Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.20637

72 (2017) 78–84
April

Identification of the most probable causes for filamentous bacteria over-proliferation in Riqqa wastewater treatment plant, Kuwait

A. Abusam[a,*], A. Mydlarczyk[a], F. Al-Salameen[b], M.I. Ahmed[a]

[a]*Wastewater Treatment and Reclamation Technology Program, Water Research Center, Kuwait Institute for Scientific Research, P.O. Box 24885, Safat 13109 Kuwait, email: aabusam@kisr.edu.kw (A. Abusam), amydlarczyk@kisr.edu.kw (A. Mydlarczyk), miaahmed@kisr.edu.kw (M.I. Ahmed)*

[b]*Department of Biotechnology, the Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait. P.O. Box 24885, Safat 13109, Kuwait, e-mail: fslamian@ kisr.edu.kw.*

Received 9 September 2016; Accepted 23 February 2017

ABSTRACT

Excessive growth of filamentous bacteria in an activated sludge system is caused by a large number of factors that are related to the influent quality, the system's operating variables and/or the environmental conditions. Identification of the most probable cause(s) is very difficult from a large dataset that contains all of the information about these factors. Riqqa wastewater treatment plant (WWTP), which treats mainly domestic wastewater, is experiencing excessive growth of filamentous bacteria. Mixed liquor samples were collected from the aeration tank of Riqqa WWTP. The dominant filaments were identified using a molecular method called Vermicon Identification Technology (VIT). The significant associations between the filament growth, influent qualities, and operating conditions were then identified using multivariate statistical analysis, namely correlation analysis (CA) and principal component analysis (PCA). Interestingly, CA has indicated that the abundance of the filaments was directly related to the temperature of wastewater. PCA has identified six factors that account for about 75% of the total variance in the dataset. In addition to confirming CA results, PCA has also indicated that abundance of *Nocardioforms actinomycetes* and *Microthrix parvicella* was inversely related to the concentrations of TSS and VSS in the influent. These results are of great importance for controlling the proliferation of filamentous bacteria not only in Riqqa WWTP, but also in other WWTPs situated in hot climates since very little is published about the dynamics of filamentous bacteria in such conditions.

Keywords: *Activated* sludge; Bulking; Filamentous bacteria; Multivariate statistics; Correlation analysis; Principal component analysis

1. Introduction

Filamentous microorganisms constitute a natural part of the microorganisms in an activated sludge system [1]. They create the backbone for the flocs [2,3]. However, the excessive growth of filamentous bacteria cause sludge bulking and foaming problems which usually leads to poor effluent quality, odor nuisances and sludge management problems in activated sludge plants [4–7]. If not properly controlled, the excessive growth of filamentous bacteria can even cause a complete failure of the entire activated sludge process [8,9]. Numerous studies have been done about activated sludge bulking and foaming problems. Published literature, however, is almost limited to the temperate developed countries such as European countries, United States of America, South Africa and Japan, while very little is published about the problem in countries located in tropical and desert climatic zones [10,11].

Filamentous sludge bulking and foaming problems are complex problems caused by a multitude of interrelated factors. To solve sludge bulking and foaming problems it needs, on one hand, accurate identification of the types of the dominant filaments and, on the other hand, determination of the causes that have led to their over-proliferation.

---

*Corresponding author.

Conventionally, the identification methods of filamentous bacteria were based on the morphological characteristics of the filaments, which have been proven to be tedious and unreliable [9,12,13]. Fortunately, molecular techniques, which have been developed in the last decades, have revolutionized the procedures of filamentous bacteria identification and have tremendously increased the reliability of the identification results [13,14]. However, the determination of probable causes is not an easy task, since the overgrowth of filamentous bacteria is often related to many interrelated factors, including influent quality parameters, activated sludge operating variables and/or the environmental conditions [3,15]. The correlation of the filamentous bacteria types with specific causative conditions is very useful in developing specific control measures for bulking episodes [16]. However, it is often very difficult to interpret and draw meaningful conclusions from vast and complex datasets that contain huge information about the influent qualities, the system operating conditions, the effluent qualities and the information about the abundance of the identified filaments. Therefore, statistical methods, such as multivariate analysis, can help in pinpointing the most probable causes. Multivariate statistical methods have been widely used to evaluate numerous complex environmental datasets [17]. However, the use of multivariate analysis for assessing activated sludge bulking and foaming problems is relatively limited [18,19].

