processes

MDPI

Article

# Scaling-Up and Long-Term Operation of a Full-Scale Two-Stage Partial Nitritation-Anammox System Treating Landfill Leachate

**Albert Magrí [1,*,†], Maël Ruscalleda [1,2,†], Albert Vilà [1], Tiago R. V. Akaboci [1,3], M. Dolors Balaguer [1], Josep M. Llenas [4,5] and Jesús Colprim [1]**

[1] Laboratory of Chemical and Environmental Engineering (LEQUIA), Institute of the Environment, University of Girona, Campus Montilivi, Carrer Maria Aurèlia Capmany 69, E-17003 Girona, Catalonia, Spain; mael.ruscalleda@createch360.com (M.R.); albert.vila@lequia.udg.cat (A.V.); ea.tiagovitor@gmail.com (T.R.V.A.); dolors.balaguer@udg.edu (M.D.B.); jesus.colprim@udg.edu (J.C.)
[2] Createch360°, Costa d'en Paratge 22, E-08500 Vic, Catalonia, Spain
[3] Department of Civil Engineering, Santa Catarina State University, Rua Getúlio Vargas 2822, Ibirama CEP 89140-000, Santa Catarina, Brazil
[4] CORSA Landfill Site, Ferrovial Servicios, Camí Vell de Riudoms a Vilaseca 65, E-43205 Reus, Catalonia, Spain; josepmaria.llenas@ferrovial.com or ingenieriaeinnovacion.medioambiente@ferrovial.com
[5] Departament of Innovation, Area of Engineering and Strategic Projects, Ferrovial Servicios, Paseo de la Zona Franca 111, Planta 19-20, E-08038 Barcelona, Catalonia, Spain
* Correspondence: albert.magri@udg.edu or albert.magri@gmail.com; Tel.: +34-972-41-95-42
† Both authors contributed equally to this manuscript.



**Citation:** Magrí, A.; Ruscalleda, M.; Vilà, A.; Akaboci, T.R.V.; Balaguer, M.D.; Llenas, J.M.; Colprim, J. Scaling-Up and Long-Term Operation of a Full-Scale Two-Stage Partial Nitritation-Anammox System Treating Landfill Leachate. *Processes* **2021**, *9*, 800. https://doi.org/10.3390/pr9050800

Academic Editor: George Z. Kyzas

Received: 8 April 2021
Accepted: 28 April 2021
Published: 1 May 2021



**Abstract:** (1) Background: Biological treatment of leachate in landfill sites using anaerobic ammonium oxidation (anammox) is challenging because of the intrinsic characteristics of this complex wastewater. In this work, the scale-up and subsequent full-scale implementation of the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain) are presented as a case study to achieve long-term nitrogen (N) removal from mature leachate mostly through a completely autotrophic pathway. (2) Methods: The treatment system consists of two sequencing batch reactors (SBRs) running in series to individually operate partial nitritation (PN) and anammox (A). Following biological treatment, physicochemical oxidation (i.e., Fenton-based process) was used to remove the remaining non-biodegradable organic matter. A cost analysis comparative was conducted in relation to the former technology used on-site for treating the leachate. (3) Results: The scale-up of the process from pilot- to full-scale was successfully achieved, finally reaching an average removal of 7.4 kg N/d. The composition of the leachate changed over time, but especially once the landfill site stopped receiving solid waste (this fact involved a marked increase in the strength of the leachate). The adjustment of the alkalinity-to-ammonium ratio before feeding PN-SBR helped to improve the N-removal efficiency. Values of conductivity above 25 mS/cm in A-SBR could negatively affect the performance of the anammox process, making it necessary to consider a dilution strategy according to the on-line monitoring of this parameter. The analysis of the operational costs showed that by implementing the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain) in the landfill site, savings up to 32% were achievable. (4) Conclusions: Treatment of mature landfill leachate in such a two-stage PN-A system was demonstrated as feasible and economically appealing despite the complexity of this industrial wastewater. Accurate expert supervision of the process was a key factor to reaching good performances.

**Keywords:** anammox; nitrogen removal; landfill leachate; industrial wastewater treatment; sequencing batch reactor; pilot-scale; full-scale; cost analysis

## 1. Introduction

Sanitary landfilling is one of the most commonly used methods for the disposal of municipal solid waste. In landfills, the release of inherent water from the disposed materials, the occurring biochemical transformation processes, and the rainwater percolating through the waste layers, taken together, lead to the formation of leachates which may contain large amounts of organic matter—partly biodegradable, but also refractory to biodegradation—including ammonium, metals, chlorinated organics, and inorganic salts [1]. Typically, as the age of the landfill increases, the leachate formed is characterized by a lower bCOD-to-N ratio (bCOD: biodegradable chemical oxygen demand, N: nitrogen) [2]. To prevent negative impacts on the environment, these hazardous streams must be treated properly, in accordance with the current regulatory standards, before being discharged into water bodies. Multiple processes can be applied when treating landfill leachate [3], often consisting of physicochemical methods [4,5]. However, such techniques are mostly associated with a large demand of chemicals and the production of concentrated rejection streams that will need further specific management and treatment.

Completely autotrophic anammox-based processes (anammox: anaerobic ammonium oxidation), nowadays mostly applied to side-streams resulting from dewatering anaerobically digested sludge in wastewater treatment plants (WWTPs) [6], represent a cost-effective way of removing the N contained in landfill leachates [7,8]. The main advantages of this alternative in comparison with conventional biological N removal are the lower energy demand for aeration, no need for an organic carbon source, and the reduction in the amount of sludge produced [9,10]. Yet, treatment of such a complex and highly loaded wastewater with the anammox process is challenging because of the inherent local- and time-dependent compositional variability. Changes in the availability of organic carbon [11], N substrates [12], toxic substances [13] as well as conductivity [14], are expected to affect biological activity. Even though the unplanned occurrence of the anammox reaction in leachate treatment plants applying biological methods has been reported previously [15–17], only a small number of experiences at the full-scale, according to new or retrofitted setups, have been described in the scientific literature achieving stable and robust performance in the long-term [18,19].

At the University of Girona, LEQUIA research group effectively combined partial nitritation (PN) and anammox processes (PN-A) for treating highly N-loaded landfill leachates (up to 6 kg $N/m^3$) on the basis of coupling two independent sequencing batch reactors (SBRs) operated in series. In the early works, aerobic ammonium oxidation to nitrite was shown to be strongly dependent on the pH value measured in the bulk of PN-SBR. The pH determines the ionization factor for these two N-compounds (TAN: $NH_4^+ + NH_3$; TNN: $NO_2^- + HNO_2$), and consequently, the availability of the unionized forms (i.e., $NH_3$ and $HNO_2$, respectively) as suppressors of the activity of the nitrite-oxidizing bacteria (NOB), but also as potential limiting agents of the activity of the ammonium-oxidizing bacteria (AOB) [20,21]. The availability of inorganic carbon (TIC: $H_2CO_3^* + HCO_3^- + CO_3^{2-}$) was proved as a key factor controlling the nitrite-to-ammonium ratio (TNN/TAN) of the resulting effluent [22,23]. Moreover, the interactions between aeration, carbon dioxide stripping, alkalinity, pH, and nitrification kinetics were assessed numerically using mathematical modelling [24]. Partial denitrification of the nitrite formed in PN-SBR was studied on-site by stopping aeration during the leachate feeding events but at the risk of increasing the amount of nitrous oxide ($N_2O$) emitted [25–27]. Concerning the anammox reactor (A-SBR), it was shown that both anammox and heterotrophic denitrification could coexist while concomitantly contributing to the removal of N as nitrogen gas ($N_2$) [28,29]. The exposure to high nitrite concentrations resulted in a partially reversible inhibition of the anammox bacteria (AnAOB) activity [30]. Additional downstream treatment after the PN-A system using advanced oxidation processes (AOPs) allowed removing refractory non-bCOD [31]. The know-how gained in such researches by operating bench-scale reactors lead to the development and scaling-up of the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain), which was first tested at the pilot-scale, and finally implemented at the full-scale in a landfill site for treating the leachate produced there. The aim of this paper is to describe for the first time such a successful case study.

## 2. Materials and Methods

### 2.1. Description of the PANAMMOX® Technology

The PANAMMOX® technology was developed by the LEQUIA research group in view of treating highly N-loaded mature landfill leachate under high conductivity levels. Based on the experience gained in the laboratory using bench-scale systems, the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain) (Figure 1) was first prototyped at the pilot-scale for demonstrative purposes and finally implemented at the full-scale in the CORSA landfill site according to a two-stage PN-A layout. Both reactors constituting the system (i.e., PN-SBR and A-SBR) are equipped with probes for the on-line monitoring of the pH, dissolved oxygen (DO), redox potential (ORP), temperature, and water level. In view of reaching an appropriate PN of the raw leachate in PN-SBR, the alkalinity-to-ammonium (ALK/TAN) molar ratio of the leachate is expected to be adjusted in the pre-treatment tank by adding bicarbonate or acid, thus regulating TIC availability before aeration. The effluent from PN-SBR is stored in a buffer tank before feeding A-SBR. This buffer tank also allows for the settling of most of the suspended solids eventually contained in the effluent from PN-SBR. The routinary purge of this tank is needed to prevent an undesired transference of solids to A-SBR.

