification start characteristics and start before and after flora structure change in order to for rapid start short-range nitrification reactor of sludge source select provide basis.

1. Material and Methods

1.1 Experimental Device

This experiment using MBR (Figure 1) Reactor by organic glass made long 10 cm Wide 8 cm Effective Height 24 Length Effective Volume 1.9 L. MBR The hollow fiber microfiltration membrane module membrane hole 0.1 μm Membrane area 0.2 m^2 . Reactor by peristaltic pump continuous water by membrane module suction continuous water. Reactor at the bottom of the use of exposure Gas sand head Oxygen Supply, DO Control 0.6~1.0 mg in L^{-1} By timing socket control stop exposure time. The whole operation process in reactor place in water tub in keep run temperature in (30 ± 1) .

1.2 Inoculation sludge

This experiment using 30 of sludge Reactor 1 (R1): From Suzhou a wastewater treatment plant A²/O Process of good oxygen nitrification sludge; Reactor 2 (R2): From laboratory long-term place 1 More than of anaerobic Nitrite Of sludge; Reactor 3 (R3): 1:1 Mixed inoculation the above of anaerobic nitrification sludge and anti-nitrification sludge from Suzhou a wastewater treatment plant A²/O Process of hypoxia pool. Early inoculation sludge properties such as table 1 Shown in.

1.3 Experimental water and run conditions

Reactor experimental water same were the Artificial Water Distribution. To sodium acetate as an organic carbon source ammonium chloride as an nitrogen source potassium dihydrogen phosphate as an phosphorus sodium bicarbonate provide alkalinity regulation water COD, NH_4^+ -N, TP Concentration and PH Value and add magnesium sulfate, Calcium chloride and biological required nutrition elements and other trace elements specific content see

2. The whole experimental operation process in, 3A reactor PH Value were control in 7.5~8.0. Reactor of specific operation conditions are shown in Table 3.

2. Results and discussions

2.1 MBR Start-up analysis of shortcut nitrification

Influent control by Reactor NH_4^+ -N And COD Load in 72~1.20 $\text{kg} \cdot (\text{M}^3 \cdot \text{D})^{-1}$, PH For 7.5~8.0, Do $\bar{0}$ Du is 0.6~1.0 $\text{mg} \cdot \text{L}^{-1}$ Intermittent Aeration and gradual reduction HRT The way 56 d All obtained stable short-cut nitrification. In this experiment, the nitrite accumulation rate is continuous. 7 d Stable in 85% The above marks the success of shortcut nitrification, R1, R2, and R3. Run separately 46, 8, and 30 d Achieve short-cut nitrification. Inlet and outlet water quality of each reactor during startup, Ammonia Nitrogen Removal Rate and nitrite accumulation rate 2. The whole running process is divided 4. Stages.

Stage, using continuous aeration, gradually activated, Augmentation AOB The activity, 3. Ammonia Nitrogen Removal Rate in each reactor gradually increased 95% Above. In this phase, R1, R2, and R3. Reactor time consumption 16, 10 and 12 d This is associated with the inoculation of sludge contained in AOB Quantity is closely related. Running until the end of this phase, R1, and R3. Inside the reactor NO_3^- -N The concentration showed an upward trend, because NH_4^+ -N Reduction of free ammonia NO_b The inhibitory effect NO_2^- -N More NO_3^- -N. It is worth mentioning that, R2. Effluent from Reactor NO_3^- -N Concentration stabilized 13 $\text{mg} \cdot \text{L}^{-1}$ About, Average nitrite accumulation rate 90% And successfully started short-cut nitrification. The analysis shows that: On the one hand MBR The Interception Effect of the reactor was effectively avoided. AOB Stable membrane environment is very conducive AOB Enrichment and proliferation; on the other hand, the inoculation of anaerobic AOB And lower orders of magnitude NO_b Bacteria, coupled with the effective regulation of the outside environment, that is, high temperature, Low Do And fitting PH Successful suppression under conditions NO_b Short-cut nitrification.

Stage In keeping HRT Under the same conditions, using hypoxia/ Aerobic bi 1.: 3. (15 min: 45 min) Operating mode of Intermittent Aeration. R1, and R3. Inside the reactor NO_3^- -N The overall concentration showed a downward trend, indicating that hypoxia/ Aerobic alternate operating mode can effectively inhibit nitrate nitrogen.