In this study, filamentous bacteria that dominate the Riqqa activated sludge system, in Kuwait, were identified and quantified using Vermicon Identification Technology (VIT), which is a molecular biology method [20]. To gain more insight into what had most probably led to the excessive growth of the identified filaments, Pearson's correlation analysis and principal component analysis (PCA) were then conducted.

2. Materials and methods

2.1. Plant description

Riqqa WWTP is located in the West of Al-Zahr residential area of Kuwait. It was constructed in 1981, with a treatment capacity of 85,000 m^3/d. However, the rehabilitation and upgrading in 1997 has increased its capacity to 180,000 m^3/d. Riqqa WWTP is an activated sludge plant which has no primary clarifiers. It treats mainly medium strength domestic wastewater up to a tertiary level. The plant's treatment process units consist of grit removal, aeration, clarification, sand filtration, chlorine disinfection and solids handling processes. The treated effluents are used in landscape irrigation.

2.2. Sampling and analytical techniques [20]

2.2.1. Sample collection and handling

1000 ml Grab samples of wastewater and sludge were collected weekly for 29 weeks during the period from January to August 2014. Flow meters were read and then wastewater and sludge samples were collected in sterile bottles from the aeration tank and the influent, secondary effluent and tertiary effluent streams. After in-situ measurement of temperature (Temp), electrical conductivity (EC) and hydrogen ion concentrations (pH), all of the samples were placed in an ice-box and transported for analysis within 24 h at the laboratories of Sulaibiya Research Plant (SRP) of Kuwait Institute for Scientific Research (KISR) [20].

2.2.2. Filament identification and quantification

Filamentous bacteria were identified and quantified using VIT, which is based on molecular biology principles. The following VIT kits were purchased from Vermicon Inc., Munich, Germany: VIT-1851, VIT- *H. hydrossis*, VIT-*Nocardiaform*, VIT-021N/*Thiothrix*, VIT-N. *Limicola II* and VIT-*M. parvicella.* Identification and quantification of the dominant filaments were carried out according to the manufacturer's instructions.

Leica LS2 fluorescence microscope was used to investigate the presence or absence of filamentous bacteria. Images of the filaments were captured and visualized using Leica DFC295 digital colored camera system at standard resolution of 2048 × 1536 pixels (3 megapixels). The abundance of the identified filaments was then quantified using VIT proposed scoring scale which ranges from zero to five (0: None, 1: few, 2: some, 3: many, 4: abundant and 5: excessive). To confirm the filament identification and abundance level, all of the microscopic investigations and scoring of identified filaments were conducted independently by two observers [20].

2.2.3. Determination of wastewater quality and operating variables

Water and wastewater examination standard methods [21] were used to determine the following 16 parameters for the wastewater samples that were collected from the influent and tertiary effluent streams: Temperature (Temp.), pH, electrical conductivity (EC), dissolved oxygen (DO), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), five-days biochemical oxygen demand (BOD_5), volatile fatty acids (VFAs), oil and grease concentration (O&G), total nitrogen (TN), ammonia nitrogen (NH_4), nitrate nitrogen (NO_3), nitrite nitrogen (NO_2), total phosphorous (TP) and hydrogen sulfide (H_2S). Also, the concentration of dissolved oxygen in the aeration tank (DO_{AT}) and sludge volume index (SVI) were determined from the samples that were collected from the aeration tank [20].

Portable meters were used for in-situ measurement of Temp., pH, EC and DO. An analytical balance, a Muffle furnace and an oven were used in the determination of TSS and VSS concentrations. SVI was measured using an Imhoff cone. COD concentrations were determined using an open reflux apparatus (heating mantle and reflux condenser), while BOD_5 concentrations were estimated using a DO meter and an incubator. HACH Spectrophotometer (DR 2800) was used to measure the concentrations of TN, NH_4, NO_3, NO_2, TP and H_2S.

Finally, the following operating variables of the activated sludge system were estimated from the laboratory results: hydraulic retention time (HRT), organic load (OL) and food to microorganism ratio (F/M).

