

V. Romero, A. Zornoza, and J.L. Alonso

 Instituto Universitario de Ingeniería del Agua y Medio Ambiente, (IIAMA). Universitat Politècnica de València, Spain (e-mail: jalonso@ihdr.upv.es).

INTRODUCTION

Bacteria that belong to the genus *Thiothrix* are characterized by distinctive morphological features, such as filamentous growth and accumulation of sulphur granule when incubated in the presence of sulphide or thiosulphate. The *Thiothrix* morphotypes occur in municipal and industrial WWTPs with and without nutrient removal [1]. Traditionally, the problems of biological bulking have been monitored by classical microscopy such as morphological observation and polychromatic staining [2]. Few works have been carried out for the detection of *Thiothrix* in WWTP samples with molecular procedures such as the fluorescence in situ hybridization (FISH) technique. In this study, we investigated several Spanish WWTPs with the FISH technique to analyze the composition of *Thiothrix* species and their relationships with physico-chemical and operational parameters.

MATERIAL AND METHODS

The sampling period was conducted between February to November 2012 in 3 urban WWTPs with conventional activated sludge system (CAS) and 1 urban WWTP with extended aeration system. Fortnightly samples of mixed liquor were analyzed by light microscopy and quantitative FISH.

In the FISH analysis a set of 8 rRNA-targeted nucleic acid probes covering *Thiothrix* genus and species were used (Table 1). The abundance of filamentous bacteria in the 139 activated sludge samples was measured according to the subjective scoring method of Eikelboom (2000) where observations are rated on scale from 0 (none) to 5 (extensive growth). The hybridizations were performed at 46 °C for 2 hours. All the probes (Table 1), labelled at the 5' end with Tamra, were purchased from TibMobiol, Germany.

Table 1: FISH probes used for the identification of <i>Thiothrix</i> filamentous bacteria				
Specificity	Probe	Sequence (5' – 3')	%FA	Reference
<i>Thiothrix</i>	G123T	CCTTCGATCTATGCA	40	Kanagawa <i>et al.</i> , (2000)
Comp. <i>Thiothrix</i>	G123TC	CCTTCGATCTTACGCA	40	Kanagawa <i>et al.</i> , (2000)
<i>T. disciformis</i>	G1B	TGTGTCGAGTTCCCTGC	30	Kanagawa <i>et al.</i> , (2000)
<i>T. eikelbomii</i>	G2M	GCACCAACGACCCCTAG	35	Kanagawa <i>et al.</i> , (2000)
<i>T. defluvii</i>	G3M	CTCAGGGATTCTGCCAT	30	Kanagawa <i>et al.</i> , (2000)
<i>T. nivea</i>	TNI	CTCCTCTCCCACATTCTA	45	Wagner <i>et al.</i> , (1994)
<i>T. fructosivorans</i>	TFR	CTCCTCTCCCACACTCTA	35	Kim <i>et al.</i> , (1994)
Comp. TNI y TFR	TEI	TCCCTCTCCCACATTCTA	45/35	Kim <i>et al.</i> , (2002)
C. "Meganema perideroedes"	Meg 983	CGGGATGTCAAAGGTGG	35	Kragelund <i>et al.</i> , (2005)
C. "M. perideroedes"	Meg 1028	CTGTCACCGAGTCCTTG	35	Kragelund <i>et al.</i> , (2005)

