



Article Effect of Chemical Oxygen Demand Concentration on Nutrient Removal in Simultaneous Nitrification, Denitrification and Phosphorus Removal System in High-Altitude Areas

Yani Zhao¹, Liling Zhang², Meng Zhang², Jingya Wu², Shuping Li¹, Douzhi Ran¹, Liwei Sun^{2,*} and Guangcan Zhu^{1,2,*}

- ¹ Xizang of Water Pollution Control and Ecological Restoration Engineering Laboratory, School of Information Engineering, Xizang Minzu University, Xianyang 712082, China; zhaoyani2000@163.com (Y.Z.); lishuping@xzmu.edu.cn (S.L.); ranwei199802@163.com (D.R.)
- ² School of Energy & Environment, Southeast University, Nanjing 210096, China; ll-zhang2021@163.com (L.Z.); 220180612@seu.edu.cn (M.Z.); 220200551@seu.edu.cn (J.W.)
- * Correspondence: liwei-sun@seu.edu.cn (L.S.); gc-zhu@seu.edu.cn (G.Z.)

Abstract: The application of biological nitrogen and phosphorus removal processes in high-altitude areas faces severe challenges due to low temperature, low atmosphere pressure and low oxygen concentration. In this study, a simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system was operated under low atmosphere pressure. The chemical oxygen demand (COD) concentrations in influent were decreased from 300 mg/L (stage I) to 200 mg/L (stage II), corresponding to the low COD concentration of sewage in high-altitude areas. The removal of COD and total phosphate was efficient at the H1 reactor (72 kPa). The removal rates of COD and total phosphate were 94.08% (stage I), 90.66% (stage II) and 98.43% (stage I), 99.34% (stage II), respectively, which were similar to L1 (100 kPa). The removal rates of total inorganic nitrogen and simulation nitrification and denitrification were from 81.21% (stage I) and 59.48% (stage I) to 72.86% (stage II) and 31.95% (stage II), respectively, which were also improved compared to L1. Cycle experiment results indicated that the activity of phosphorus accumulating organisms was enhanced, while the ammonia oxidation process was inhibited under low atmosphere pressure.

Keywords: SNDPR; low atmosphere pressure; low COD; phosphorus accumulating organisms

1. Introduction

Environmental characteristics of high-altitude areas are low temperature, low atmosphere pressure and low-oxygen content [1]. Annual average pressure, oxygen content and atmospheric density at Lhasa are 50%, 60% and 66% of those at sea level, respectively. At present, commonly used sewage treatment processes in high-altitude areas in China include anaerobic-anoxic-oxic (A²O) and cyclic activated sludge systems [2]. In high-altitude areas, the actual dissolved oxygen (DO) content in water is only about 50% of that in low-altitude areas under the same aeration conditions, which decreases microbial growth, nitrification, and aerobic phosphorus uptake in biological treatment processes [3], ultimately reducing treatment efficiency and discharged water quality. Therefore, the application of biological treatment processes for sewage nitrogen (N) and phosphorus (P) removal in high-altitude areas faces severe challenges [4,5].

The simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system realizes the simultaneous removal of organic matter, N and P, in the same reactor. In 2003, Zeng R.J. et al. [6] successfully achieved SNDPR reactions in a single-stage reactor under low DO conditions (0.5 mg/L) in an alternating anaerobic/aerobic mode. Currently, SNDPR has been studied and applied in aerobic granular sludge systems [7,8], biofilm reactors [9–11] and mixed sludge systems [12]. Compared with traditional A²O



Citation: Zhao, Y.; Zhang, L.; Zhang, M.; Wu, J.; Li, S.; Ran, D.; Sun, L.; Zhu, G. Effect of Chemical Oxygen Demand Concentration on Nutrient Removal in Simultaneous Nitrification, Denitrification and Phosphorus Removal System in High-Altitude Areas. *Water* **2021**, *13*, 2656. https://doi.org/10.3390/ w13192656

Academic Editor: Layla Ben Ayed

Received: 24 August 2021 Accepted: 20 September 2021 Published: 26 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). processes, a SNDPR system needs lower DO and can remove N and P with internal carbon sources [13–15], and therefore saves 50% carbon source and 30% aeration consumption [16,17]. It is expected to be suitable for domestic sewage treatment in high-altitude areas [18].

Studies have shown that the organic concentration (calculated by COD) in influent is an important factor affecting the performance of biologically removing N and P from wastewater. The organic matter concentration in influent serves as an electron donor in the denitrification process and affects the denitrification process efficiency and metabolism activity of associated microorganisms, so the COD concentration in influent significantly affects the simultaneous nitrification-denitrification process [19]. Pochana et al. [20] concluded that the demand for complete denitrification can be met when COD and total Kjeldahl nitrogen ratio is seven, while when the COD and total Kjeldahl nitrogen ratio is nine, the system is able to achieve simultaneous N and P removal. Han and Wang [21] reported that when the COD increased to $4.0 \text{ kg/(M}^3 \cdot \text{d})$, the content of polyhydroxy fatty acids in the sequencing batch moving bed biofilm reactor system significantly increased, while the efficiency of N and P removal in the system also increased. Zhou et al. [22] found that low carbon nitrogen ratios in the SNDPR system were not conducive to the N removal process and affected the composition of the microbial community. The investigations of the present study showed that COD concentrations of municipal sewage in Tibet are low, ranging from 80 mg/L to 150 mg/L [23]; however, the content of N and P is relatively high. The reason may be that the residents in plateau areas ingest more meat, milk and other high-protein foods, resulting in insufficient carbon source and high N and P content in the influent of sewage treatment plants.