2.3. Statistical analysis

The statistical analysis was performed using Matlab™ software version 7.0. Multivariate statistics techniques, namely correlation analysis (CA) and principal component analysis (PCA) were conducted. PCA was purposely used to reduce the size of the dataset in order to help identify the most probable associations between the parameters. The size of the dataset used in the analysis was a matrix of 29 rows by 28 columns. The rows represented the twenty nine sampling weeks. The 28 columns were as follows: 13 columns for the influent quality parameters (Temp., pH, EC, TSS, VSS, COD, VFAs, O&G, NH_4, NO_3, TN, TP, H_2S), 5 columns for the operating conditions (DO_{AT}, HRT, SVI, OL, F/M), 4 columns for the tertiary effluent quality parameters (TSS, COD, TN, TP) and 6 columns for the scores of the six identified filaments (*Nocardiaform actinomycetes, Microthrix parvicella, Haliscomenobacter hydrossis, Type 1851, Type 021N/Thiothrix and Nostocoida limicola II*).

CA was carried out to identify the correlations among various parameters of the dataset. It was conducted using Pearson's method, for which p-values less than or equal to 0.05 were considered to be significant.

PCA is a powerful pattern recognition technique that is commonly used to explain the variance of a large dataset of intercorrelated variables with a smaller set of independent variables (principal components, PCs) while retaining the present variations as much as possible [22]. To avoid dimensionality problems, the dataset was standardized (zero mean and unit variance) before performing the PCA. The minimum numbers of PCs that explain about 75% of total variance were extracted. Loadings (communalities) of the extracted PCs that were equal to or greater than 0.6 were considered to be significant [23]. The extracted PCs were then subjected to orthogonal rotation of axes, according to the Varimax rotation criteria in order to reduce the number of variables that exhibit a high loading. The number of iterations during the rotation of PCs was limited to 25 iterations.

3. Results

3.1. Abundance of identified filaments

Fig. 1 presents the profile of the filamentous bacteria that were identified in the samples collected from the aeration tanks of Riqqa activated sludge system [20]. This figure clearly shows that the growth of filamentous bacteria was triggered by the sharp drop in the temperature of wastewater during the first weeks of the winter season (January), but it also shows that the filaments continued to dominate the system even during the summer period (May to August). This indicates clearly that not only temperature caused the proliferation of filaments, but there were also other factors that contributed to the excessive growth of the filaments.

3.2. Correlation results

Results of the correlation analysis are presented in Table 1. Most of the filaments were found to have significant correlations (p-value > 0.5) to the influent qualities and operating conditions of the activated sludge system, except for *Nocardioform* and *Microthrix*, which were found to have