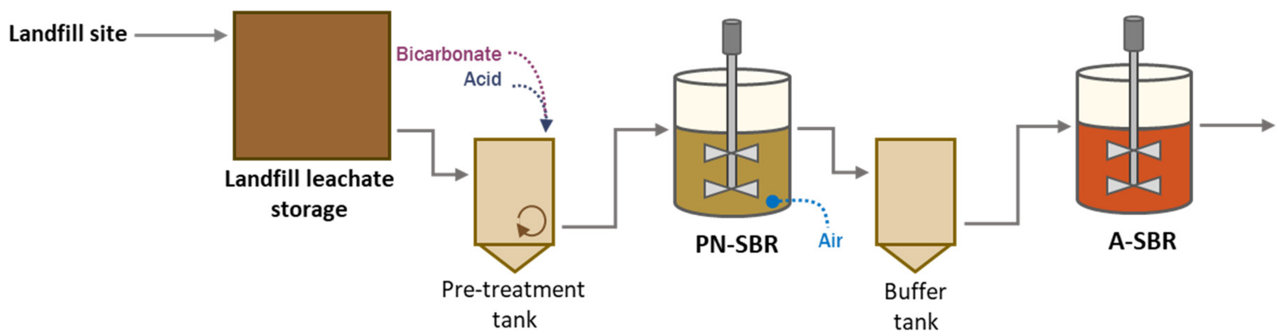



**Figure 1.** Descriptive scheme for the PANAMMOX® technology.

### 2.2. The Pilot Plant

Both SBRs constituting the pilot plant had an individual total volume of 250 L. The PN-SBR ran at 25 °C and the A-SBR ran at 35 °C. Before biological treatment, in the pre-treatment tank, the ALK/TAN molar ratio of the leachate was adjusted to a nominal value of 1.14 according to the anammox stoichiometry given by Strous et al. [32]; i.e., this is equivalent to 57% of the theoretical alkalinity requirements for achieving complete nitritation. The PN-SBR was operated by considering a step feed strategy and intermittent aeration, allowing heterotrophic denitrification [21,27]. The DO concentration during the oxic periods was controlled to a set-point value (2 mg/L). Previously, this reactor had been processing leachate from different landfill sites for a long time in view of feeding different bench-scale anammox reactors (i.e., adapted nitrifying sludge was already available). The A-SBR was inoculated with granular sludge from a bench-scale anammox reactor treating synthetic wastewater. The strategy applied for promoting the growth of anammox sludge adapted to the characteristics of the leachate consisted in start feeding the reactor with synthetic wastewater, and subsequently, supplying nitritated leachate in the inlet at an increasing blending ratio until feeding it undiluted. In this case, feeding and mixing were provided under the absence of aeration. The length of the feeding events was adjusted according to the N-loading rate (NLR) applied. After biological treatment, the photo-Fenton process was assessed in a 1-L reactor equipped with a UV lamp [31]. The data analyzed concerning the operation of the pilot-scale plant falls within the period from July 2012 to December 2014.

*2.3. The Industrial Plant at the CORSA Landfill Site*

The PANAMMOX® technology was subsequently implemented at the full-scale (Figure 2) in the CORSA landfill site (Mas Calbó, Reus, Spain). When it was operative, the landfill site was receiving about 7500 t/mo. of municipal equivalent solid waste. The annual rainfall in the geographical area where the landfill is located is around 500 mm. The treatment plant was designed for processing a maximum leachate flow rate of ca. 20 $m^3$/d. In the pre-treatment tank, the ALK/TAN molar ratio is adjusted by adding sodium bicarbonate ($NaHCO_3$) when it is measured below the desired value, or by adding sulfuric acid ($H_2SO_4$) when the ratio reaches higher values. The total volume of the reactors is 27 $m^3$ for PN-SBR and 40 $m^3$ for A-SBR (i.e., for safety reasons, this reactor was oversized in relation to the PN-SBR). The reaction temperature is monitored but usually not controlled if it is above 20 °C (i.e., the reaction temperature must fall within the range 20 to 35 °C; when the temperature is below 20 °C, an electric resistance heating system is activated according to a given set-point). In PN-SBR, the air supply blower is controlled through a set-point value for the concentration of DO in the range 1.5 to 3.5 mg/L (e.g., 2 mg/L). A set-point value is also considered for the pH (eligible values fall within the range 6 to 8); i.e., the filling pump stops working if the pH value is above the set-point (e.g., pH 7.9), which makes the dynamic autoregulation of the load applied while preventing biomass inhibition due to the accumulation of unionized $NH_3$ feasible. Additional details concerning the description and operation of the treatment plant and SBRs working cycle configuration are provided as Supplementary Materials.


**Figure 2.** Views of the PANAMMOX® system running at the CORSA landfill site.

The landfill site is managed by the company Ferrovial Servicios (Madrid, Spain). Before implementing the biological system, the leachate had been treated by combining an AOP (Fenton) with a $NH_3$ stripping–scrubbing unit. Once the PN-A plant started running regularly, the application of the Fenton process was revised, becoming used for the downstream degradation of the refractory non-bCOD. This landfill site stopped receiving solid waste by December 2018, after 27 years of uninterrupted activity. The data analyzed regarding the performance of the full-scale treatment plant concern two different periods: (i) start-up (from April 2014 to December 2014) and (ii) long-term operation (from September 2017 to March 2020).

### *2.4. Characteristics of the Synthetic Wastewater*

The synthetic wastewater used in the pilot plant was prepared according to López et al. [32]. The composition of this mineral media was $NaHCO_3$ (1.05 g/L), $KH_2PO_4$ (0.00625 g/L), $CaCl_2 \cdot 2H_2O$ (0.3 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $FeSO_4 \cdot 7H_2O$ (0.0125 g/L), EDTA·$2H_2O$ (0.0125 g/L), HCl 1 M (1.25 mL/L) and trace element solution (1.25 mL/L). The trace element solution was prepared following van de Graaf et al. [33]. Ammonium, nitrite and nitrate were added as $NH_4Cl$, $NaNO_2$ and $NaNO_3$, and their concentration was adjusted according to the performance of A-SBR.

### *2.5. Characteristics of the Leachate*

During the development of the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain), the leachate generated in the CORSA landfill site was first used as feeding in the pilot plant, and later treated in-situ. Table 1 summarizes the composition of the raw leachate treated throughout the study. For the pilot plant, raw leachate was regularly transported by truck in 1-$m^3$ tanks to the LEQUIA facilities. The main characteristics of the leachate could vary significantly over time. At the full-scale, a noticeable increase in the strength of the leachate was detected once the landfill site stopped receiving solid waste. Yet, the full-scale treatment plant is still planned to work in the next years.

**Table 1.** Main characteristics of the landfill leachate treated in the pilot- and full-scale implementations [1].

| Parameter | Pilot-Scale (Active Landfill) | Full-Scale (Active Landfill) | Full-Scale (Closured Landfill) |
|---|---|---|---|
| pH | 8.4 (0.4) | 7.3 (0.3) | 7.9 (0.4) |
| Conductivity (mS/cm) | 26.4 (7.6) | - | 44.7 (4.1) |
| ALK (kg $CaCO_3/m^3$) | 7.1 (2.6) | 6.9 (1.6) | 9.4 (2.1) |
| COD (kg $O_2/m^3$) | 3.5 (1.2) | 2.7 (0.9) | 4.5 (1.4) |
| TAN (kg $N/m^3$) | 2.1 (0.8) | 1.5 (0.4) | 2.4 (0.5) |
| ALK/TAN (molar ratio) | 1.2 (0.4) | 1.3 (0.2) | 1.1 (0.3) |

[1] Values are means (standard deviation in brackets). Abbreviations: ALK, alkalinity; COD, chemical oxygen demand; TAN, total ammonium nitrogen.

### *2.6. Analytical Methods*

Samples were mostly analyzed following the *Standard Methods for the Examination of Water and Wastewater* [34]. In the laboratory, pH measurements were carried out electrometrically using a bench pH meter, and conductivity measurements were carried out using a conductivity meter. Alkalinity (ALK) was determined by acid titration to an endpoint pH of 4.5 (reported as $CaCO_3$). Mixed liquor suspended solids (MLSS) were determined gravimetrically after sample filtration and drying to constant weight at 105 °C, and mixed liquor volatile suspended solids (MLVSS) were determined after further ignition in a muffle furnace at 550 °C. Total COD was determined through the dichromate method using a spectrophotometer, and bCOD was calculated after conducting the five-day biochemical oxygen demand ($BOD_5$) incubation test. TAN in leachate was analyzed using the distillation method, whereas in more diluted samples, it was analyzed using ion chromatography (IC) and spectrophotometry. Similarly, TNN and nitrate ($NO_3^-$) were both analyzed using IC and spectrophotometry. The microbial community structure was analyzed by high-throughput sequencing using the Illumina MiSeq platform as detailed elsewhere [35].