In this study, two SNDPR systems were operated under different atmospheric pressures (100 kPa and 72 kPa). The COD concentrations in influent were decreased from 300 mg/L to 200 mg/L, which corresponded to a low concentration of COD influent of sewage in high-altitude areas. The N and P removal performance of SNDPR systems for different COD concentrations under different atmospheric pressures was explored. The mechanism of the removal of N and P was studied in the cycling experiment. The cycle experiment of an SNDPR system consists of four stages: anaerobic, aerobic, sedimentation and drainage. Cycle experiments are conducted to study the changes in concentrations of COD, N and P during one single operating cycle of the reactor, to analyze the major pollutant conversion and removal pathways and to evaluate the applicability of SNDPR systems under different atmospheric pressure environments. The research results provide a technical basis for applying an SNDPR system to domestic sewage treatment processes in high-altitude areas.

2. Materials and Methods

2.1. Experimental Set-Up and Operational Approaches

Two cylindrical sequencing batch reactors (SBR) were used in this experiment. One reactor (denoted as L1) was set in an atmospheric pressure environment (100 ± 1 kPa) and the other (denoted as H1) was set in a low atmospheric pressure environment (72 ± 1 kPa). The low-pressure environment was provided by a high-altitude environment simulation capsule [24]. The water temperatures of L1 and H1 were controlled at 20 ± 2 °C. These reactors were run for 120 days at different atmospheric pressure environments (100 kPa and 72 kPa) after stabilization. The data for 60 days were used in the paper. The effective volume of the reactor was 5L, and volume exchange ratios were 40%. The SNDPR system operated in anaerobic stirring/low oxygen aeration for four cycles every day. Each single cycle lasted 6h and consisted of an anaerobic stirring phase of 130 min, a 210 min of low oxygen aeration, a sedimentation of 3 min, a 10 min draining and a standing of 7 min. A 50 mL mud-water mixture was discharged by the peristaltic pumps in the last minute of the aeration stage to keep mixed liquor suspended solids (MLSS) in the system at 2200 \pm 200 mg/L. The SBR reactor kept the sludge age of the system at 25 days. In the low oxygen aeration stage, the DO was controlled below 1.0 mg/L. To achieve the same

DO conditions, the aeration rate at 72 kPa was 1.34 times of that at (100 kPa: 40 mL/min, 72 kPa: 60 mL/min).

The operation of the reactor was divided into two stages according to different COD concentrations in influent (Table 1).

Item	Stage I	Stage II
Operating stage (day)	1–26	27–53
Anaerobic time (min)	120	120
Aerobic time (min)	210	210
COD influent concentration (mg \cdot L ⁻¹)	300	200
NH_4^+ -N influent concentration (mg·L ⁻¹)	30	30

Table 1. Influent concentration of SNDPR systems at different stage.

2.2. Test Water and Inoculated Sludge

The test water was set up to simulate urban domestic sewage in high-altitude areas. Influent quality is shown in Table 2. Organic matter was expressed in COD concentration and provided by sodium acetate (CH₃COON_a). Ammonia nitrogen (NH₄⁺-N) was provided by ammonium chloride (NH₄CL). Total phosphorus (TP) was provided by potassium dihydrogen phosphate (KH₂PO₄). Calcium-ion (Ca²⁺) and magnesium-ion (Mg²⁺) were provided by calcium chloride (CaCL₂) and magnesium sulfate heptahydrate (MgSO₄ 7H₂O) respectively, and the alkalinity was provided by sodium bicarbonate (NaHCO₃). Trace elements necessary for microbial growth of 0.5 mL were added to each liter of simulated sewage sodium acetate (CH₃COON_a) and ammonium chloride (NH₄CL) were produced by Tianjin Tianli Chemical Reagent Co. (TL TJ CHN), potassium dihydrogen phosphate (KH₂PO₄), calcium chloride (CaCL₂), magnesium sulfate heptahydrate (MgSO₄7H₂O) and sodium bicarbonate (NaHCO₃) were produced by Shanghai Maikelin Biochemical Technology Co. (ML, SH, CHN). The compositions are shown in Table 2.