%FA: Formamide percentage; G123TC y Comp. TNI y TFR: Competitor probes

RESULTS

Thiothrix filaments were present in the four WWTP analyzed. In 92 (66,2 %) of the samples, *Thiothrix* filaments were observed with the probe G123T. It was evident that mixed populations of *Thiothrix* (figure 1) were present in the activated sludge samples investigated, the observed differences were in the relative abundance of the various groups. These findings were supported by the results obtained using conventional microscopy. *T. eikelbomii* (figure 2), *T. fructosivorans* (figures 3) and *T. nivea* (figure 4) were common ($F_1 > 3$) in two of the four WWTP analyzed. No fluorescence signal was detected with the probes G1B (*T. disciformis*), G3M (*T. flexilis*) and Meg1028+Meg 983 (*Meganema perideroedes*). Redundancy analysis (RDA) ordination diagram (biplot) suggested different behaviour among species within genus *Thiothrix*. *T. eikelbomii* showed a positive relationship with the reactor temperature (Tr) (figure 5). Conversely, *T. fructosivorans* was negatively related with reactor temperature (Tr). *T. nivea* and *T. fructosivorans* were associated with high sludge retention time (SRT) values and low values of organic loading rate (OLR). *T. nivea* showed a positive relationship with the hydraulic retention time (HRT) and negative with the influent carbohydrates and proteins. *T. eikelbomii* and *T. nivea* were associated with nitrogen and phosphorus deficit (BOD5/TN/TP) (figure 6).

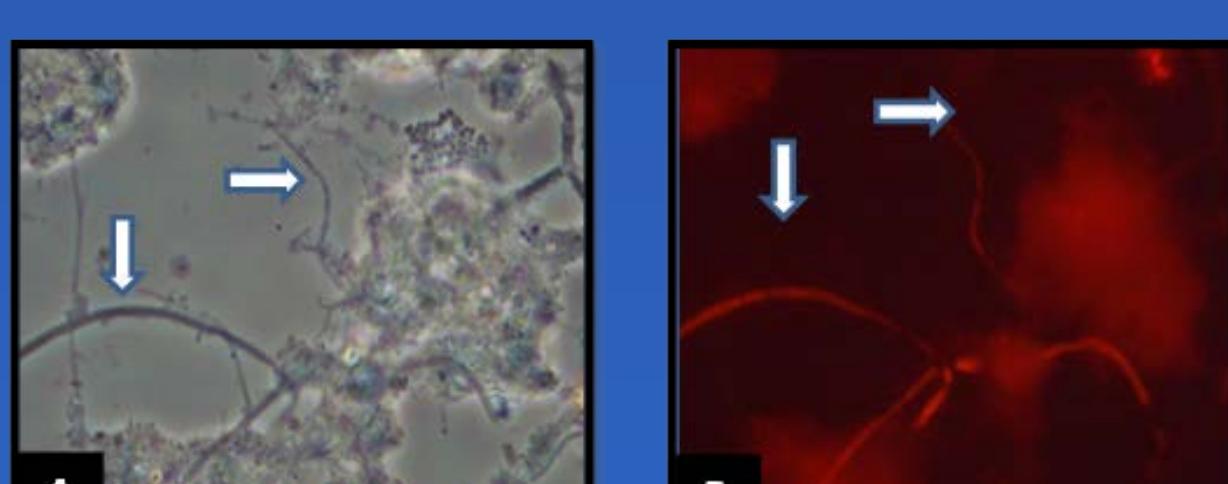


Figure 1: *Thiothrix* spp. (G123T probe), A: phase contrast, B: epifluorescence 1000X