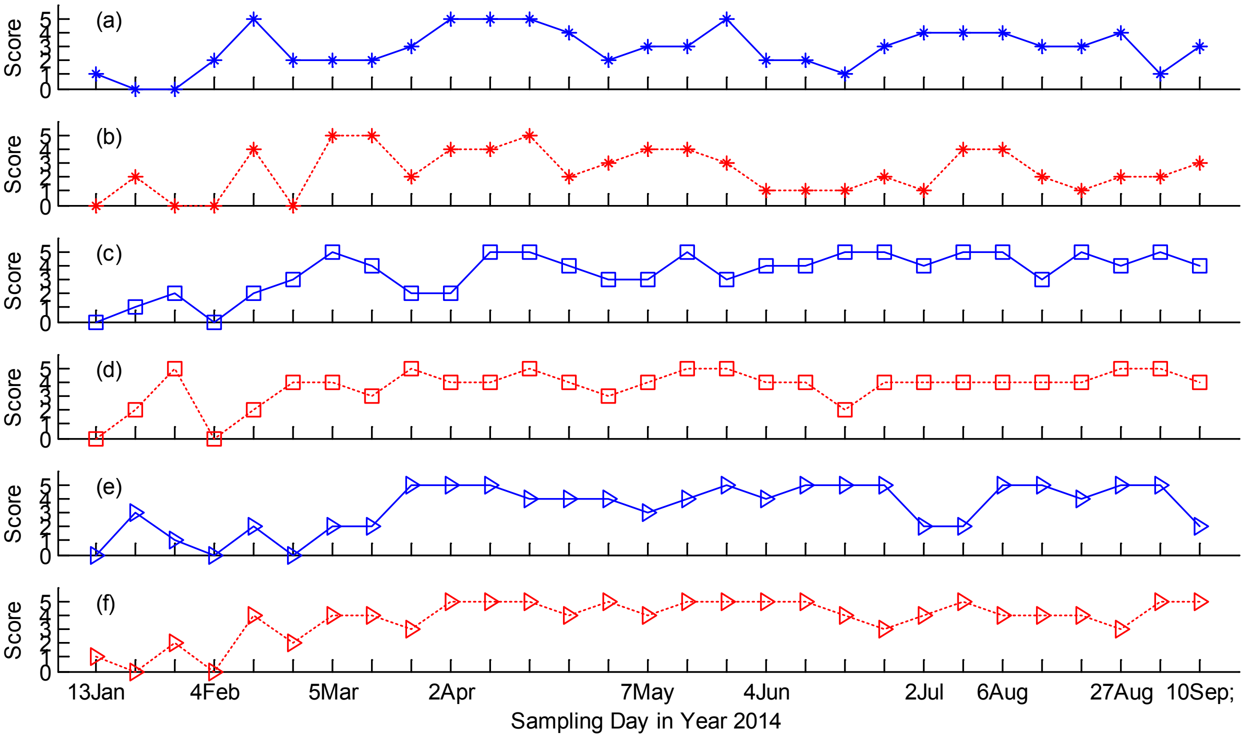

Fig. 1. Score of filamentous bacteria identified in the aeration tank Riqqa WWTP: (a) *Nocardiaform actinomycetes,* (b) *Microthrix parvicella,* (c) *Haliscomenobacter hydrossis,* (d) *Type 1851,* (e) *Type 021N/Thiothrix* and (f) *Nostocoida limicola II.* Source [20].

Table 1
Results of the correlation analysis (CA)

	Nocardia form actinomycetes	*M. parvicella*	*H. hydrossis*	*Type 1851*	*Type 021N/ Thiothrix*	*N. limicola II*
Influent quality						
Temp	0.23	0.11	0.74	0.51	0.62	0.65
pH	−0.11	0.04	−0.08	0.18	−0.05	0.12
EC	−0.02	0.01	0.5	0.21	0.2	0.28
TSS	−0.41	−0.24	−0.36	−0.1	−0.39	−0.19
VSS	−0.4	−0.26	−0.34	−0.11	−0.33	−0.15
COD	0.1	0.21	0.05	0.11	0.23	0.28
VFAs	−0.23	0.35	0.01	−0.21	−0.12	−0.05
O&G	−0.27	−0.15	−0.35	−0.43	−0.44	−0.3
NH_4–N	0.02	0.27	0.11	0.32	0.13	0.06
NO_3–N	0.02	0.16	0.02	0.26	0.11	−0.04
TN	0.21	0.11	0.33	0.57	0.4	0.38
TP	−0.41	−0.48	−0.7	−0.68	−0.59	−0.69
H2S	0.21	0.25	0.75	0.5	0.43	0.49
Operating variables						
DO_{AT}	0.13	−0.16	0.25	−0.04	0.45	0.1
HRT	0.28	0.12	0.39	0.16	0.14	0.37
SVI	−0.37	−0.09	−0.48	−0.45	−0.72	−0.54
OL	0	0.13	−0.12	0.05	0.13	0.08
F/M	0	0.26	−0.21	0.02	−0.07	−0.03
Effluent quality						
TSS	−0.16	0.17	−0.1	0.1	−0.36	−0.08
COD	−0.07	0.28	−0.04	0.1	−0.27	0.02
TN	−0.1	0.23	−0.08	−0.21	−0.4	−0.11
TP	−0.11	0.32	0.05	0.03	−0.24	−0.03

weak associations (p-value < 0.5). *H. hydrossis* abundance was found to be strongly related to the increase in Temp and H_2S, but inversely related to TP concentration of the influent. Type 1851 occurrence was found to correlate positively to the influent's Temp., TN and H_2S, while correlating negatively to the influent's TP. Growth of *Type 021N* and *Thiotrix* were also positively correlated to Temp but negatively correlated to SVI and influent's TP. The association between *N. limicola* to Temp, SVI and TP were found to be similar to that of *Type 021N* and *Thiotrix*.

Eight PCs, with eigenvalues > 1, were found to explain 83.34% of the total variance of the dataset. However, Table 2 presents six PCs, each explains 5% or more of the variance. The six PCs together explain 74.82% of variability in the dataset. As shown in Table 2, the high communality (0.7) of most parameters indicates that the selected PCs explain the variability in the dataset without losing significant information.