Water Quality of Influent		Trace Element Solution Composition [25]			
	Main Medium	Concentration (mg·L ⁻¹)	Component	Concentration (g·L ⁻¹)	
	COD	200 or 300	F _e Cl ₃ .6H ₂ O	1.5	
	NH_4^+-N	30	MnCl2.4H2O	0.12	
	TP	5	C _o Cl ₂ .6H ₂ O	0.15	
	Ca ²⁺	10	H_3BO_3	0.15	
	Mg ²⁺	10	NaMoO ₄ .2H ₂ O	0.06	
	NaHCO ₃	50	CuSO ₄ .5H ₂ O	0.03	
			KI	0.18	
			$Z_nSO_4.7H_2O$	0.12	
				10	

Table 2. Composition of influent in the SNDPR system.

The inoculated sludge of the SNDPR system at 100 kPa and 72 kPa came from the returning sludge of the improved A²O process of Xianyang Dongjiao sewage treatment plant in Shaanxi Province. The activated sludge was elutriated and filtered in fine gravel through a 100-mesh sieve and then inoculated into the SBR reactor. After domestication, the MLSS concentration in the system was 3453 mg/L, the 5 min sludge sedimentation ratio was 20.9% and the sludge volume index was 60.53 mL·g⁻¹.

2.3. Water Quality Measurement Method

The water sample to be tested was filtered by a 0.45 um needle filter and then stored in a 4 °C refrigerator. All water quality indexes were measured according to relevant standard methods [26]. COD was determined by an intelligent multiparameter tester (LB-3B, Lihua, Beijing, China) and DO was determined with a DO meter (HQ30d, HACH, Loveland,

USA). TP was determined by ammonium molybdate spectrophotometry, NO_2^- -N by N-(1-naphthyl)-ethylenediamine spectrophotometry, NO_3^- -N by ultraviolet spectrophotometry, NH_4^+ -N by Nessler reagent spectrophotometry and MLSS was determined by the drying and weighing method.

2.4. Simultaneous Nitrification and Denitrification Efficiency

Simultaneous nitrification and denitrification (SND) efficiency was defined as the loss of N in the system during the aerobic stage and it was an important parameter to characterize the SND efficiency of the system [27]. The specific calculation Equations (1) and (2) were as follow [28]:

$$SND(\%) = \left(1 - \frac{\Delta NO_x^-}{\Delta NH_4^+}\right) \times 100\%$$
(1)

$$\Delta NO_{x}^{-} = \left(NO_{3,ana}^{-} - NO_{3,eff}^{-}\right) + \left(NO_{2,ana}^{-} - NO_{2,eff}^{-}\right)$$
(2)

where ΔNH_4^+ and ΔNO_x^- are the variations standing for the concentration of NH_4^+ -N and NO_x^- -N between the end of the anaerobic and aerobic phases, respectively, in mg/L; ΔNO_x^- was calculated from the concentration changes of NO_3^- and NO_2^- ; $NO_{3,ana}^-$ and $NO_{2,ana}^-$ stood for the concentration values of NO_3^- -N and NO_2^- -N at the end of the anaerobic stage, respectively, in mg/L; $NO_{3,eff}^-$ and $NO_{2,eff}^-$ stood for the concentration of effluent NO_3^- -N and NO_2^- -N, respectively.

2.5. COD Consumption in Anaerobic Stage

 COD_{ana} was defined as the COD consumed by the SNDPR system in an anaerobic stage, mainly including two parts [29]: the COD (COD_{dn}) consumed by denitrification, and the COD (COD_{intra}) consumed by phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) in the system for intracellular carbon storage.

The specific calculation Formulae (3)–(5) were as follows [30]:

$$COD_{ana} = COD_{dn} + COD_{intra}$$
(3)

$$COD_{dn} = 1.71 \times \Delta NO_2^- - N_{an} + 2.86 \times \Delta NO_3^- - N_{an}$$

$$\tag{4}$$

$$COD_{intra} = COD_{PAO} + COD_{GAO}$$
(5)

where ΔNO_2^- -N_{an} and ΔNO_3^- -N_{an} represented the consumption of NO₂⁻-N and NO₃⁻-N during the anaerobic stage, respectively, in mg/L; 1.71 and 2.86 are the theoretical values of COD consumed by denitrification per unit NO₂⁻-N and NO₃⁻-N respectively [31]; COD_{PAO} and COD_{GAO} was the COD transformed and stored by PAOs and GAOs in anaerobic stage in mg/L.

3. Results and Discussion

3.1. Effects of COD Concentrations on Organic Matter Removal in a SNDPR System under Different Atmospheric Pressures

According to different COD concentrations in influent, the operation of the SNDPR system was divided into two stages (divided by dotted lines in the figure). Organic matter removal in the SNDPR system with different COD concentrations in influent at different atmospheric pressures is shown in Figure 1.



Figure 1. Organic matter removal and the COD_{intra}, COD_{dn} and SND_{ratio} in SNDPR system with different COD concentrations in influent. ((**a**,**b**): 100 kPa; (**c**,**d**,**b**): 72 kPa).