## 3. Results and Discussion

### 3.1. The PANAMMOX® Pilot Plant

#### 3.1.1. PN-A Process Performance

The availability in the lab of nitrifying sludge well-adapted to a complex matrix such as the landfill leachate made it possible that the PN process could easily be operated from the beginning. Previous details regarding the enrichment of the nitrifying sludge using activated sludge from an urban WWTP can be found in Gabarró et al. [21]. The pilot reactor was directly fed with raw leachate producing a suitable effluent for the downstream A-SBR. The NLR applied in a 500-d period averaged 0.6 kg N/($m^3$·d) and nitrite was produced at a rate of ca. 0.35 kg N/($m^3$·d) (Figure 3). The TNN/TAN ratio of the effluent averaged 1.22, well-approaching the targeted value of 1.32 mol TNN per mol TAN [36]. No nitrate was produced after such a long-term operation, owing to the successful suppression of the NOB activity, allowing for a high N-removal efficiency in A-SBR. High bCOD removal efficiency was also achieved in PN-SBR (ca. 95% of the total bCOD; bCOD in the leachate was ca. 25% of the total COD). Such bCOD removal helped in preventing uncontrolled heterotrophic denitrification in A-SBR. By the end of the experimental period, PN-SBR was coupled with A-SBR.

The pilot A-SBR was operated for 900 days, encompassing two main running phases—*PHASE I*: start-up and sludge enrichment with synthetic wastewater (478 days; Figure 4a) and *PHASE II*: sludge adaptation to landfill leachate and routinary operation (422 days; Figure 4b). In *PHASE I*, synthetic wastewater was used for feeding the reactor (the concentration of the N species was progressively increased). The A-SBR was initially seeded with a low amount of anammox sludge, which was available from a bench reactor also fed with synthetic wastewater (i.e., the pilot reactor started running with an MLVSS content as low as 0.02 kg/$m^3$). The reactor was not perfectly sealed and no intensive strategy for preventing the presence of DO in the bulk liquid was implemented, neither in the inlet nor inside the reactor (i.e., liquid bubbling or headspace flushing with $N_2$ gas was not considered), mimicking the conditions in which the full-scale reactor would be started up. Initially, the occurrence of microaerophilic conditions within the reactor, together with the low amount of sludge added, led to stoichiometric ratios far from those values typically expected for the anammox reaction. Later on (from day 150 onwards), the AnAOB activity became prevalent, outcompeting the nitrifying bacteria in an exponential growth period in which the MLVSS content increased rapidly up to a value of ca. 3 kg/$m^3$; the MLVSS content was equivalent to about 85 wt% of the total MLSS. The non-ideal conditions applied for the start-up of A-SBR (e.g., inoculation of the bioreactor with a small amount of sludge enriched in anammox cells, changes in the working conditions linked to bioreactor design and operation, anaerobic conditions not guaranteed) resulted in a long lag phase. In this initial period, the anammox activity rose slowly because of the slow net growth rate of the anammox cells (low MLVSS contents were experimentally measured inside the reactor—i.e., below 0.03 kg/$m^3$). It is speculated that the achievement of effective solids retention, the evolution of the microbial community towards a more complex structure (including nitrifiers, certain heterotrophs, etc.), and even an eventual acclimation of the anammox cells to the operational conditions could led to the exponential growth of the anammox sludge finally observed.

As the NLR was increased in accordance with the N removal rate (NRR) measured—maximum values above 1 kg N/($m^3$·d) were reached—the conductivity of the influent was also progressively increased by adding marine salt—from 17 mS/cm (day 269) to 50 mS/cm (day 330)—to test the effect of conductivity on biological activity. A new increase in conductivity usually induced a transient decrease in the anammox activity. Overall, high conductivity values (below 40 mS/cm) were well-tolerated by the system. It is known that the effect of salinity (and conductivity) on AnAOB is dependent on factors such as the type of salts that are present, dominant AnAOB species, exposure pattern, or process temperature, making data from different sources hardly comparable [14]. Once arrived at this point, the enrichment of the anammox sludge was considered accomplished and the excess of sludge began to be purged to maintain the MLSS content at a stable value (i.e., 3 to 4 kg MLVSS/$m^3$ corresponding to about 80 wt% of the MLSS). On day 375, the concentration of the N species in the synthetic wastewater used for feeding the reactor was adjusted to match the characteristics of the effluent produced in the PN-SBR. Preliminary tests in A-SBR were also started using diluted nitritated leachate.

In *PHASE II*, the A-SBR was fed with an influent generated by blending synthetic wastewater with partially nitritated leachate at an increasing rate since the 100% of the wastewater fed to the reactor was PN-effluent—i.e., the relative amount of leachate supplied increased from 25% to 100% in approximately 150 days (*PHASE II-a* and *II-b*). Subsequently, PN-SBR and A-SBR were operated in series (day 630), achieving maximum NRR values of ca. 0.6 kg N/($m^3$·d), later on (day 675)—following a conservative risk prevention criterion—diminished to ca. 0.2 kg N/($m^3$·d) according to a decrease in the inflow rate supplied. A stable performance was reached despite potential perturbations occurring in the composition of the raw leachate. The results obtained proved that the two-stage PN-A system could successfully be used for treating mature landfill leachate, overcoming challenges such as starting up with little amount of anammox sludge, the potential impact on the process of the high conductivity levels, and direct coupling PN-SBR + A-SBR. The next step was to transfer 85% of the MLSS from the pilot A-SBR to the full-scale A-SBR (day 806). In the following months, the pilot A-SBR continued to be operated, now with a low MLVSS content (ca. 0.4 kg/$m^3$), to discard eventual, previously non-reported impacts of the leachate on the biological activity in the long-term, and as a reservoir of anammox sludge adapted to the leachate matrix. High N removal efficiencies (88% as mean value) were reached in this last sub-phase. The specific anammox activity was periodically determined in short-term tests based on manometric measurements [30], obtaining maximum values with leachate (*PHASE II-c*) of ca. 0.1 g $N_2$-N/($g_{MLVSS}$·d). These values were much lower than those previously measured with synthetic wastewater in *PHASE I-a* (>1 g $N_2$-N/($g_{MLVSS}$·d)), indicating a significant change in the anammox activity due to the complex nature of the leachate. Thus, to reach high NRRs in full-scale reactors treating landfill leachate, high MLVSS contents within the bioreactor (i.e., high sludge retention capacity) should be ensured.

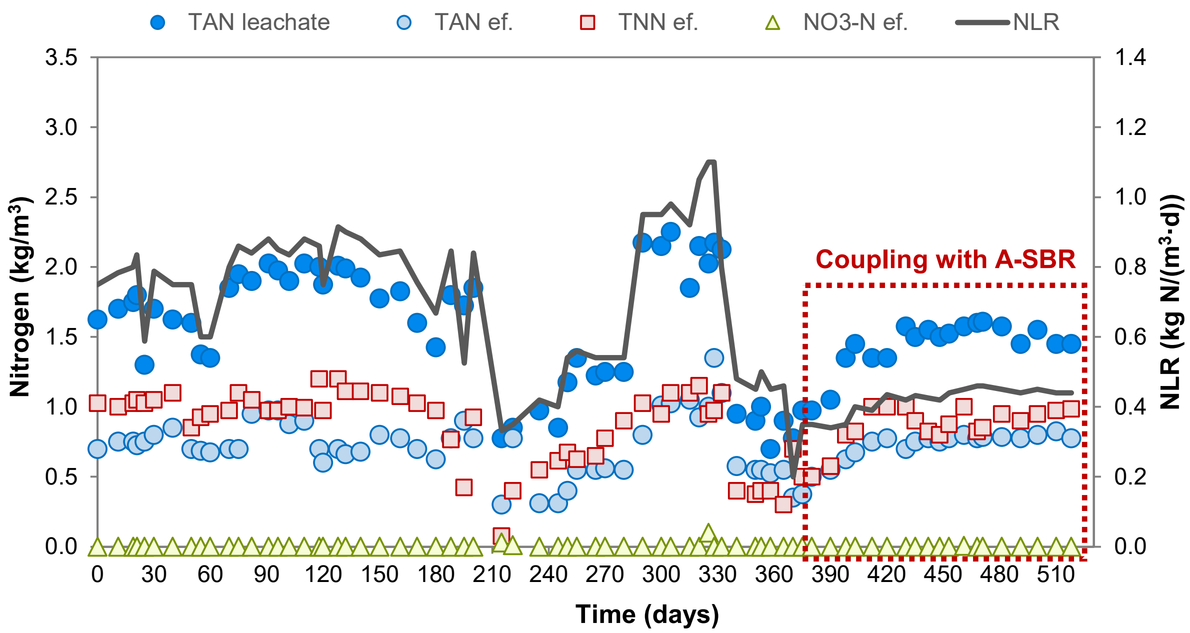



**Figure 3.** Long-term performance of the pilot-scale PN-SBR treating raw landfill leachate. By the end of the running period, PN-SBR was coupled with A-SBR (see Figure 4, *PHASE II-c*).