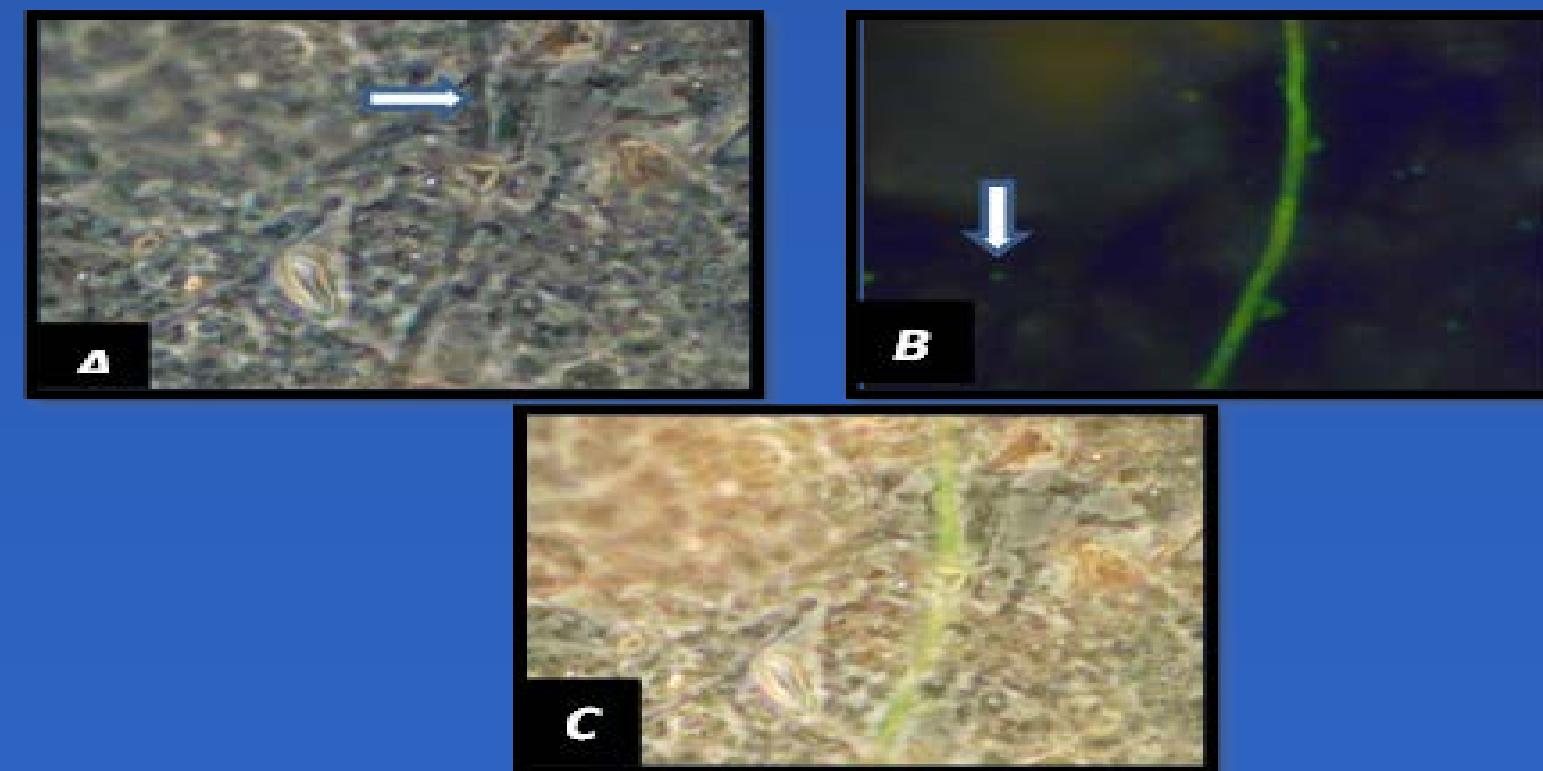


Figure 3: *T. fructosivorans* (TFR probe), A: phase contrast, B: epifluorescence, 1000X