In stage I (Figure 1a COD = 300 mg/L, 1–26 d), the average COD concentration in effluent of L1 was 16.63 mg/L and the average COD concentration at the end of the anaerobic stage was 17.14 mg/L, showing a good COD removal performance. The average amount of internal carbon source stored by PAOs and GAOs in the anaerobic stage COD_{intra} was 95.98 mg/L (Figure 1b) [32], accounting for 91.88% of COD_{ana}. The average COD removal rate was 94.38%. H1 also demonstrated good COD removal performance in stage I. The average COD concentration in effluent and COD concentration at the end of the anaerobic stage were 17.87 mg/L and 20.25 mg/L, respectively (Figure 1c). COD_{intra} accounted for 90.09% of COD_{ana} in the anaerobic stage (Figure 1d), and the average COD removal rate was 94.08%. These results showed that the two SNDPR systems under different atmospheric pressures had effective COD removal in stage I, and most COD was removed at the anaerobic stage.

In stage II (COD = 200 mg/L, 27–53 d), the average COD concentration in the effluent of L1 and the COD concentration at the end of the anaerobic stage were 17.52 mg/L and 20.21 mg/L, respectively; the result still showed high removal efficiency. The average COD removal rate was 91.24%. However, COD_{intra} decreased to 47.65 mg/L, and the proportion of COD_{intra} in COD_{ana} was reduced to 76.08% (Figure 1b). The main reason may be that when the COD in influent concentration decreased, the available carbon sources for PAOs and GAOs were reduced in the anaerobic stage, resulting in the decrease of the amount of internal carbon sources stored in the system. Similar results were also observed in the H1 reactor. The average COD concentration in effluent of H1 was 18.63 mg/L, and the average removal rate was 90.66%; the average COD_{intra} was 41.70 mg/L, and the proportion of COD_{intra} in COD_{ana} was reduced to 77.73%. These results indicate that when decreased COD concentrations in the influent were placed under low atmospheric pressure, the exogenous denitrifying bacteria preferentially used COD to degrade nitrate, and the exogenous carbon source used for internal carbon source storage and P released were reduced [33].

The decrease in COD concentrations of influent under low atmospheric pressure (72 kPa) did not decrease COD removal in the SNDPR system; this result was consistent with the results of Du et al. [34]. When the COD in influent decreased from 300 mg/L to 200 mg/L under 100 kPa, the total inorganic nitrogen (TIN) removal rate of the L1 system dropped from 80.55% to 69.60% (Figure 2). The data indicated that the SND of the system was weakened in the aeration stage. The COD consumed by exogenous denitrification in the anaerobic stage increased from 8.48 mg/L to 14.98 mg/L, accounting for 8.12% and 23.91% of COD_{ana} respectively. With the decrease of COD concentrations in influent, the proportion of COD consumed by exogenous denitrifying bacteria increased and the storage of internal carbon source decreased, which also affected the subsequent TIN removal performance. On the other hand, under low atmospheric pressure (72 kPa), the TIN removal rate of the H1 system in stage I (COD = 300 mg/L) and in stage II (COD = 200 mg/L) was 81.22% and 72.84%, respectively, both higher than that occurring in the L1 system under normal pressure. It may show that, although the DO content from the air into the water in the aerobic aeration stage was reduced, the SND often occurred efficiently under low oxygen pressure [35], thus the TIN removal rate was higher under lower atmospheric pressure. Similarly, under lower atmospheric pressure, the COD consumed by the external denitrification in H1 (COD_{dn}) was 8.36 mg/L and 13.30 mg/L, respectively, corresponding to the COD at 300 mg/L and 200 mg/L, which accounted for 9.39% and 31.89% of COD_{ana}, Compared with that of L1, the amount of COD_{dn} decreased. Such a result may be caused by the decrease of residual nitrate content in the H1 system from the previous stage.



Figure 2. Variations of influent NH_4^+ -N, effluent NH_4^+ -N, NO_2^- -N and NO_3^- -N and TIN removal efficiency under different COD in influent ((**a**): 100kPa, (**b**): 72 kPa).

3.2. Effect of COD on TIN Removal in an SNDPR System under Different Atmospheric Pressures

In stage I (Figure 2), both L1 and H1 systems showed efficient nitrification performance. The average concentrations of NH_4^+ -N, NO_2^- -N and NO_3^- -N in the effluent are 0.49 mg/L, 0.05 mg/L, 5.29 mg/L and 0.54 mg/L, 0.12 mg/L, and 5.13 mg/L, respectively. The average NH_4^+ -N removal rates in L1 and H1 reactors reached 98.41% and 98.24%, respectively.

The average removal TIN rates reached 80.54% and 81.21%, respectively. The average SND rate in L1 was 58.00% and 59.41% in H1. There was no significant difference in NH_4^+ -N concentrations in effluent and removal rate between H1 and L1; both maintained a low level, while the TIN removal rate in H1 was slightly higher than that in L1. He et al. [36] concluded that in a SNDPR system with low COD in influent, the reduction of aeration rate would reduce the DO level in the aerobic stage and maintain a low DO concentration (0.5–1.0mg/L), which would improve the denitrification rate and strengthen simultaneous nitrification and denitrification. In the present study, H1 maintained a low DO concentration in the system during the aeration stage, conducive to TIN removal in the system.