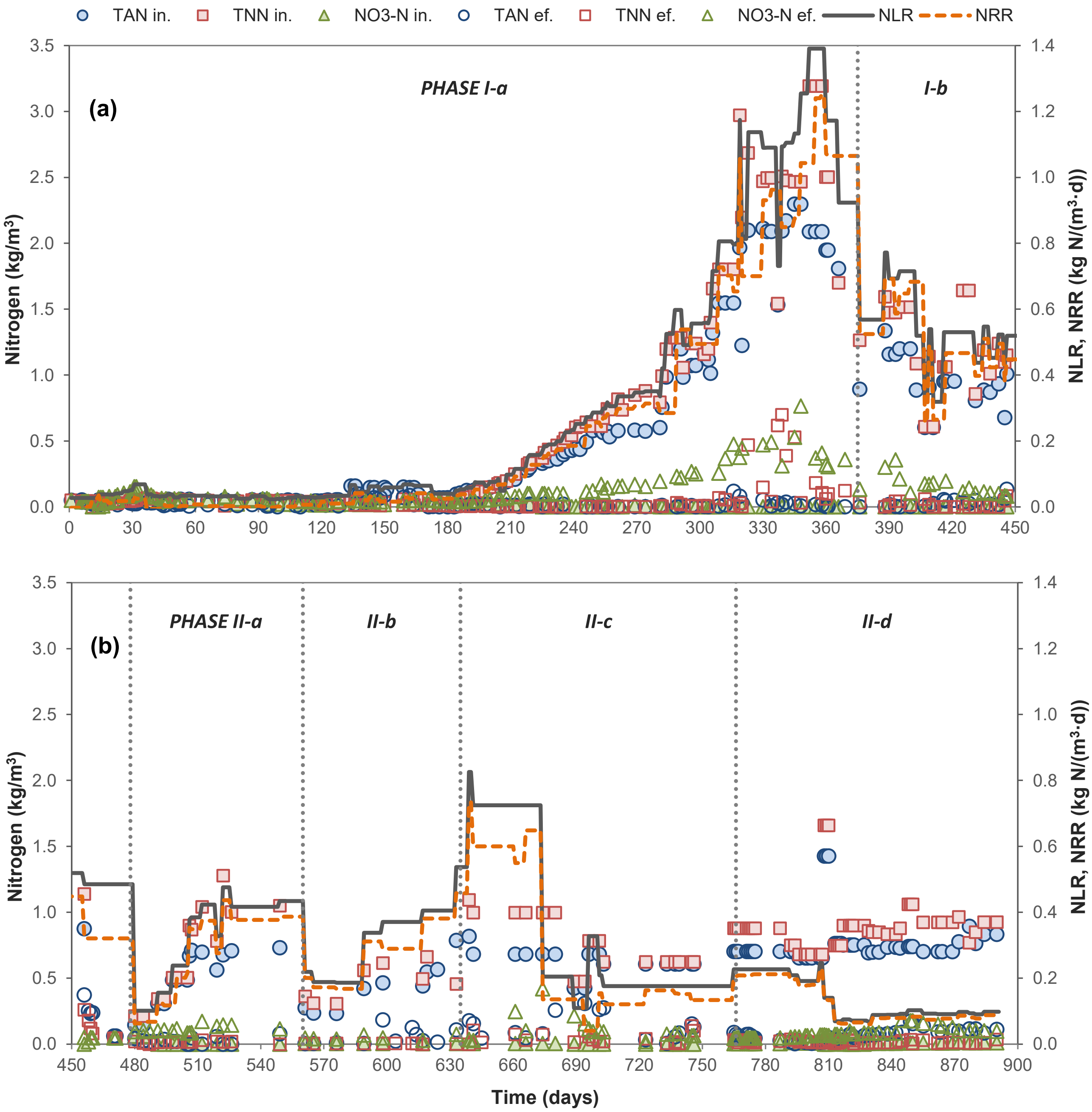



**Figure 4.** Performance of the pilot-scale A-SBR; (**a**) *PHASE I*: Start-up and enrichment with synthetic wastewater; (*I-a*) influent with increasing N content; (*I-b*) influent with N content similar to that in the PN-SBR effluent and preliminary tests partially adding nitritated leachate; (**b**) *PHASE II*: adaptation to landfill leachate and routinary operation; (*II-a*) influent with 25% to 50% of leachate treated in PN-SBR; (*II-b*) influent with 50% to 100% of leachate treated in PN-SBR; (*II-c*) coupling of PN-SBR with A-SBR treating raw leachate; (*II-d*) operation with raw leachate once transferred 85% of the MLSS to the full-scale A-SBR.

### 3.1.2. Coupling of PN-A with AOP

Samples from the outlet of the PN-A process were collected during the period in which both reactors were working in series treating raw leachate (*PHASE II-c*) to test the capability of an AOP—i.e., Fenton-based process (photo-Fenton)—for degrading remaining refractory COD. By applying the photo-Fenton process, after acidification to pH 2.98 and the addition of hydrogen peroxide (5.8 kg $H_2O_2/m^3$) and ferrous iron (5.98 g $Fe^{2+}/m^3$), the COD removal efficiency attained values as high as 98% (final COD was measured below 0.2 $kg/m^3$). The total N content was not modified, but eventually, available nitrite was fully oxidized to nitrate. The amount of chemical sludge produced was ca. 0.6 kg/m$^3$. Thus, the results obtained at the pilot-scale demonstrated feasibility for combining PN-A and AOP for an effective removal of N and COD from landfill leachate and the readiness of the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain) to be implemented at the full-scale.

### 3.2. The PANAMMOX® Industrial Plant

#### 3.2.1. Start-Up of the PN-A System

The full-scale PN-SBR was seeded with activated sludge (7 m$^3$) from a nearby urban WWTP. The start-up of the process was fast. In approximately 60 days, TAN was satisfactorily partially converted to nitrite and no nitrate was significantly found in the effluent (Figure 5). In the following days, the inflow rate was increased progressively, finally resulting in NLRs above 1.5 kg N/(m$^3$·d), and stable PN was achieved despite the variability in the composition of the leachate depending on its origin within the landfill site. The NOB activity was mostly suppressed (a maximum of 4% of the total-N in the effluent was found as nitrate), and suitable effluent for feeding A-SBR was produced in continuous. An optimized management of the sectorial cells existing in the landfill site and used for storing the leachate was shown as helpful in balancing the composition of the blended leachate in the pre-treatment tank. According to this fact, the use of chemicals could be reduced. Moreover, by regularly characterizing the leachate in such sectorial cells, it was feasible to decrease the potential risk of biomass inhibition events in the reactors due to the presence of toxic compounds. Once the start-up period finished, the PN-SBR was able to treat mature landfill leachate at a daily flow rate of about 20 to 25 m$^3$/d according to N loads of 30 to 40 kg N/d. The nitritated leachate was then used to start up A-SBR, processing the excess flow directly by AOP. The pH value of the effluent was close to neutrality (6.5 to 7), indicating an appropriate use in nitritation of the alkalinity available. An example of an on-line monitoring profile is shown as Supplementary Materials.

Four months after starting operating PN-SBR, A-SBR was inoculated with the sludge harvested from the pilot plant described in Section 3.1.1. The initial working volume of the bioreactor was set to 3 m$^3$ and the MLVSS content was as low as 0.07 kg/m$^3$. The main concerns during the start-up regarding reactor operation were (i) to reach anoxic conditions without purging with synthetic $N_2$, (ii) to avoid high nitrite concentrations and long exposure of the sludge to this N compound, and (iii) to ensure nitrate availability to prevent hydrogen sulfide formation (i.e., by adding sodium nitrate ($NaNO_3$) to the influent stream). During the first 60 days (data not shown), the presence of DO traces in the mixed liquor led to the accumulation of nitrite up to concentrations of 50 g N/m$^3$ (with ORP values around 80 mV). To minimize nitrite accumulation, the TNN/TAN ratio in the inlet was controlled close to 1 and/or raw leachate was punctually supplied. Once anoxic conditions could be ensured, the ORP values decreased below 0 mV (an example of an on-line monitoring profile is shown as Supplementary Materials). By the end of this initial period, the TNN/TAN removal ratios approached well the stoichiometric value expected for the anammox reaction (i.e., 1.32 mol/mol [36]), averaging 1.36 $\pm$ 0.12 in the next four months. Otherwise, nitrate production, typically linked to the anammox reaction (i.e., 0.26 mol/mol [36]), could not consistently be measured in the effluent during this period because of the coexistence of heterotrophic denitrification. Overall, in six months, the treatment capacity of A-SBR increased from 0.03 kg N/d to 0.28 kg N/d and the MLVSS content was raised from 0.07 kg/m$^3$ to 0.28 kg/m$^3$. Once at this point, the working volume of the reactor was progressively increased, and the integrated two-stage PN-A system was considered as ready for routine operation, ensuring high N removal efficiencies.