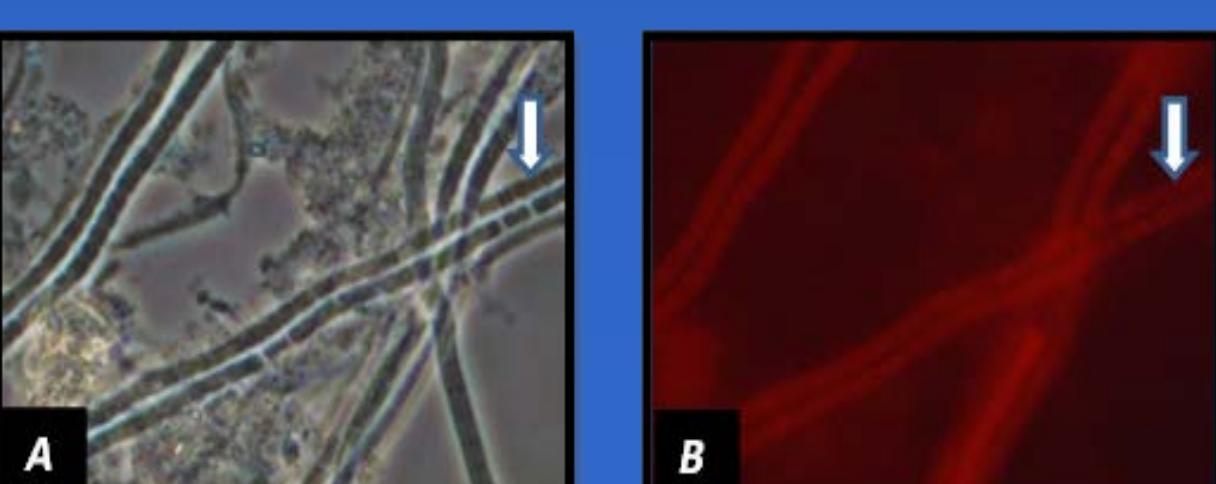


Figure 2: *T. eikelbomii* (G2M probe), A: phase contrast, B: epifluorescence 1000X