At stage II, the NH_4^+ -N and NO_2^- -N concentrations in effluent of L1 and H1 remained stable, but the NO_3^- -N concentration in effluent increased with the decrease of COD in influent, and the NO_3^- -N concentration of L1 and H1 increased to 8.75 mg/L and 7.78 mg/L, respectively. Shown in Figure 1, the SND rates of L1 and H1 decreased compared with the previous stage I, with an average of 24.52% and 31.95% respectively. The main reason was due to the decrease of COD in influent. The carbon source available in the denitrification process was reduced, resulting in the decline of the denitrification performance of the system. In this stage, the NO_3^- -N concentration in effluent of L1 was higher than that of H1, but the SND rate was lower than that of H1. He et al. [36] found that in an SNDPR system, a higher DO level would cause high NO_3^- -N residue. In this study, the DO level of L1 operating at 100 kPa in the aerobic stage was higher than that of H1 at 72 kPa (Figure 3a,b), resulting in the higher nitrate nitrogen concentration in effluent of L1. Although the high DO level in the aerobic stage guaranteed the oxidation process of ammonia N, it inhibited the denitrification process, leading to the lower SND rate of L1 compared to H1. This result was consistent with the research results by Meyer et al. [37].



Figure 3. Removal of PO_4^{3+} -P in a SNDPR system under different COD concentrations in influent ((a): 100 kPa, (b): 72 kPa).

3.3. Effect of COD Concentration on TP Removal in an SNDPR System under Different Atmospheric Pressures

In stage I, the high COD concentration in influent provided a sufficient carbon source and high activity of PAOs in the system, with the result that the PAOs exhibited good P release and absorption performance. The average TP concentration in effluent of L1 and H1 were less than 0.09 mg/L, and the removal rates were greater than 99% (Figure 3). Beyond this, the TP concentration of L1 at the end of the anaerobic stage was 21.74 mg/L, and it was observed that the phosphorus release amount (PRA) during the anaerobic stage was 16.44 mg/L, lower than the TP concentration (26.12 mg/L) and PRA (21.03 mg/L) of H1 at the end of the anaerobic stage. The same result was also observed in stage II. Chen et al. [24] found that decreasing atmospheric pressure would enhance the activity of PAOs in an SNDPR system in batch test. Our results also showed that P release in reactor H1 operating at 72 kPa was higher due to the enhancement of PAOs activity.

At the beginning of stage II, due to decreased COD concentration in influent, the TP concentrations in L1 and H1 during the anaerobic stage decreased to the lowest concentration of 13.47 mg/L and 6.75 mg/L, respectively. With the continuous operation of the reactors, the P release in L1 and H1 during the anaerobic stage increased to an average of 20.92 mg/L and 27.34 mg/L, respectively. The reason may be that the PAOs in the system gradually adapted to the water quality of this stage and took advantage of carbon source utilization when the COD in influent was limited.

In conclusion, the change of COD concentrations in influent significantly impacted on PO_4^{3+} -P concentration and PRA during the anaerobic stage of the system. At low atmospheric pressure, PAOs activity in the SNDPR system was higher.

3.4. The Pollutant Removal Pathways in a SNDPR System in Typical Cycles under Different Atmospheric Pressures

The concentrations of C, N and P in a typical cycle under 100 kPa are shown in Figure 4a. At the first 20 min of the anaerobic stage, NH_4^+ -N concentration in influent decreased from 30 to 14.37 mg/L in the SNDPR system due to dilution, and the COD concentration rapidly decreased to 32.71 mg/L. The residual $NO_x^- - N$ in the previous stage was utilized by exogenous denitrifying bacteria, resulting in the COD_{dn} consumption of about 16.99 mg/L. Hence, more COD (about 91.56%) in the influent was stored as an internal carbon source by PAOs and GAOs in the form of polyhydroxy fatty acids, which were used for P absorption and SND in the next aerobic stage. During the 60 min of the anaerobic stage, PO_4^{3+} -P concentration rose rapidly to 19.10 mg/L and PRA reached 13.89 mg/L. In the subsequent anaerobic period (60–120 min), PO_4^{3+} -P concentration remained unchanged at around 19.10 mg/L, while COD decreased slightly from 32.71 mg/L to 18.69 mg/L, the results indicating that the decrease of COD at this stage was mainly caused by the participation of GAOs in the reaction of the system. During the first 120 min anaerobic stage, NH_4^+ -N concentration was slowly nitrated from 15.80 mg/L to 1.00 mg/L, while the accumulation of NO_x^- -N in the system was 6.91 mg/L, less than the content of oxidized NH₄⁺-N, resulting in the loss of total inorganic N of 5.69 mg/L. The results indicated that 46.69% of SND occurred in the aerobic stage. In the subsequent aerobic stage (120–210 min), the DO content in the system increased rapidly from 0.54 mg/L to 6.47 mg/L, and the concentrations of main pollutants COD, PO₄³⁺-P and NH₄⁺-N changed little.