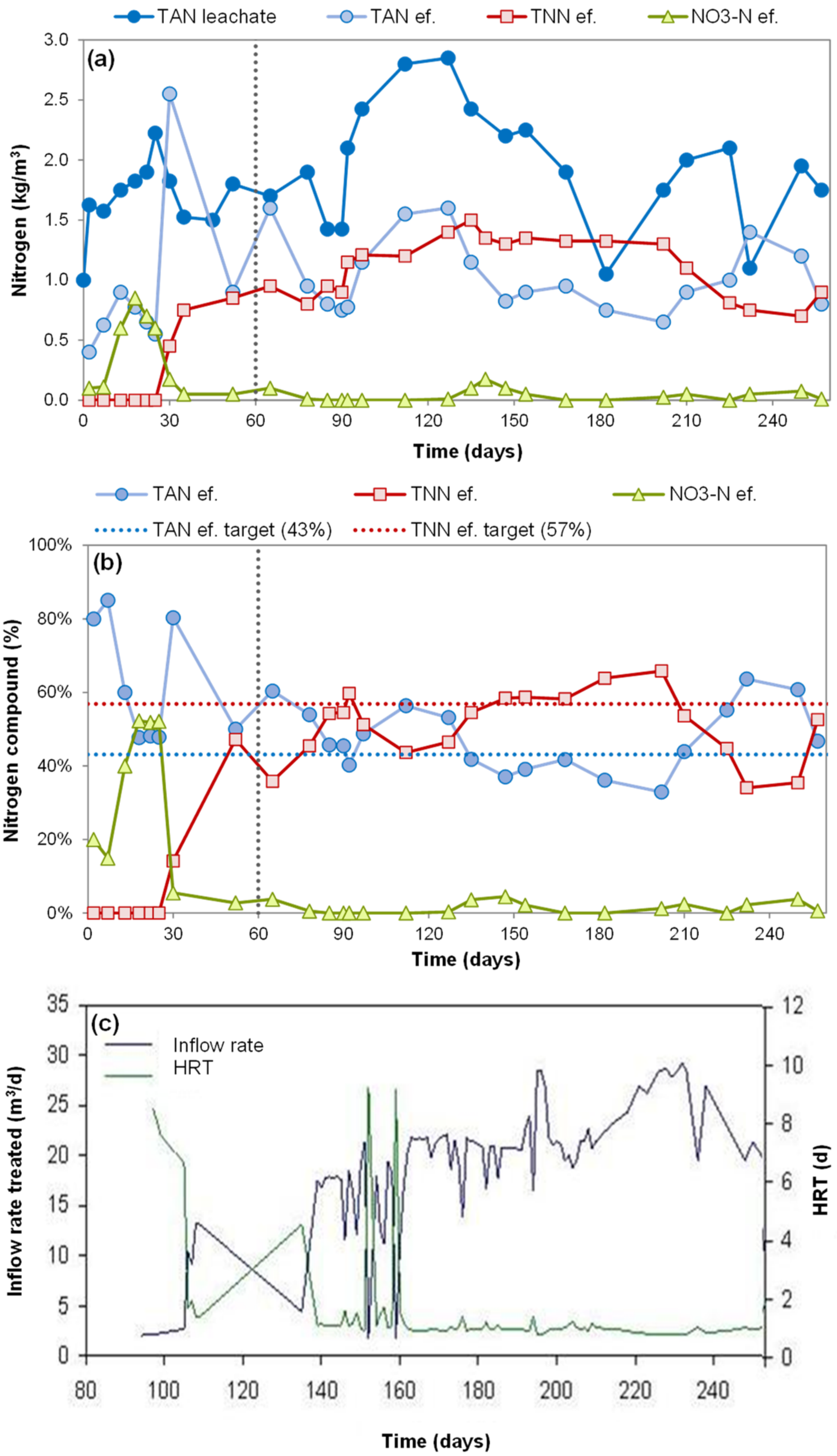



**Figure 5.** Performance of the full-scale PN-SBR during the start-up; (**a**) evolution of the concentration of the N compounds (TAN: total ammonium N, TNN: total nitrite N); (**b**) relative content of N compounds in the effluent; (**c**) inflow rate and hydraulic residence time (HRT) applied.

### 3.2.2. Long-Term Operation of the PN-A System

The adjustment of the alkalinity in the pre-treatment tank (ALK/TAN molar ratio as 1.14) allowed successful PN at the long-term despite the high conductivity of the leachate (i.e., PN was not affected by the high conductivity). Under regular operation, the NLR applied to PN-SBR roughly ranged from 0.4 to punctually >3 kg TAN/($m^3$·d) (mean: 1.2 ± 0.8 kg TAN/($m^3$·d)). The effluent TNN/TAN molar ratio averaged 1.4 ± 0.6 (Figure 6; Table 2), most of the time reaching appropriate values in view of the anammox process. The high values attained between days 600 and 700 were caused by an exploratory campaign to achieve complete nitritation. The MLVSS content within the PN-SBR at the beginning of a new cycle ranged from 1 to 6 kg/$m^3$ (mean: 3.2 ± 1.4 kg/$m^3$; it corresponded to 64 wt% of the MLSS). In specific terms, the nitrite production rate averaged 0.31 g N/($g_{MLVSS}$·d). Conductivity was reduced by ca. 10% in this first stage.

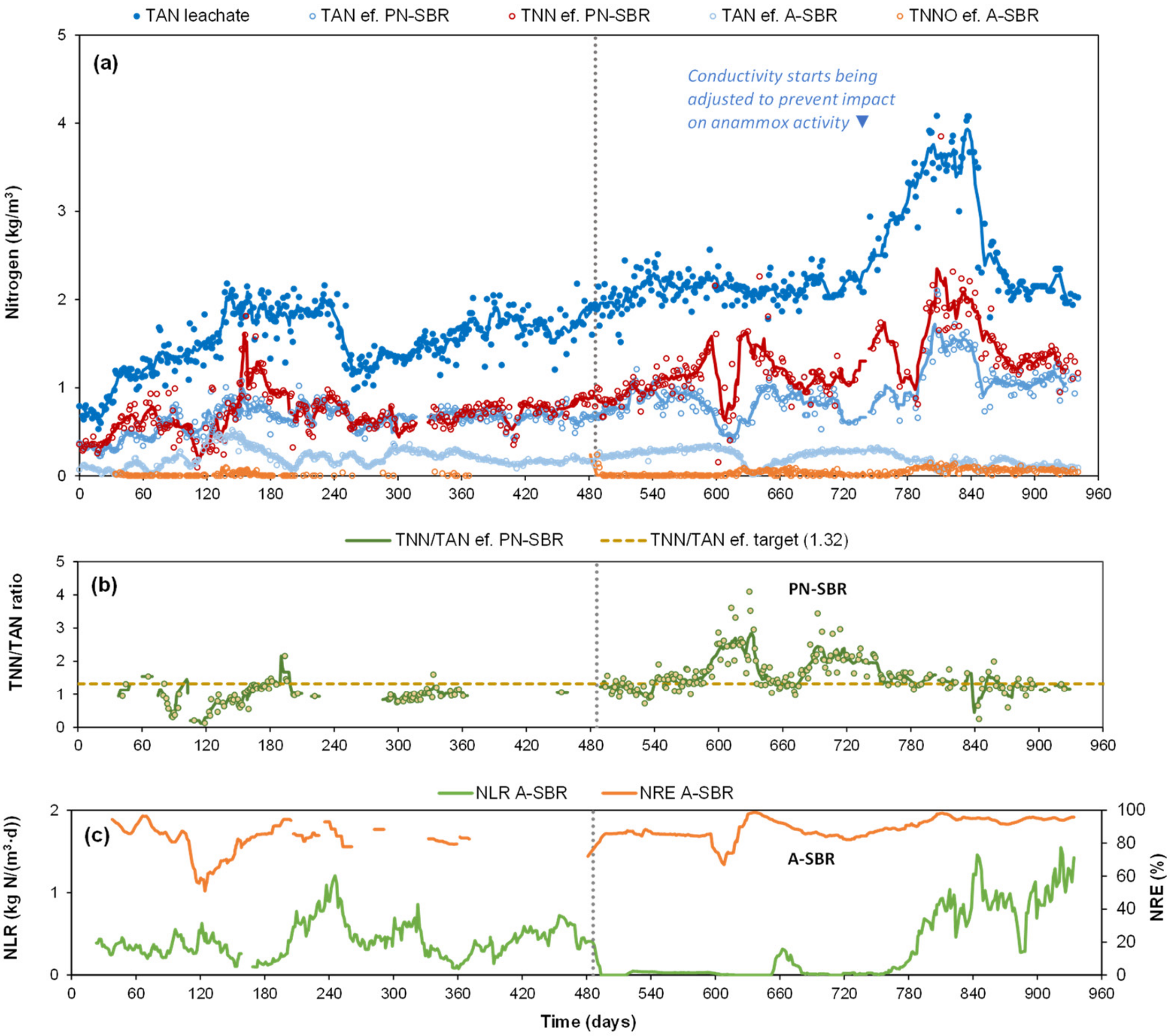



**Figure 6.** Performance of the full-scale system at the long-term; (**a**) Evolution of the concentration of the N species in both reactors (TAN: total ammonium N, TNN: total nitrite N, TNNO: TNN+ nitrate N); (**b**) TNN/TAN ratio in the PN-SBR effluent; (**c**) N loading rate and removal efficiency in A-SBR. Day 0 is 1 September 2017. The vertical dashed lines indicate when the landfill site stop receiving solid waste (on day 486).

