Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Characterization of nutrient-removing microbial communities in two full-scale WWTP systems using a new qPCR approach

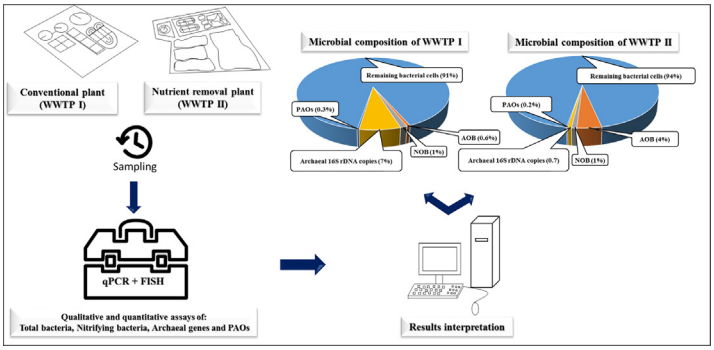
Tarik Abzazou [a,*], Humbert Salvadó [b], Yexenia Cardenas Youngs [a], Alberto Becerril Rodríguez [a], Eva Mª. Ciriero Cebirán [c], Anna Huguet [c], Rosa Mª. Araujo [a]

[a] Department of Microbiology, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[b] Department of Animal Biology, Faculty of Biology, Unversity of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[c] Empresa Mixta d'Aigües de la Costa Brava S.A., Roses WWTP, Camí Arenes s/n 17480 Roses, Girona, Spain

HIGHLIGHTS

- Microbial composition of two WWTPs was characterized using qPCR and FISH techniques.
- gBlocks were used as a rapid and easy technique for elaboration of DNA standards.
- The log-linear range of DNA standards was improved at least one order of magnitude.
- Microbial composition of WWTPs was in accordance with the operational performance.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 November 2016
Received in revised form 22 August 2017
Accepted 23 August 2017
Available online xxxx

Keywords:
gBlocks
FISH
AOB
NOB
Archaeal gene
PAOs

ABSTRACT

Biological wastewater treatment processes involve very complex microbial communities. Culture-independent molecular methods are feasible tools used to analyze and control the structure of different microbial communities, such as bacterial communities that remove nutrients. Here, we used the gBlocks gene fragments method, a new real-time PCR approach for the development of DNA standards, to quantify total bacterial cells, AOB, NOB, and Archaeal genes at two different WWTPs. PAOs were also quantified using the FISH technique. Our findings highlight a significant improvement in real-time PCR detection for the microorganisms studied. The qPCR and FISH technique applied allowed characterization of the microbial composition of two WWTPs operated as a conventional WWTP and a biological nutrient-removal WWTP. The results revealed a significant difference in the microbial profiles of the WWTPs, with a higher abundance of nitrifying bacterial communities and PAOs in the nutrient removal plant, which were in accordance with operational performance.

1. Introduction

Biological processes are widely used at the main bioengineering facilities for the treatment of domestic wastewater. All such processes necessarily depend on a very complex microbial composition, especially its bacterial composition. Nevertheless, the exploration of this microbial composition in the full-scale WWTPs is still in discovery phase.

* Corresponding author.
E-mail addresses: tabzazou@ub.edu (T. Abzazou), hsalvado@ub.edu (H. Salvadó), emciriero@aiguescb.com (E.M.ªC. Cebirán), amhuguet@aiguescb.com (A. Huguet), raraujo@ub.edu (R.M.ª Araujo).

https://doi.org/10.1016/j.scitotenv.2017.08.241
0048-9697/

Therefore, understanding the ecology that governs the biological processes is of great importance. By determining the underlying principles dictating structure and function in these complex microbial communities, process failures may be avoided and treatment systems optimized. Biological nutrient removal systems have been widely used in most WWTPs to prevent ecological problems such as eutrophication of water sources (Semerci and Hasilci, 2016). Biological nutrient removal processes are usually based on anaerobic, anoxic and aerobic phases linked in series (Cosenza et al., 2013; Lu et al., 2015).

Nitrogen is one of the most important contaminants of water bodies and is mainly present in wastewaters in its reduced form as ammoniacal nitrogen. In the biological nitrogen removal (BNR) process, the nitrogen is removed in two steps through nitrification and denitrification processes. In the nitrification step, the ammonium (NH_4^+) is first oxidized metabolically to nitrite (NO_2^-) under aerobic conditions: the step known as nitritation. This process is carried out mainly by ammonia-oxidizing bacteria (AOB), as the principal oxidizing organisms. However, other organisms can be involved in this process, such as ammonia-oxidizing archaea (AOA) (Gao et al., 2013). In the second step of nitrification, the step called nitritation, the nitrite is oxidized rapidly to nitrate by nitrite-oxidizing bacteria (NOB) in the presence of molecular oxygen.