Figure 4. Variations of N, P and organic carbon in cyclic study in stage II (COD = 200 mg/L) ((a): 100 kPa, (b): 72 kPa).

The concentration changes of C, N and P elements in a cyclic test at 72 kPa are shown in Figure 4b. In the first 20 min of the anaerobic stage, COD content decreased rapidly and finally fluctuated around 23.65 mg/L, similar to the system at 100 kPa, in which the concentration of PO_4^{3+} -P increased from 5.72 mg/L to 28.13 mg/L. The P release was higher than that of the system at 100 kPa and the PRA content was 22.34 mg/L, which was also higher than that of the system at 100 kPa. These results indicated that the PAOs activity in the SNDPR system was enhanced under low atmospheric pressure. Compared with GAOs, PAOs preferentially utilized COD in influent and released intracellular phosphate for metabolism. This result also supported the results of Chen et al. [24]. During the first 120 min anaerobic stage, the concentration of PO_4^{3+} -P decreased rapidly from 28.58 mg/L to 0 mg/L, and the removal rate was as high as 100%. Moreover, the TIN concentration decreased from 13.05 mg/L to 9.88 mg/L, indicating that denitrifying phosphorus accumulating organisms may participate in the reaction of N and P removal. According to Wang et al. [38], under low oxygen conditions (0.5-1.0 mg/L), denitrifying phosphorus accumulating organisms often adopted NO_x^- -N as the electron acceptor for P absorption reaction, so the N and P elements in the system could be removed cooperatively; this result was also consistent with the present results. In the aerobic stage, the ammonia oxidation rate (AOR) was 0.06 mg N/L/min, lower than the AOR (0.08 mg N/L/min) at 100 kPa. This indicated that the ammonia oxidation process was inhibited under low atmospheric pressure, thus affecting the activity of ammonia oxidizing bacteria. In addition, the concentration of main pollutants in the H1 system did not change at the end of the aerobic stage, and the DO level increased to 5.16 mg/L, lower than that in L1 at below 100 kPa (6.47 mg/L). This indicated that the efficiency of oxygen transferring from air to water was lower at low atmospheric pressure.

4. Conclusions

(1) H1 reactor showed efficient COD and TP removal performance at 72 kPa when the COD in influent was decreased from 300 mg/L to 200 mg/L. The average effluent COD and TP concentrations of the system at 53 days were 18.26 mg/L and 0.06 mg/L, respectively, similar to those of L1 operating at 100 kPa (COD: 17.08 mg/L; TP: 0.18 mg/L).

- (2) The TIN removal rate in the H1 reactor decreased from 81.21% to 72.86%, and the SND rate decreased from 59.48% to 31.95% when the COD in influent was changed from 300 mg/L to 200 mg/L. Compared with L1 operating at 100 kPa, the TIN removal rate of the SNDPR system increased in stage I (COD = 300 mg/L) and stage II (COD = 200 mg/L).
- (3) PRA in the H1 reactor at 72 kPa was higher than that in the L1 reactor; this indicated that the PAOs activity was higher at low atmospheric pressure. However, in the aerobic stage, the AOR in H1 reactor was 0.06 mg N/L/min, lower than that of L1 at 100 kPa, indicating that the ammonia oxidation process in SNDPR system was inhibited at low atmospheric pressure.

These results showed that the SNDPR system exhibited efficient COD and TP removal performance under low atmospheric pressure. Compared with the reactor operating under normal pressure, the TIN removal rate was improved, although the AOR of the system was reduced. The lower COD in influent under low atmospheric pressure did not affect COD and TP removal efficiency. In conclusion, the SNDPR system was a process suitable for domestic sewage treatment in high-altitude areas.