The first PC explains 28.07% of the total variance and has positive strong loading (> 0.6) on Temp., EC, H_2S, DO_{AT}, *H. Hydrossis* and *Type 021N/Thiothrix*. But it also has a strong negative loading on the influent's TP. The second PC account for 18.58% of the variability and has a strong positive loading on the influent's COD and the F/M ratio. The third PC, explaining 8.95% of the total variance, has a strong positive loading on influent TSS, COD, and effluent TP. It also has a strong negative loading on HRT. The fourth PC accounts for 8.10% of the total variance and has strong negative loading on NH_4–N and TN of the influent. It also has moderate negative loading on influent pH. The fifth PC explains 6.01% of the variability and has a moderate positive loading on influent TSS and VSS, but it has a negative loading on *Nocardioform* and *Microthrix*. The sixth PC, explaining 5.12% of the total variance, has moderate loading on VFAs and O&G of the influent and Effluent TN.

4. Discussion

Fig. 1 shows that the excessive growth of filamentous bacteria in Riqqa activated sludge system had started at the beginning of the winter (January). As shown in Fig. 2 there was a sharp drop in wastewater temperature from 27°C to 37°C (mean 33°C) during summer (May to August) to about 22°C at the beginning of the winter season (January to April). From these figures, it can be concluded that filamentous bacteria started to dominate Riqqa activated sludge system when wastewater temperature dropped to around 22°C. In contrast, over-proliferation of filamentous bacteria the activated sludge systems located in temperate countries

Table 2
Results of the principal component analysis (PCA)

	PC1	PC2	PC3	PC4	PC5	PC6	Communality
Variance (%)	28.07	18.58	8.95	8.10	6.01	5.12	
Cum. variance (%)	28.07	46.65	55.60	63.70	69.71	74.82	
Influent quality							
Temp.	0.80	–0.27	–0.38	0.02	–0.11	–0.25	0.93
pH	–0.03	–0.08	–0.11	–0.65	0.14	0.13	0.48
EC	0.72	–0.18	–0.31	0.20	0.14	0.08	0.71
TSS	–0.21	0.36	0.30	–0.08	0.75	–0.03	0.84
VSS	–0.20	0.38	0.24	–0.07	0.74	–0.07	0.80
COD	0.16	0.90	0.03	–0.08	0.08	–0.03	0.85
VFAs	0.02	0.15	0.30	–0.27	–0.15	0.63	0.61
O&G	–0.23	0.04	–0.01	–0.13	0.25	0.56	0.45
TN	0.07	0.22	0.16	–0.88	–0.08	0.08	0.87
NH_4–N	–0.02	0.17	–0.00	–0.87	–0.04	0.02	0.79
NO_3–N	0.19	0.35	0.26	–0.37	–0.03	–0.53	0.65
TP	–0.79	–0.16	–0.06	–0.00	0.30	0.23	0.80
H_2S	0.87	–0.05	–0.12	0.02	–0.12	0.02	0.78
Influent quality							
DO_{AT}	0.87	–0.05	–0.12	0.02	–0.12	0.02	0.63
HRT	0.21	0.05	–0.74	0.04	–0.17	0.04	0.57
SVI	0.37	–0.58	–0.26	0.02	–0.13	–0.13	0.73
OL	–0.37	–0.06	0.42	0.05	0.27	0.58	0.96
F/M	–0.03	0.96	0.14	–0.11	0.09	–0.00	0.94
Effluent quality							
TSS	–0.01	0.29	0.88	0.12	0.14	0.12	0.90
COD	0.07	0.33	0.83	0.12	0.01	0.19	0.86
TN	–0.07	0.09	0.36	–0.26	–0.08	0.74	0.77
TP	–0.10	0.12	0.66	–0.20	–0.10	0.15	0.53
Filaments							
Nocardiaform actinomycetes	0.14	0.06	–0.01	0.02	–0.71	–0.34	0.64
M. parvicella	0.34	0.15	0.36	–0.16	–0.64	0.10	0.71
H. hydrossis	0.85	–0.08	0.03	–0.08	–0.24	–0.15	0.82
Type 1851	0.59	–0.01	0.23	–0.36	–0.09	–0.57	0.86
Type 021N/Thiothrix	0.44	0.28	–0.38	–0.13	–0.40	–0.44	0.78
N. limicola II	0.70	0.13	0.06	–0.09	–0.30	–0.31	0.70

is usually triggered by a sharp drop in wastewater temperate to a degree that is well below 20°C [1,24].