Phosphorus, as well as being an important macronutrient, is considered an important pollutant that contributes directly to the eutrophication of aquatic systems as a key limiting nutrient. The biological process used for phosphorus removal processes is known as enhanced biological phosphorus removal (EBPR). EBPR is based on the capacity of certain microorganisms, such as polyphosphate-accumulating organisms (PAOs), to take up excess orthophosphate and store it as polyphosphate. Then, the stored phosphorus is removed with the biomass from the WWTP (Bao et al., 2007). The features of EBPR, high P-removal efficiency, lower operational costs, lower sludge production and the potential recovery of phosphorus, have contributed to its widespread use (Nielsen et al., 2012).

Since all the aforementioned biological nutrient removal processes are necessarily dependent on microorganisms, understanding the community dynamics of the different microorganisms involved in these processes is becoming essential for performance optimization of wastewater treatment. To gain this understanding, we require accurate, rapid and easy to perform microbial quantification techniques. This has led to the recent use of advanced biotechnology, especially molecular techniques, in different environmental fields (Gilbride et al., 2006). Generally, the molecular techniques used to explore wastewater microbial communities can be roughly grouped into four categories: clone libraries, molecular fingerprinting, hybridization and quantitative real-time PCR (qPCR) (Kim et al., 2013). In this work, we focused our study on the use of qPCR and fluorescence *in situ* hybridization (FISH) techniques, which are adequate for the characterization, quantification and monitoring of the microbial communities at WWTPs. The potential of qPCR as a routine microbial monitoring tool is recognized due to its rapidity, specificity and accuracy. These features mean that qPCR is suitable to apply in different wastewater treatment processes, including processes with fixed and suspended microbial communities under both aerobic and anaerobic conditions (Hall et al., 2002; Kindaichi et al., 2006; Lee et al., 2011; Limpiyakorn et al., 2005). Usually, qPCR involves the use of DNA plasmids or pure cell cultures for the preparation of standard curves of a known quantity of the target DNA; however, these methods can be expensive and time consuming. One alternative method that can resolve these inconveniences is the chemical synthesis of a known concentration of double-stranded DNA fragments with DNA targets as qPCR standards known as Double-stranded gBlocks Gene Fragments.

The objectives of this work were: (i) to design DNA standards for qPCR using **Double-stranded gBlocks Gene Fragments;** (ii) to set up a qPCR technique for the quantification of total bacterial cells, AOB, NOB, and Archaeal 16S rDNA gene in two different WWTPs; (iii) to set up and apply FISH technique for the quantification of PAOs; and (iv) to compare the microbial communities at a conventional WWTP and a biological nutrient removal WWTP, and their operational performance.

2. Material and methods

2.1. Sampling

Activated sludge samples (1 L) were collected fortnightly for three months from two WWTPs in the province of Girona (Spain). The samples were collected and processed within 24 h of collection. The

Table 1
Average values and standard deviations of the measured operational parameters for each WWTP.

Parameter		WWTP's	
		WWTP I	WWTP II
Volume capacity (m^3)		7500	7700
Flow rate (m^3 day^{-1})		7691 ± 2363	5023 ± 1061
HRT (days)		0.86 ± 0.08	1.50 ± 0.23
F/M (kg DBO_5 kg $MLSS^{-1}$ day^{-1})		0.17 ± 0.13	0.06 ± 0.05
Sludge age (days)		5.5 ± 2.3	14.68 ± 6.9
O_2 (mg L^{-1})		0.62 ± 0.29	2.16 ± 0.14
TSS (mg L^{-1})		1698 ± 360	4816 ± 801
VSS (mg L^{-1})		1347 ± 420	3460 ± 643
BOD_5 (mg L^{-1})	Influent	140.33 ± 32.88	303.17 ± 121.53
	Effluent	4.33 ± 2.16	3.00 ± 0.00
	Removal rate (%)	96.9	99
COD (mg L^{-1})	Influent	419.16 ± 152.57	748.67 ± 216.11
	Effluent	103.16 ± 19.80	41.83 ± 10.07
	Removal rate (%)	75.38	94.41
NH_4^+ (mg L^{-1})	Influent	41.17 ± 3.37	53.17 ± 5.70
	Effluent	29.50 ± 6.35	3.41 ± 5.40
	Removal rate (%)	28.34	93.58
TN (mg L^{-1})	Influent	53.16 ± 5.98	68.50 ± 5.2
	Effluent	38.15 ± 10.42	6.85 ± 4.50
	Removal rate (%)	28.23	90
TKN (mg L^{-1})	Influent	53.16 ± 5.98	68.50 ± 5.2
	Effluent	38.00 ± 10.50	3.31 ± 2.17
	Removal rate (%)	28.51	95.16
P (mg L^{-1})	Influent	5.41 ± 1.55	9.68 ± 2.89
	Effluent	1.80 ± 1.87	2.08 ± 2.63
	Removal rate (%)	44.5	72

TN: total nitrogen. TKH: Total Kjeldahl nitrogen.

Please cite this article as: Abzazou, T., et al., Characterization of nutrient-removing microbial communities in two full-scale WWTP systems using a new qPCR approach, Sci Total Environ (2017), https://doi.org/10.1016/j.scitotenv.2017.08.241