Author Contributions: L.S., G.Z. and Y.Z. conceived and designed the experiments; Y.Z. performed the experiment; L.Z., M.Z. and D.R. partially performed the experiments; Y.Z. and L.S. analyzed the data; L.Z., J.W. and S.L. partly analyzed the data; G.Z. revised the manuscript; Y.Z. and L.S. wrote the paper version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Xizang Natural Science Foundation Project of the Xizang Science and Technology Department (XZ 2018 ZR G-89(Z)); Xizang key Research and Development Project (XZ202001ZY0052G).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be obtained by contacting the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Cuo, L.; Zhang, Y. Spatial patterns of wet season precipitation vertical gradients on the Tibetan Plateau and the surroundings. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
- Zhou, H.; Li, X.; Xu, G.; Yu, H. Overview of strategies for enhanced treatment of municipal/domestic wastewater at low temperature. *Sci. Total. Environ.* 2018, 643, 225–237. [CrossRef] [PubMed]
- 3. Nur, S.; Johan, S.; Khalidaet Mika, S.; Norelyza, H. Effect of magnetic field on biomass properties and their role in biodegradation under condition of low dissolved oxygen. *Appl. Water Sci.* 2021, *11*, 1–12. [CrossRef]
- 4. Zhang, P.; Li, Y.; Wang, L. Brief discussion on existing problems and prospects of plateau wastewater treatment plants in China. *Environ. Eng.* **2019**, *37*, 82–86, 91. [CrossRef]
- Kim, T.; Hite, M.; Rogacki, L.; Sealock, A.W.; Sprouse, G.; Novak, P.J.; LaPara, T.M. Dissolved oxygen concentrations affect the function but not the relative abundance of nitrifying bacterial populations in full-scale municipal wastewater treatment bioreactors during cold weather. *Sci. Total. Environ.* 2021, 781, 146719. [CrossRef] [PubMed]
- 6. Zeng, R.J.; Lemaire, R.; Yuan, Z.; Keller, J. Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. *Biotechnol. Bioeng.* 2003, *84*, 170–178. [CrossRef]
- He, Q.; Wang, H.; Chen, L.; Gao, S.; Zhang, W.; Song, J.; Yu, J. Elevated salinity deteriorated enhanced biological phosphorus removal in an aerobic granular sludge sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. *J. Hazard. Mater.* 2019, 390, 121782. [CrossRef]
- 8. He, Q.; Song, J.; Zhang, W.; Gao, S.; Wang, H.; Yu, J. Enhanced simultaneous nitrification, denitrification and phosphorus removal through mixed carbon source by aerobic granular sludge. *J. Hazard. Mater.* **2019**, *382*, 121043. [CrossRef]
- Iannacone, F.; Di Capua, F.; Granata, F.; Gargano, R.; Esposito, G. Simultaneous nitrification, denitrification and phosphorus removal in a continuous-flow moving bed biofilm reactor alternating microaerobic and aerobic conditions. *Bioresour. Technol.* 2020, 310, 123453. [CrossRef]