Once aeration is restored, a long-term experience "Hunger" Of AOB Ammonia production capacity can be more used to make its own proliferation. AOB Of

Effluent from Reactor NO_3^- -N The concentration showed a trend of first decline and then rise, reaching the highest 21 $\text{mg} \cdot \text{L}^{-1}$, The corresponding nitrite accumulation rate fell 85%. Pre-Analysis NO_3^- -N The decreased and hypoxia/ Good oxygen alternating run style about late NO_3^- -N Of rise because on the one hand with the reactor in AOB Enrichment degree more and more high water NH_4^+ -N Matrix can't meet AOB Of growth, AOB Activity by

suppression; On the other hand HRT is too long reactor in there NO₂⁻ leads to an accumulation NO₂⁻ - N further oxidation NO₃⁻ - N.

Stage And stage Respectively in keep hypoxia/Good oxygen 1:3 (15 min:45 min) The same of conditions under shorten HRT to 4 h And 3 H. With HRT of shorten, R1, R2 And R3 Reactor, Stage of ammonia nitrogen removal rate mean respectively 87%, 93%, 91% And 85%, 91%, 88%. Show that with HRT of shorten the ammonia nitrogen removal rate gradually reduce. Stage stable run R1, R2 And R3 Reactor ammonia nitrogen average removal load respectively 1.04, 1.07 kg in $\cdot (M^3 \text{In D})^{-1}$ R2 Reactor show is high Of Ammonia Nitrogen Removal load (R2 Reactor in AOB Activity is good. At the same time, R1, R2 And R3 Reactor water NO₃⁻ - N Mean respectively 9, 9 And 7/Mg IN L⁻¹ Nitrite accumulation rate average 92%, 93% And 94% Show that 3A reactor were success start short-range nitrification and R3 Reactor show more stability of short-range nitrification.

2.2 High Flux sequencing results analysis

2.2.1 MBR Reactor microbial abundance and diversity analysis

Use MiSeq High Flux sequencing platform R1, R2 And R3

Reactor of inoculation sludge and start success stable run the first 56 d Of sludge sequencing by single sample of Diversity Analysis (α Diversity) reflecting microbial community of abundance and diversity specific situation such as table 4 Shown in. The sequencing 6A sample of coverage were greater 99% Show that the sequencing results can representative sample in microbial of real situation. ACE And Chao Index can estimation community in OTU Number of objective index characterization microbial population of rich of in ecology often used to estimate the species total number its value the greater the show that species total ACE, Chao Index performance $A_0 > C_0 > B_0$ (R1 Reactor in microbial population of abundance highest, R3 Reactor secondly, R2 Reactor at least. Analysis R1 Reactor inoculation of Wastewater Treatment Plant A²/O Good oxygen nitrification sludge has certain of nitrogen removal carbon removal function microbial population of rich of relative is high; And R2 Reactor inoculation of laboratory place 1 More than of anaerobic nitrification sludge after long-term of endogenous respiratory and anaerobic digestion role microbial population of abundance decreased; R3 Reactor 1:1 Mixed inoculation anaerobic nitrification sludge and anti-nitrification sludge microbial population of abundance than both moderate. In addition, R1, R2 And R3 Reactor start success after, ACE And Chao Index were decreased show that species total number reduce its decline respectively 45.4%, 30%, 36% And 45.3%, 28.5%, 38.3% And the two index of Change Law and OTU The number of change consistent. Shannon And Simpson Index is used to estimate sample in microbial of diversity, Shannon Value the greater the (microbial population diversity the higher, Simpson Index the greater the (microbial population diversity the low,

At the same time also (advantage microbial accounted for total biomass of the greater the proportion. 4 Display, R1, R2 And R3 Reactor in Shannon Index of Change Trend and ACE, Chao Index change trend consistent decreased trend. And R1 And R2 Reactor decline is big respectively 42.3% And 16.1% R3 Reactor decline is only 5.1%. Corresponding, R1 And R2 Reactor Simpson Index increase is big, R3 Reactor Simpson Index keep the same. Show that R1 And R2 Reactor in short-range nitrification start before and after microbial population diversity significantly reduce,

And R3 Reactor in microbial diversity change not. Short-range nitrification of START process essentially is Ammonia Oxidation Bacteria of enrichment disadvantages Strain

Of out. In this process in with the system in advantage microbial accounted for than gradually increase usually will makes microbial population diversity reduce.