**Table 2.** Summary of the operational conditions applied in PN-SBR and A-SBR at the long-term.

| Parameter | Average ± SD |
|---|---|
| PN-SBR | |
| IFR ($m^3/d$) | 14.2 ± 10.2 |
| HRT (d) | 1.9 ± 0.6 |
| MLVSS ($kg/m^3$) | 3.2 ± 1.4 |
| NLR (kg TAN/($m^3$·d)) | 1.2 ± 0.8 |
| TNN/TAN ratio effluent (−) | 1.4 ± 0.6 |
| A-SBR | |
| IFR ($m^3/d$) | 5.8 ± 8.1 |
| HRT (d) | 4.1 |
| MLVSS ($kg/m^3$) | 0.8 ± 0.5 |
| NLR [1] (kg N/($m^3$·d)) | 0.4 ± 0.5 |
| NRE [1] (%) | 86 ± 9 |
| N removed (kg N/d) | 7.4 |

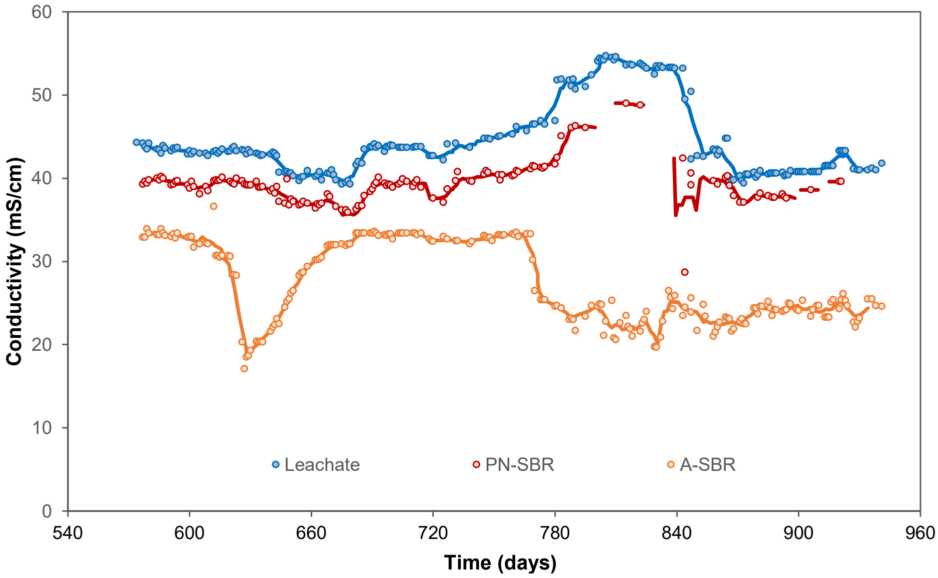
Abbreviations: HRT, hydraulic residence time; IFR, influent flow rate; MLVSS, mixed liquor volatile suspended solids; N, nitrogen; NLR, N loading rate; NRE, N removal efficiency; SD, standard deviation; TAN, total ammonium N, TNN, total nitrite N. [1] TAN + TNN.

The anammox process performed well when treating the nitritated leachate coming from PN-SBR, but particularly when conductivity was below 25 mS/cm. The MLVSS content in the A-SBR roughly ranged from 0.1 to 2 $kg/m^3$ (mean: 0.8 ± 0.5 $kg/m^3$; it corresponded to 46 wt% of the MLSS). The NLR applied to the A-SBR was 0.4 ± 0.5 kg N/($m^3$·d) with 86 ± 9% N removal efficiency—equivalent to a specific NRR ca. 0.4 g N/($g_{MLVSS}$·d)—leading to the removal of 7.4 kg N/d (i.e., according to this value, the treatment plant has been working below its maximum capacity). Conductivity was additionally reduced by approximately 15% in this second stage. Yet, the progressive increase of the leachate strength over time led to higher conductivity values in the final effluent, surpassing 30 mS/cm, which could negatively impact on the AnAOB activity. Once arrived at this point, the implementation of some kind of action to maintain the good performance of the anammox sludge in A-SBR became indispensable to reach the internal standards fixed in the landfill site for the effluent (e.g., 0.1 to 0.3 kg TAN/$m^3$), as will be discussed later on in Section 3.2.3. The PN-A effluent was further treated by an AOP (Fenton) to remove the refractory non-bCOD, attaining final COD levels of 1.8 ± 0.9 $kg/m^3$ (according to a removal efficiency of 50 ± 24%) before being transferred to a nearby urban WWTP.

Overall, the configuration in two stages [37] characteristic of the PANAMMOX® technology (LEQUIA Research Group, Girona, Catalonia, Spain) allowed for controlling the TNN/TAN ratio in the PN-effluent according to the adjustment of the alkalinity in the pre-treatment step. This fact made it feasible to correct punctual mismatches in the availability of ammonium and nitrite in view of the subsequent anammox step. Moreover, it also helped to soften the impact of eventual fluctuations in the composition of the leachate in terms of bCOD. As the last option, in the case that the sludge become seriously damaged, the PN-SBR could easily be re-inoculated with new activated sludge.

#### 3.2.3. Facing Increasing Leachate Conductivity after the Closure of the Landfill Site

Conductivity above 25 to 30 mS/cm negatively affected the anammox activity, finally becoming necessary to be adjusted in the pre-treatment tank. The impact of the conductivity on the process was more significant after closing the landfill site, when the conductivity of the leachate reached maximum values as high as 55 mS/cm (days 800 to 840, as shown in Figure 7a). In order to reduce conductivity, reverse osmosis was temporarily applied to the PN effluent before feeding A-SBR. Yet, to reduce costs, other strategies were finally considered, such as the dilution of the leachate using rainwater collected in the landfill, or physicochemically oxidized PNA effluent, according to the on-line monitoring of the conductivity within A-SBR. Once the optimized conditions regarding the adjustment of conductivity (25 mS/cm) were regularly implemented, the anammox sludge showed high activity (Figure 7b) and NRRs above 1 kg N/($m^3$·d) were feasible in A-SBR.




(**a**)

(**b**)

**Figure 7.** (**a**) Evolution of the conductivity in the treatment plant once the landfill site stopped receiving solid waste (day 486); (**b**) Detailed view of the biomass growing in A-SBR after decantation in an Imhoff cone (January 2020).

### *3.3. Microbial Community in the Reactors*

The analysis of the microbial community in the bioreactors at two different times (August 2016 and November 2018) (Figure 8) showed *Proteobacteria* as the dominant phylum in PN-SBR. Several taxonomic subgroups affiliated with the class *Betaproteobacteria*, and which can be associated with denitrification [38], were found abundantly (e.g., *Thauera*, *Burkholderiaceae*). *Nitrosomonas* (which also belongs to *Betaproteobacteria*) was the only ammonium-oxidizing genus identified. No nitrite-oxidizing genera were detected.

In A-SBR, the phylum *Planctomycetes* reached a similar abundance as *Proteobacteria*. Other phyla, such as *Bacteroidetes*, *Actinobacteria* and *Chloroflexi*, may also reach significant percentages. The dominant anammox genus was Candidatus *Kuenenia* (20 to 28% relative abundance; species Ca. *K. stuttgartiensis*). Although the genus Ca. *Brocadia* was also detected, its relative abundance was < 1%. In this regard, Ca. *K. stuttgartiensis* has frequently been identified as the dominant anammox species in other PN-A systems treating high-strength landfill leachate [39–41] and its enrichment has been linked to the operational conditions applied.

### *3.4. Cost Analysis*

Savings achieved in the CORSA landfill site by the implementation of the new treatment system consisting of PN-A plus AOP in substitution of the old technology consisting of AOP plus stripping–scrubbing were assessed. The analysis was conducted by considering the operational costs linked to the use of chemicals ($H_2O_2$, $FeCl_2$, $H_2SO_4$ and NaOH), the handling of the waste streams resulting from the treatment (taxes, water management and ammonium sulfate ($(NH_4)_2SO_4$) management), and also the consumption of electricity (Table 3). Without considering the electricity supply, the running cost of treating the leachate using the new configuration was estimated at 18.3 €/$m^3$, which was 22% cheaper than for the original scenario (23.5 €/$m^3$). When electricity was also included in the analysis, a 32% reduction in the total cost was estimated (19.7 €/$m^3$ vs. 28.8 €/$m^3$). Savings in electricity costs were estimated as high as 73%. Reasons justifying this reduction in costs are the lower alkalinity (involving less consumption of reagents to adjust the pH value) and the lower COD (thus requiring shorter $H_2O_2$ addition) entering the AOP, which, in turn, has a positive side-effect: the decrease in the effluent flow rate to be managed. Moreover, the production of $(NH_4)_2SO_4$—which in this case was managed as a waste and not as a by-product because its origin made valorization as source of N by the fertilizer industry unfeasible—could be completely discarded. The cost for electricity was accounted for separately in the analysis since a power generation unit might be installed in the landfill site using the biogas formed as an energy source, which could ensure energy self-sufficiency of the treatment facility.