Fig. 1 also shows that the filaments continued to dominate the activated sludge system despite the increase in wastewater temperature to above 35°C in summer. Thus, in contrast to what has been reported for temperate countries [19,24–26], the growth rate of the filaments in Riqqa activated sludge system was not slowed down by the increase in wastewater temperature. This implies that raising wastewater temperature, if it would be feasible, will not be an effective method to suppress the overgrowth of filamentous bacteria in Riqqa activated sludge system once it had started.

Value of sludge volume index (SVI) equal to or greater than 200 l/g is usually considered as an indication of sludge bulking [24–26]. Fig. 2 shows that at the beginning of the winter (January) SVI values were much higher than 200 l/g. However, SVI values had dropped to around 200 l/g, or even less, with the increase in wastewater temperature in April. Fig. 2 also shows that concentrations of the mixed liquor suspended solids (MLSS) had increased from about 2500 mg/l in winter to about 4000 mg/l in the summer. Accordingly; the performance of the activated sludge had significantly improved, as evident from the low COD concentration of the secondary effluent. This indicates that despite the dominance of the filaments (Fig. 1), the non-filamentous (floc-forming) bacteria had regained activity with the increase in wastewater temperature, and that in turn had led to improvement in the performance of the system.

In agreement with the above discussion, the results of correlation analysis (CA) presented in section 3.2 indicate that

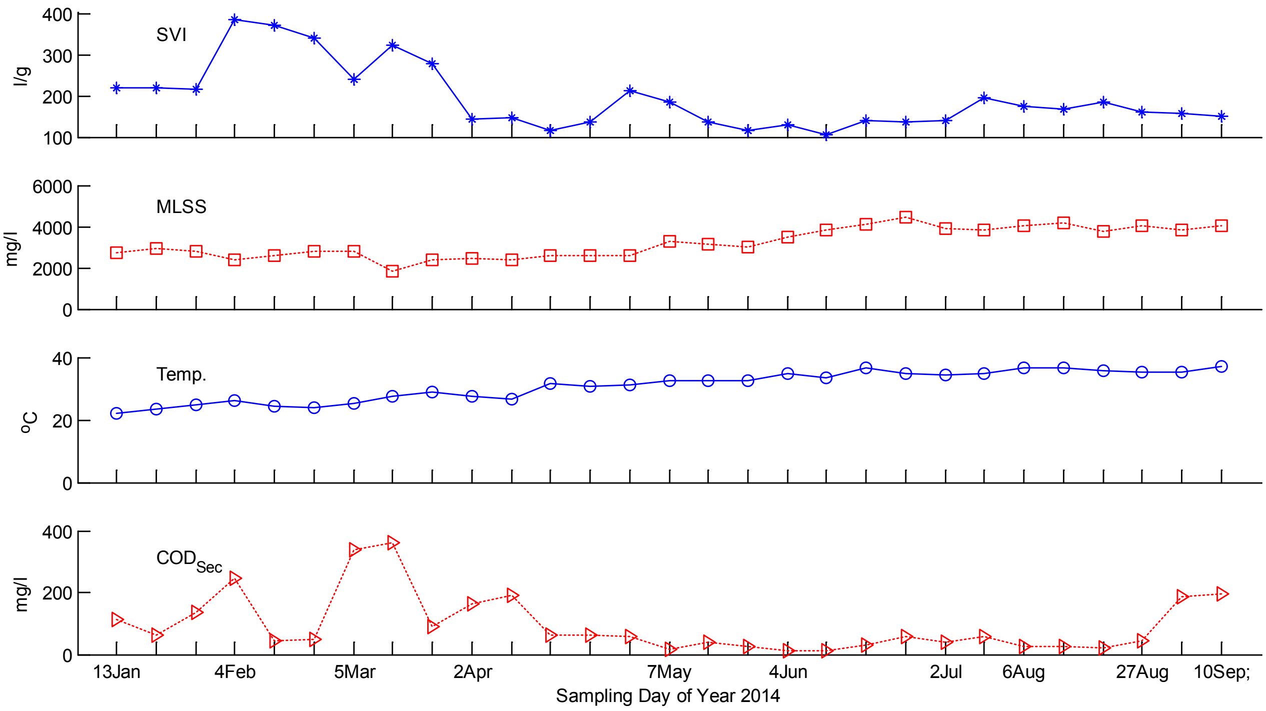

Fig. 2. Profiles of sludge volume index (SVI), mixed liquor suspended solids (MLSS), temperature (Temp) and chemical oxygen demand of the secondary effluent (COD_{Sec}) during the sampling period.