From this point of view, R1 And R2 Reactor short-range nitrification start before and after of microbial community structure change in line with this a trend. Compared in R2 Reactor, R3 Reactor start time slightly longer but nitrification stability performance is strong this May and reactor in microbial community of diversity have Should be a stress of complementary unit to makes in through stress after community of Rapid Repair Ability stronger improve community of recovery force ensure that community of stability.

2.2.2 MBR Reactor in door classification level of Distribution Law 3 For 6A sample in door level under microbial community structure of distribution situation from can see main including deformation bacteria door (Proteobacteria), Quasi-of door (Bacteroidetes), Green bending bacteria door (Chloroflexi), Actinomycetes door (Actinobacteria), Thick-walled bacteria door (Firmicutes) And acid of door (Acidobacteria) And and Shaped bacteria door and quasi-of door main of proportion respectively up 6%~58.9% And 3.9%~35%. In addition, 6A sample also detection to floating mold door (Planctomycetes) And nitrification bacterium door (Nitrospirae) Of proportion relative is small.

And nitrosation screw bacteria (Nitrospira) System not detection to nitrosation screw bacteria of so inference the system in AOB Belongs to nitrosation single. This and most research results consistent the Sewage Processing 6 Is MBR Reactor inoculation different mud source in short-range nitrification start before and after nitrogen removal bacteria of the genus distribution characteristics from can see, 3

A reactor start short-range nitrification after nitrosation single of were in excellent

Research indicate that the in organic short-range nitrification System in nitrogen removal bacteria of main for anti-nitrification bacteria and nitrification single of not found nitrification screw bacteria of this and this experimental results phase consistent. Analysis show that in a certain amount of carbon source conditions under, AOB and NOB Produce DOC COMPETITION MECHANISM AND AOB OKAY DOOF affinity ability higher than that NOB Makes AOB In favorable status. Combined with this experiment the Intermittent Aeration and shorten HRT Of Style gradually Suppression, Washing reactor in NOB. At the same time only in R3 Reactor start success of samples in detection to a small amount of anti-nitrifying bacteria (Denitratisoma) And anti-nitrifying bacteria (Pseudomonas) Of specific reason still need to be further study here not explain. In addition contrast inoculation sludge, R2 And R3 Reactor in the Tao, the genus, In P. Of and thermal single (Thermomonas) Was reduce Trend. Analysis Three are of heterotrophic bacteria with the reactor in advantage strain nitrosation single cell bacteria of the genus enrichment disadvantages bacteria of abundance gradually reduction The Study on Nitrogen found to sodium acetate for carbon source no matter good oxygen or anaerobic conditions all have nitrite of cumulative this is because anti-nitrifying bacteria to sodium acetate for carbon source to nitrate nitrogen for electronic receptor anti-nitrification, to lead to the nitrite of accumulation so the author think reactor in anti-nitrifying bacteria of the genus there promote the short-range nitrification of implementation.

3. Conclusion

D All of them successfully started short-cut nitrification, R2. Minimum reactor start-up time. During the stable operation period, R1, R2, And R3. The accumulation rate of nitrite in the reactor was stable. 90% Above, and R3. The reactor showed more stable short-cut Nitrification performance..

(2.) Ace, Chao, Shannon And Simpson The index results show that compared with the inoculated sludge, R1, And R2. The microbial abundance and diversity of the reactor were significantly lower than that of the initial inoculation.

Level, R3. The abundance of reactor species decreased slightly while the diversity level changed little..

(3.) After stable operation, 3. The main bacteria in the reactor were Change, bacteria, door (Proteobacteria) Kazuo, bar, fungus, door Bacteroidetes) And the abundance of the Main Nitrogen Removal Function bacteria is higher than that of the inoculated sludge.. Second, 3. Short-range Reactor

After the success of nitrification Beta-Proteus is the dominant bacteria in the system.

(4.) After the success of shortcut nitrification, R1, R2, And R3. Reactor, internal, superior, potential, bacteria, genus, all sub, nitrate, chemical, Mono, cell, bacteria, genus Nitrosomonas) Respectively, the proportion 12. 8%, 20. 2%

19. 7%. Compare R1. Reactor, R2, And R3. There is a certain proportion of denitrifying bacteria in the reactor inoculated sludge, which is more conducive to the realization of short-cut Nitrification in the system..

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