| | PN-SBR | | A-SBR | |
|---|---|---|---|---|
| | Aug-2016 | Nov-2018 | Aug-2016 | Nov-2018 |
| ***Proteobacteria*** | **77%** | **62%** | **25%** | **35%** |
| ***c_Alphaproteobacteria*** | | | | |
| *Rhizobiaceae* unclassified | 1% | 0% | 1% | 4% |
| *Paracoccus* | 4% | 0% | 0% | 0% |
| ***c_Betaproteobacteria*** | | | | |
| *Thauera* | 37% | 0% | 1% | 0% |
| *Burkholderiaceae* unclassified | 1% | 23% | 0% | 1% |
| *Pusillimonas* | 0% | 11% | 0% | 0% |
| *Limnobacter* | 1% | 0% | 6% | 3% |
| *Ottowia* | 5% | 0% | 1% | 0% |
| *Comamonadaceae* unclassified | 5% | 0% | 1% | 0% |
| *Nitrosomonas* | 4% | 8% | 0% | 0% |
| ***c_Gammaproteobacteria*** | | | | |
| *Luteibacter* | 0% | 8% | 1% | 2% |
| *Arenimonas* | 0% | 0% | 0% | 8% |
| ***Planctomycetes*** | **1%** | **1%** | **23%** | **30%** |
| Ca. *Kuenenia* | 0% | 0% | 20% | 28% |
| ***Bacteroidetes*** | **7%** | **19%** | **26%** | **12%** |
| *Saprospiraceae* uncultured | 0% | 15% | 1% | 3% |
| *IheB3-7* | 0% | 0% | 14% | 0% |
| *Lentimicrobiaceae ge* | 0% | 0% | 3% | 2% |
| *Moheibacter* | 3% | 0% | 1% | 1% |
| ***Actinobacteria*** | **7%** | **6%** | **17%** | **3%** |
| *Actinomarinales* uncultured | 0% | 0% | 14% | 0% |
| *Microbacteriaceae* unclassified | 2% | 4% | 0% | 0% |
| ***Chloroflexi*** | **1%** | **3%** | **5%** | **9%** |
| *SBR1031 ge* | 0% | 0% | 1% | 5% |
| ***Armatimonadetes*** | **0%** | **5%** | **0%** | **0%** |
| *Armatimonadetes* unclassified | 0% | 5% | 0% | 0% |
| ***Acidobacteria*** | **0%** | **0%** | **0%** | **3%** |
| *Bryobacter* | 0% | 0% | 0% | 3% |
| **Other** | **6%** | **4%** | **4%** | **7%** |

| **% Relative abundance** |
|---|
| < 5 |
| 5–10 |
| 10–15 |
| 15–20 |
| 20–25 |
| 25–30 |
| > 30 |

**Figure 8.** Relative abundance of the main bacterial representatives at the phylum and genus levels in both SBRs (PN-SBR and A-SBR) at two different times (August 2016 and November 2018). The community was analyzed by high-throughput sequencing using the Illumina MiSeq platform. *Nitrosomonas* as the dominant genus for AOB and Ca. *Kuenenia* as the dominant genus for AnAOB are indicated in red color.

**Table 3.** Operational cost analysis for the treatment of the leachate produced in the CORSA landfill site. Comparison of the old technology (based on AOP + stripping-scrubbing) with the new technological approach (based on PN-A + AOP).

| **Treatment System** | **AOP + Stripping** | **PN-A + AOP** | **Savings** |
|---|---|---|---|
| **Units** | **(€/m$^3$)** | **(€/m$^3$)** | **(%)** |
| Running cost | 23.5 | 18.3 | 22% |
| *Chemicals* | *10.69* | *9.23* | *14%* |
| *Effluent management* | *12.64* | *9.01* | *29%* |
| *Sludge management* | *0.12* | *0.05* | *58%* |
| Electricity | 5.3 | 1.4 | 73% |
| **Total Cost** | **28.8** | **19.7** | **32%** |

On the other hand, expenses incurred on the construction and purchase of equipment installed in the PANAMMOX® treatment plant at the CORSA landfill site were about 275 k€. By a rough estimation of the equivalent annual cost, a value of 3.5 €/m$^3$ was obtained. Finally, by adding capital and operating costs, the total cost for the treatment of the leachate produced in the landfill site was estimated as 23.2 €/m$^3$.

## 4. Conclusions

The PANAMMOX® technology (which is based on the coupling of PN and anammox processes in dedicated SBRs) was presented as an attractive option to achieve long-term N removal from mature landfill leachate at a full-scale. Its configuration in two stages allowed for a distinguished design and operation of the bioreactors. The pre-treatment step targeting adjusting alkalinity before biological treatment was essential to produce a good-quality nitritated effluent in view of anammox. The heterotrophic activity in PN-SBR also favored softening the impact on the anammox process caused by the availability of bCOD in the leachate. The buffer tank following PN-SBR allowed for the rejection of most of the suspended solids before entering A-SBR. In this case study, N removal rates of ca. 7.4 kg N/d were reached. The composition of the leachate to be treated fluctuated over time, but especially once the landfill site stopped receiving solid waste (which involved a marked increase in the strength of the leachate). Values of conductivity above 25 mS/cm could negatively impact the performance of the anammox process, making it necessary to consider a dilution strategy. A cost analysis showed that, by implementing this technology in the CORSA landfill site, operational savings up to 32% with respect to the old technology were achievable.

## 5. Patents

PANAMMOX® was registered as a Spanish trademark by Ferrovial Servicios (formerly CESPA Gestión de Residuos, S.A.) in 2008.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/pr9050800/s1, additional details concerning description and operation of the industrial treatment plant: layout (Supplementary Figure S1) and P&ID (Supplementary Figure S2), SBRs working cycle configuration: PN-SBR (Supplementary Figure S3) and A-SBR (Supplementary Figure S4), and online monitoring profiles: PN-SBR (Supplementary Figure S5) and A-SBR (Supplementary Figure S6).

**Author Contributions:** Conceptualization, A.M., M.R. and J.C.; methodology, A.M., M.R. and J.C.; validation, A.M., M.R., M.D.B. and J.C.; formal analysis, A.M., M.R., A.V. and T.R.V.A.; investigation, A.M., M.R., A.V., T.R.V.A., J.M.L. and J.C.; resources, M.R. and J.M.L.; data curation, A.M., M.R., A.V. and T.R.V.A.; writing—original draft preparation, A.M. and M.R.; writing—review and editing, A.M., M.R., A.V., T.R.V.A., M.D.B., J.M.L. and J.C.; visualization, A.M. and M.R.; supervision, M.R., M.D.B. and J.C.; project administration, A.M., M.R. and J.C.; funding acquisition, J.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** The scaling up of the PANAMMOX® technology for the treatment of landfill leachate was funded by the Spanish Government through the N-OPTIMOX project [IPT-2011-1073-310000]. The Laboratory of Chemical and Environmental Engineering (LEQUIA) (http://www.lequia.udg.edu/) from the University of Girona (UdG) is member of the TECNIO network, and it was recognized as a consolidated research group by the Government of Catalonia [2017-SGR-1552]. T.R.V.A. acknowledges the PhD grant provided by the Brazilian National Council of Technological and Scientific Development (CNPq) [Grant number 246382/2012-0].

**Acknowledgments:** The support received from CESPA Gestión de Residuos S.A. (currently Ferrovial Servicios), and particularly from Ferran Ralea and Montserrat Elorduy in the initial days after starting the public-private partnership between LEQUIA-UdG and CESPA, is kindly acknowledged.

**Conflicts of Interest:** The PANAMMOX® technology was developed, scaled-up and implemented at the CORSA landfill site thanks to several UdG-CESPA (currently Ferrovial Servicios) collaborative R&D projects. The authors declare no conflict of interest.