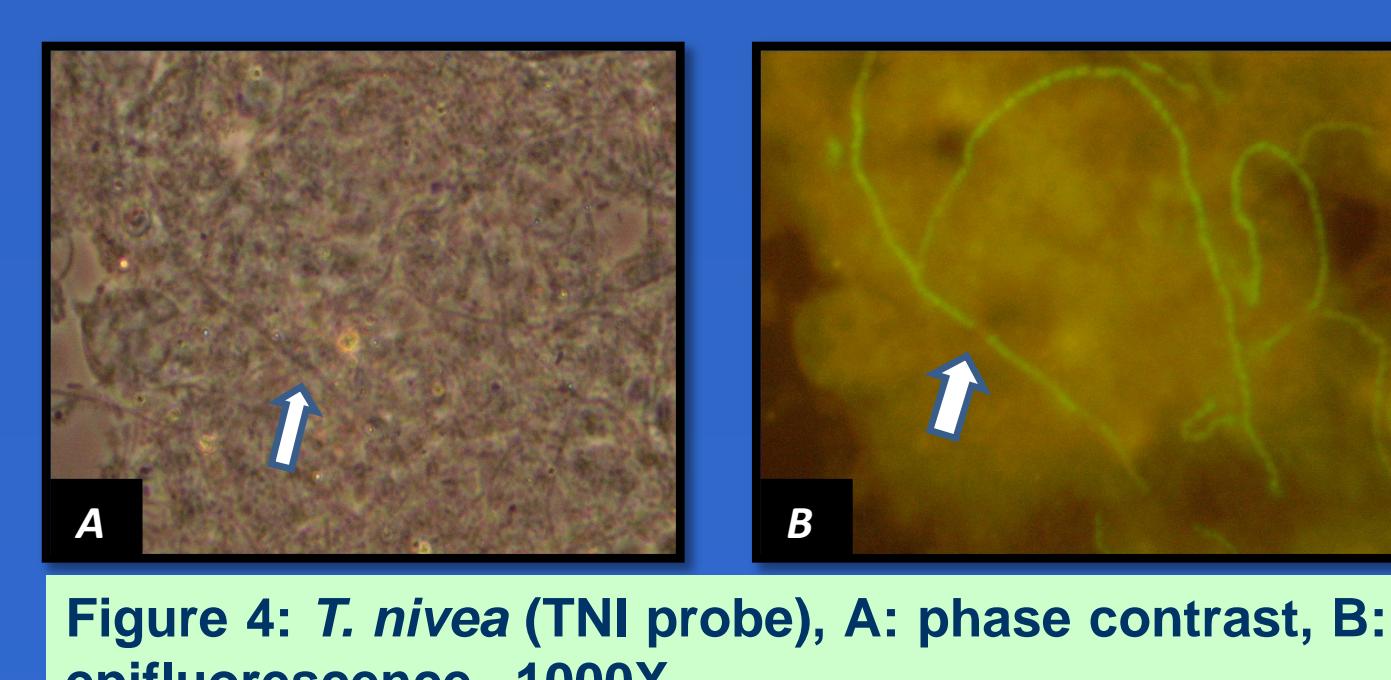


Figure 4: *T. nivea* (TNI probe), A: phase contrast, B: epifluorescence, 1000X

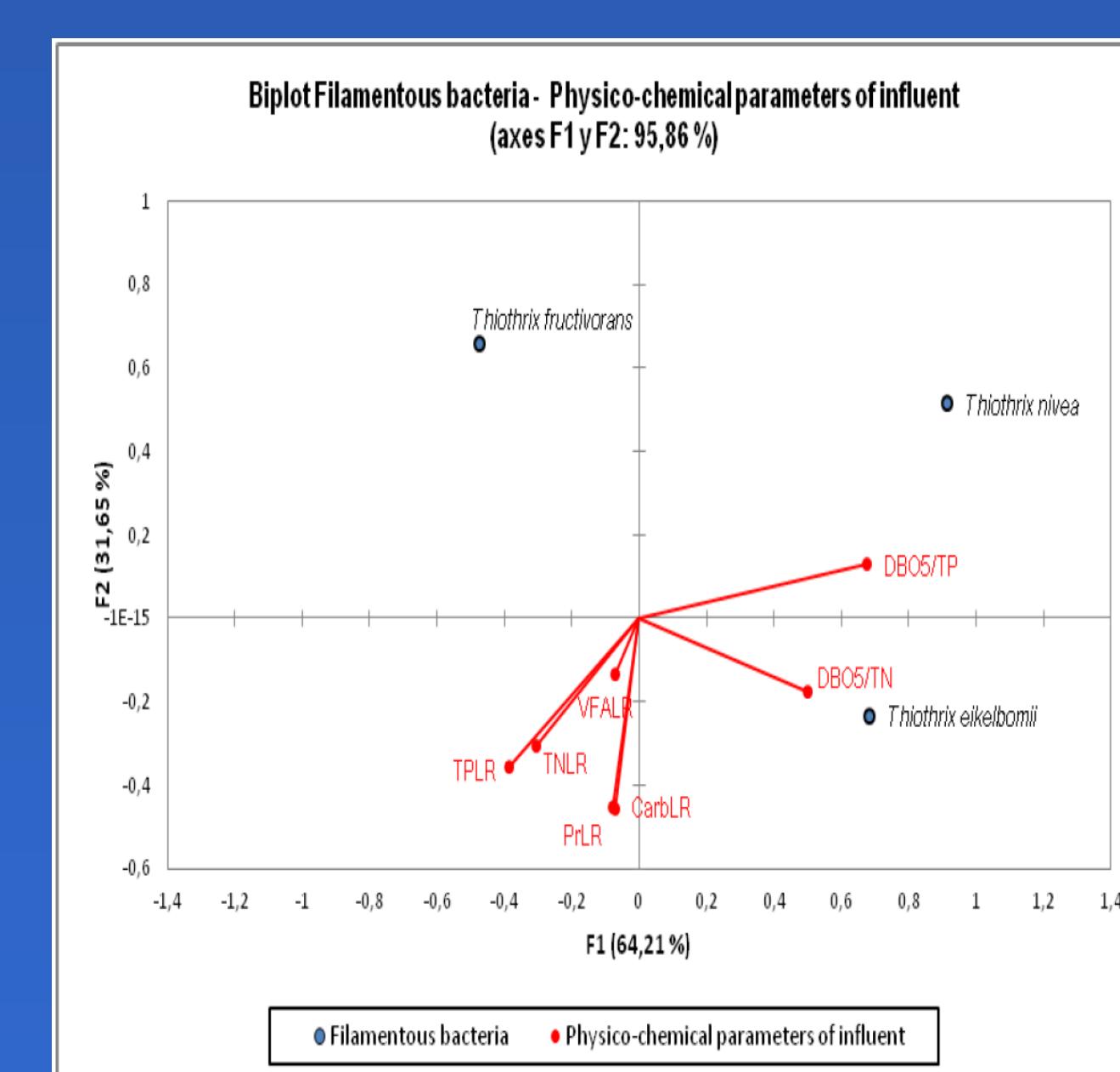


Figure 5: RDA between *Thiothrix* species and physico-chemical parameters

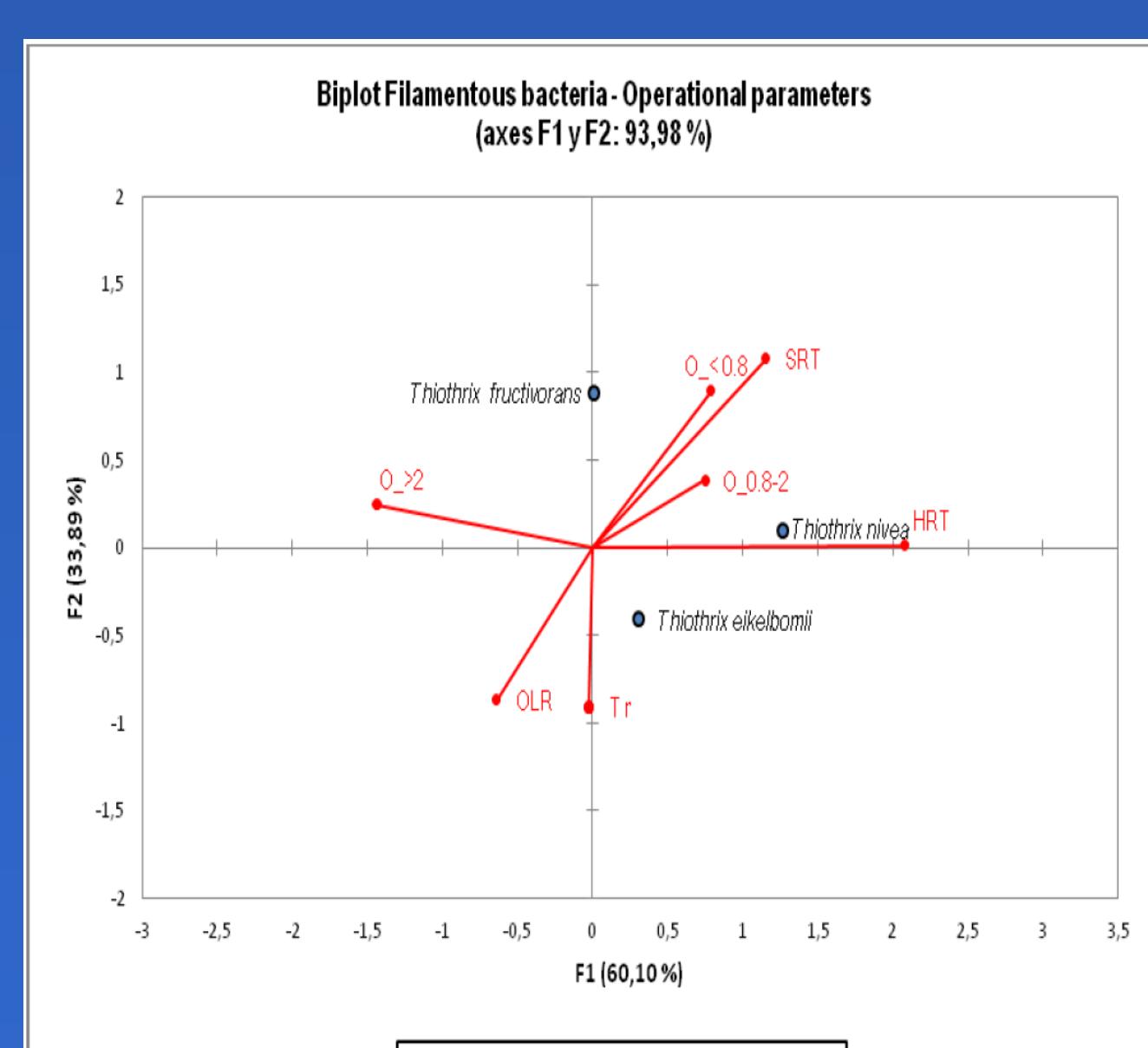


Figure 6: RDA between *Thiothrix* species and operational parameters

REFERENCES

- [1] Eikelboom D (2000) Process control of activated sludge plants by microscopic investigation. IWA Publishing, London, England.
- [2] Jenkins D., Richard M., Daigger G. (2004) Manual on cases and control of activated sludge bulking and foaming. Lewis Publishers, Chelsea, Michigan.