- Iannacone, F.; Di Capua, F.; Granata, F.; Gargano, R.; Pirozzi, F.; Esposito, G. Effect of carbon-to-nitrogen ratio on simultaneous nitrification denitrification and phosphorus removal in a microaerobic moving bed biofilm reactor. *J. Environ. Manag.* 2019, 250, 109518. [CrossRef]
- 11. Yang, S.; Yang, F.; Fu, Z.; Wang, T.; Lei, R. Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment. *J. Hazard. Mater.* **2010**, *175*, 551–557. [CrossRef]
- 12. Wang, Y.; Peng, Y.; Stephenson, T. Effect of influent nutrient ratios and hydraulic retention time (HRT) on simultaneous phosphorus and nitrogen removal in a two-sludge sequencing batch reactor process. *Bioresour. Technol.* **2009**, *100*, 3506–3512. [CrossRef] [PubMed]
- 13. Zaman, M.; Kim, M.; Nakhla, G. Simultaneous nitrification-denitrifying phosphorus removal (SNDPR) at low DO for treating carbon-limited municipal wastewater. *Sci. Total. Environ.* **2020**, *760*, 143387. [CrossRef] [PubMed]
- Liu, G.; Wang, J. Long-Term Low DO Enriches and Shifts Nitrifier Community in Activated Sludge. *Environ. Sci. Technol.* 2013, 47, 5109–5117. [CrossRef] [PubMed]
- Pamela, Y.; Brian, R.; Joseph, M.; Juan, M.; Vikram, K.; Jorge, S.; Katherine, D.; Daniel, R. Candidatus Accumulibacter phosphatis clades enriched under cyclic anaerobic and microaerobic conditions simultaneously use different electron acceptors. *Water Res.* 2016, 102, 125–137. [CrossRef]
- 16. Coma, M.; Puig, S.; Balaguer, M.; Colprim, J. The role of nitrate and nitrite in a granular sludge process treating low-strength wastewater. *Chem. Eng. J.* **2010**, *164*, 1208–1213. [CrossRef]
- 17. Zahmatkesh, S.; Pirouzi, A. Effects of the microalgae, sludge and activated carbon on the wastewater treatment with low organics (weak wastewater). *Int. J. Environ. Sci. Technol.* **2020**, 1–8. [CrossRef]
- 18. Gao, F.; Nan, J.; Li, S.; Wang, Y. Modeling and simulation of a biological process for treating different COD:N ratio wastewater using an extended ASM1 model. *Chem. Eng. J.* **2018**, 332, 671–681. [CrossRef]
- 19. Münch, E.V.; Lant, P.; Keller, J. Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. *Water Res.* **1996**, *30*, 277–284. [CrossRef]
- 20. Pochana, K.; Keller, J.; Lant, P. Model development for simultaneous nitrification and denitrification. *Water Sci. Technol.* **1999**, *39*, 235–243. [CrossRef]
- 21. Han, M.; Wang, X. SBMBBR reinforced high load of organic nitrogen and phosphorus removal in municipal wastewater in-quiry. *Water Treat.* **2019**, *45*, 127–131, 138. (In Chinese) [CrossRef]
- 22. Zhou, J.; Sun, Q. Performance and microbial characterization of aerobic granular sludge in a sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal with varying C/N ratios. *Bioprocess Biosyst. Eng.* **2019**, *43*, 663–672. [CrossRef]
- 23. Fang, D.; Zhao, G.; Xu, X.; Zhang, Q.; Shen, Q.; Fang, Z.; Huang, L.; Ji, F. Microbial community structures and functions of wastewater treatment systems in plateau and cold regions. *Bioresour. Technol.* **2018**, 249, 684–693. [CrossRef]
- 24. Chen, Y.; Li, S.; Lu, Y.; Zhu, G.; Cheng, H. Simultaneous nitrification, denitrification and phosphorus removal (SNDPR) at low atmosphere pressure. *Biochem. Eng. J.* 2020, *160*, 107629. [CrossRef]
- 25. Hu, B.; Wang, Y.; Quan, J.; Huang, K.; Gu, X.; Zhu, J.; Yan, Y.; Wu, P.; Yang, L.; Zhao, J. Effects of static magnetic field on the performances of anoxic/oxic sequencing batch reactor. *Bioresour. Technol.* **2020**, *309*, 123299. [CrossRef] [PubMed]
- The Korean Society of Civil Engineers. Standard Methods for the Examination of Water and Wastewater. J. Korean Soc. Civ. Eng. 2012, 60, 127.
- 27. Xie, S.; Zhao, J.; Zhang, Q.; Zhao, J.; Lei, S.; Ma, X.; Yan, C. Improvement of the performance of simultaneous nitrification denitrification and phosphorus removal (SNDPR) system by nitrite stress. *Sci. Total. Environ.* **2021**, *788*, 147825. [CrossRef]
- 28. Lo, I.W.; Lo, K.V.; Mavinic, D.S.; Shiskowski, D.; Ramey, W. Contributions of biofilm and suspended sludge to nitrogen transformation and nitrous oxide emission in hybrid sequencing batch system. *J. Environ. Sci.* 2010, 22, 953–960. [CrossRef]
- 29. Guerrero, J.; Guisasola, A.; Baeza, J.A. Controlled crude glycerol dosage to prevent EBPR failures in C/N/P removal WWTPs. *Chem. Eng. J.* 2015, 271, 114–127. [CrossRef]
- Wang, X.; Wang, S.; Xue, T.; Li, B.; Dai, X.; Peng, Y. Treating low carbon/nitrogen (C/N) wastewater in simultaneous nitrificationendogenous denitrification and phosphorous removal (SNDPR) systems by strengthening anaerobic intracellular carbon storage. *Water Res.* 2015, 77, 191–200. [CrossRef]
- 31. Wang, X.; Zhao, J.; Yu, D.; Chen, G.; Du, S.; Zhen, J.; Yuan, M. Stable nitrite accumulation and phosphorous removal from nitrate and municipal wastewaters in a combined process of endogenous partial denitrification and denitrifying phosphorus removal (EPDPR). *Chem. Eng. J.* **2018**, *355*, 560–571. [CrossRef]
- Dai, X.; Peng, Y.; Wang, X.; Wang, S. Effect of different anaerobic time on the nutrient removal in simultaneous nitrifica-tiondenitrification and phosphorus removal (SNDPR) systems enriched with phosphorus accumulating organisms. *Chin. Environ. Sci.* 2016, *36*, 92–99. (In Chinese)
- 33. Du, Y.; Yu, D.; Zhen, J.Y.; Wang, X.; Chen, G.; Tang, P.; Wang, J.; Bi, C.; Gong, X.; Huang, S.; et al. Effect of Influent C/N Ratio on the Nutrient Removal Characteristics of SNEDPR Systems. *Env. Sci.* **2019**, *40*, 816–822. (In Chinese) [CrossRef]
- 34. Li, H.; Gao, D. Cultivate of granular sludge based on denitrifying phosphate accumulating bacteria. *Chin. Environ. Sci.* **2014**, *34*, 383–389. (In Chinese) [CrossRef]
- 35. Li, C.; Liu, S.; Ma, T.; Zheng, M.; Ni, J. Simultaneous nitrification, denitrification and phosphorus removal in a sequencing batch reactor (SBR) under low temperature. *Chemosphere* **2019**, 229, 132–141. [CrossRef] [PubMed]

- He, Q.; Zhang, W.; Zhang, S.; Wang, H. Enhanced nitrogen removal in an aerobic granular sequencing batch reactor performing simultaneous nitrification, endogenous denitrification and phosphorus removal with low superficial gas velocity. *Chem. Eng. J.* 2017, 326, 1223–1231. [CrossRef]
- Meyer, R.L.; Zeng, R.J.; Giugliano, V.; Blackall, L.L. Challenges for simultaneous nitrification, denitrification, and phosphorus removal in microbial aggregates: Mass transfer limitation and nitrous oxide production. *FEMS Microbiol. Ecol.* 2005, 52, 329–338.
 [CrossRef]
- 38. Wang, X.; Wang, S.; Zhao, J.; Dai, X.; Peng, Y. Combining simultaneous nitrification-endogenous denitrification and phosphorus removal with post-denitrification for low carbon/nitrogen wastewater treatment. *Bioresour. Technol.* 2016, 220, 17–25. [CrossRef]