## References

1. Renou, S.; Givaudan, J.G.; Poulain, S.; Dirassouyan, F.; Moulin, P. Landfill leachate treatment: Review and opportunity. *J. Hazard. Mater.* **2008**, *150*, 468–493. [CrossRef] [PubMed]
2. Kulikowska, D.; Klimiuk, E. The effect of landfill age on municipal leachate composition. *Bioresour. Technol.* **2008**, *99*, 5981–5985. [CrossRef]
3. Mojiri, A.; Zhou, J.L.; Ratnaweera, H.; Ohashi, A.; Ozaki, N.; Kindaichi, T.; Asakura, H. Treatment of landfill leachate with different techniques: An overview. *Water Reuse* **2021**, *11*, 66–96. [CrossRef]
4. Kurniawan, T.A.; Lo, W.-H.; Chan, G.Y.S. Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate. *J. Hazard. Mater.* **2006**, *B129*, 80–100. [CrossRef] [PubMed]
5. Wiszniowski, J.; Robert, D.; Surmacz-Gorska, J.; Miksch, K.; Weber, J.V. Landfill leachate treatment methods: A review. *Environ. Chem. Lett.* **2006**, *4*, 51–61. [CrossRef]
6. Lackner, S.; Gilbert, E.M.; Vlaeminck, S.E.; Joss, A.; Horn, H.; Van Loosdrecht, M.C.M. Full-scale partial nitritation/anammox experiences—An application survey. *Water Res.* **2014**, *55*, 292–303. [CrossRef]
7. Mohammad-Pajooh, E.; Weichgrebe, D.; Cuff, G. Municipal landfill leachate characteristics and feasibility of retrofitting existing treatment systems with deammonification—A full scale survey. *J. Environ. Manag.* **2017**, *187*, 354–364. [CrossRef] [PubMed]
8. Ye, J.; Liu, J.; Ye, M.; Ma, X.; Li, Y.-Y. Towards advanced nitrogen removal and optimal energy recovery from leachate: A critical review of anammox-based processes. *Crit. Rev. Environ. Sci. Technol.* **2020**, *50*, 612–653. [CrossRef]
9. Siegrist, H.; Salzgeber, D.; Eugster, J.; Joss, A. Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Sci. Technol.* **2008**, *57*, 383–388. [CrossRef]
10. Van Hulle, S.W.H.; Vandeweyer, H.J.P.; Meesschaert, B.D.; Vanrolleghem, P.A.; Dejans, P.; Dumoulin, A. Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chem. Eng. J.* **2010**, *162*, 1–20. [CrossRef]
11. Miao, L.; Wang, S.; Cao, T.; Peng, Y. Optimization of three-stage Anammox system removing nitrogen from landfill leachate. *Bioresour. Technol.* **2015**, *185*, 450–455. [CrossRef]
12. Li, Y.; Zheng, Z.; Li, J.; Zhao, B.; Bian, W.; Zhang, Y.; Wang, X. Inhibition kinetics and granular sludge in an ANAMMOX reactor treating mature landfill leachate. *Water Sci. Technol.* **2016**, *74*, 2697–2707. [CrossRef]
13. Li, G.; Puyol, D.; Carvajal-Arroyo, J.M.; Sierra-Alvarez, R.; Field, J.A. Inhibition of anaerobic ammonium oxidation by heavy metals. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 830–837. [CrossRef]
14. Scaglione, D.; Lotti, T.; Ficara, E.; Malpei, F. Inhibition on anammox bacteria upon exposure to digestates from biogas plants treating the organic fraction of municipal solid waste and the role of conductivity. *Waste Manag.* **2017**, *61*, 213–219. [CrossRef] [PubMed]
15. Hippen, A.; Rosenwinkel, K.-H.; Baumgarten, G.; Seyfried, C.F. Aerobic deammonification: A new experience in the treatment of wastewaters. *Water Sci. Technol.* **1997**, *35*, 111–120. [CrossRef]
16. Siegrist, H.; Reithaar, S.; Kock, G.; Lais, P. Nitrogen loss in a nitrifying rotating contactor treating ammonium-rich wastewater without organic carbon. *Water Sci. Technol.* **1998**, *38*, 241–248. [CrossRef]
17. Egli, K.; Fanger, U.; Alvarez, P.J.J.; Siegrist, H.; Van der Meer, J.R.; Zehnder, A.J.B. Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Arch. Microbiol.* **2001**, *175*, 198–207. [CrossRef]
18. Wang, C.-C.; Lee, P.-H.; Kumar, M.; Huang, Y.-T.; Sung, S.; Lin, J.-G. Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant. *J. Hazard. Mater.* **2010**, *175*, 622–628. [CrossRef] [PubMed]
19. Azari, M.; Walter, U.; Rekers, V.; Gu, J.-D.; Denecke, M. More than a decade of experience of landfill leachate treatment with a full-scale anammox plant combining activated sludge and activated carbon biofilm. *Chemosphere* **2017**, *174*, 117–126. [CrossRef]
20. Ganigué, R.; López, H.; Balaguer, M.D.; Colprim, J. Partial ammonium oxidation to nitrite of high ammonium content urban landfill leachates. *Water Res.* **2007**, *41*, 3317–3326. [CrossRef]

21. Gabarró, J.; Ganigué, R.; Gich, F.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. Effect of temperature on AOB activity of a partial nitritation SBR treating landfill leachate with extremely high nitrogen concentration. *Bioresour. Technol.* **2012**, *126*, 283–289. [CrossRef] [PubMed]
22. Ganigué, R.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. Operational strategy for a partial nitritation–sequencing batch reactor treating urban landfill leachate to achieve a stable influent for an anammox reactor. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 365–371. [CrossRef]
23. Ganigué, R.; Gabarró, J.; Sànchez-Melsió, A.; Ruscalleda, M.; López, H.; Vila, X.; Colprim, J.; Balaguer, M.D. Long-term operation of a partial nitritation pilot plant treating leachate with extremely high ammonium concentration prior to an anammox process. *Bioresour. Technol.* **2009**, *100*, 5624–5632. [CrossRef]
24. Ganigué, R.; Volcke, E.I.P.; Puig, S.; Balaguer, M.D.; Colprim, J.; Sin, G. Systematic model development for partial nitrification of landfill leachate in a SBR. *Water Sci. Technol.* **2010**, *61*, 2199–2210. [CrossRef] [PubMed]
25. Ganigué, R.; Gabarró, J.; López, H.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. Combining partial nitritation and heterotrophic denitritation for the treatment of landfill leachate previous to an anammox reactor. *Water Sci. Technol.* **2010**, *61*, 1949–1955. [CrossRef] [PubMed]
26. Gabarró, J.; Hernández-del Amo, E.; Gich, F.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. Nitrous oxide reduction genetic potential from the microbial community of an intermittently aerated partial nitritation SBR treating mature landfill leachate. *Water Res.* **2013**, *47*, 7066–7077. [CrossRef]
27. Gabarró, J.; González-Cárcamo, P.; Ruscalleda, M.; Ganigué, R.; Gich, F.; Balaguer, M.D.; Colprim, J. Anoxic phases are the main $N_2O$ contributor in partial nitritation reactors treating high nitrogen loads with alternate aeration. *Bioresour. Technol.* **2014**, *163*, 92–99. [CrossRef]
28. Ruscalleda, M.; López, H.; Ganigué, R.; Puig, S.; Balaguer, M.D.; Colprim, J. Heterotrophic denitrification on granular anammox SBR treating urban landfill leachate. *Water Sci. Technol.* **2008**, *58*, 1749–1755. [CrossRef]
29. Ruscalleda, M.; Puig, S.; Mora, X.; López, H.; Ganigué, R.; Balaguer, M.D.; Colprim, J. The effect of urban landfill leachate characteristics on the coexistence of anammox bacteria and heterotrophic denitrifiers. *Water Sci. Technol.* **2010**, *61*, 1065–1071. [CrossRef]
30. Scaglione, D.; Ruscalleda, M.; Ficara, E.; Balaguer, M.D.; Colprim, J. Response to high nitrite concentrations of anammox biomass from two SBR fed on synthetic wastewater and landfill leachate. *Chem. Eng. J.* **2012**, *209*, 62–68. [CrossRef]
31. Anfruns, A.; Gabarró, J.; Gonzalez-Olmos, R.; Puig, S.; Balaguer, M.D.; Colprim, J. Coupling anammox and advanced oxidation-based technologies for mature landfill leachate treatment. *J. Hazard. Mater.* **2013**, *258*, 27–34. [CrossRef] [PubMed]
32. López, H.; Puig, S.; Ganigué, R.; Ruscalleda, M.; Balaguer, M.D.; Colprim, J. Start-up and enrichment of a granular anammox SBR to treat high nitrogen load wastewaters. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 233–241. [CrossRef]
33. Van de Graaf, A.A.; Bruijn, P.D.; Robertson, L.A.; Jetten, M.S.M.; Kuenen, J.G. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology* **1996**, *142*, 2187–2196. [CrossRef]
34. APHA; AWWA; WEF. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association; American Water Works Association; Water Environment Federation: Washington, DC, USA; Denver, CO, USA; Alexandria, VA , USA, 2005.
35. Magrí, A.; Company, E.; Gich, F.; Colprim, J. Biologically induced hydroxyapatite formation in a single-stage anammox-based batch treatment system: Reactor performance, phosphorus recovery, and microbial community. *ACS Sustain. Chem. Eng.* **2021**, *9*, 2745–2761. [CrossRef]
36. Strous, M.; Heijnen, J.J.; Kuenen, J.G.; Jetten, M.S.M. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Appl. Microbiol. Biotechnol.* **1998**, *50*, 589–596. [CrossRef]
37. Jaroszynski, L.W.; Oleszkiewicz, J.A. Autotrophic ammonium removal from reject water: Partial nitrification and anammox in one-reactor versus two-reactor systems. *Environ. Technol.* **2011**, *32*, 289–294. [CrossRef]
38. Lu, H.; Chandran, K.; Stensel, D. Microbial ecology of denitrification in biological wastewater treatment. *Water Res.* **2014**, *64*, 237–254. [CrossRef]
39. Li, H.; Zhou, S.; Ma, W.; Huang, P.; Huang, G.; Quin, Y.; Xu, B.; Ouyang, H. Long-term performance and microbial ecology of a two-stage PN–ANAMMOX process treating mature landfill leachate. *Bioresour. Technol.* **2014**, *159*, 404–411. [CrossRef]
40. Huang, Y.-T.; Chen, S.-S.; Jetten, M.S.M.; Lin, J.-G. Nanoarchitectured structure and population dynamics of anaerobic ammonium oxidizing (anammox) bacteria in a wastewater treatment plant. *J. Hazard. Mater.* **2020**, *396*, 122714. [CrossRef]
41. Wu, L.; Yan, Z.; Huang, S.; Li, J.; Su, B.; Wang, C.; Peng, Y. Rapid start-up and stable maintenance of partial nitrification-anaerobic ammonium oxidation treatment of landfill leachate at low temperatures. *Environ. Res.* **2020**, *191*, 110131. [CrossRef]