the excessive growth of the filaments was positively associated with wastewater temperature and inversely related to sludge settleability, measured as SVI (Table 1). As shown by MLSS concentrations presented in Fig. 2, the non-filamentous (floc-forming) bacteria was adversely affected by the sharp drop in the temperature of wastewater in January. That seemingly gave more chance to the filamentous bacteria to over-proliferate the Riqqa system. The same figure also shows that MLSS concentrations had started to grow with the increase in the wastewater temperature in May.

The correlation results also showed that abundance of the filaments was either directly or inversely related to concentration of TP and TN. This indicated that excess of nutrients as well as nutrients' deficiency had played significant roles in the overgrowth of filamentous bacteria. It is well-known that nutrient deficiency is related to the proliferation of some types of filamentous bacteria. However, it has been reported that presences of excess nutrients also favor certain filaments. For example, *H. hydrossis* and *Microthrix* flourish at excess NH_4-N [1, 19]. Therefore, adjustment of nutrients amounts in the influent can be an effective control for the overgrowth of filamentous bacteria in Riqqa activated sludge system.

Moreover, the correlation results also indicated that the excessive growth of the filaments in Riqqa activated sludge system can be attributed to wastewater septicity (indicated by the presence of fatty acids or reduced sulfur compounds e.g. H_2S). These particular results indicate that *H. hydrossis* and *Type 1851* were strongly correlated to H_2S, which is in agreement with several studies [2,3,13,16,27–30].

Generally, the obtained PCA results have confirmed the correlation results. Specifically, they confirmed that proliferation of the filaments was strongly related to the increase in wastewater temperature, high concentration of sulfide (septicity) and both nutrient excess and nutrient deficiency. However, PCA results have also showed that dominance of *Nocardioforms actinomycetes* and *Microthrix parvicella* was inversely related to the concentrations of TSS and VSS in the influent. Eikelboom et al. [26] also found that *Microthrix parvicella* favors hydrolyzed particulates (i.e. less concentration of solids).

Although this study is about the problem of the excessive growth of filamentous bacteria in Riqqa WWTP, the obtained results could be used as a reference for preventing excessive growth of filamentous bacteria in other WWTPs situated in hot climatic conditions since very little is published about the dynamics of filamentous bacteria in these conditions.

5. Conclusions

For having more insights into the proliferation and the probable causes of the excessive growth of filamentous bacteria at Riqqa-activated sludge system, multivariate statistical analysis, namely correlation analysis (CA) and principal component analysis (PCA), was conducted.

The results of CA indicated that the following significant correlations:

- Excessive growth of filamentous bacteria was positively associated with wastewater temperature but inversely related to sludge settleability (SVI).
- Wastewater septicity is another factor for the abundance of filamentous bacteria.

- Both excess nutrients and nutrients deficiency had played important role in the dominance of filamentous bacteria.

The results of PCA have confirmed all the results obtained from CA. Further, they have indicated that the growth of *Nocardioforms actinomycetes* and *Microthrix parvicella* was inversely related to the concentrations of TSS and VSS in the influent.

Acknowledgments

The study was supported by the Kuwait Foundation for the Advancement of Sciences (KFAS) under the Code: 2012-1405-02.

References

[1] P. Madoni, D. Davoli, G. Gibin, Survey of filamentous microorganisms from bulking and foaming activated sludge in Italy, Wat. Res., 34 (2000) 1767–1772.

[2] D. Jenkins, M.G. Richard, G.T. Daigger, Manual on the Causes and Control of Activated Sludge Bulking and Foaming, Lewis Publishers, New York, USA, 1993.

[3] H. Chua, K.Y. Le, A survey of filaments foaming in activated sludge plants in Hong Kong, Water Sci. Technol., 30 (1994), 251–254.

[4] MetCalf, Eddy, Wastewater Engineering Treatment, Disposal and Reuse, 4rd ed. New York, McGraw-Hill Publishing Company, Ltd., 2003.

[5] P.H. Nielsen, C. Kragelind, R.J. Seviour, J.L. Nielsen, Identity and ecophysiology of filamentous bacteria in activated sludge, FEMS Microbiol. Rev., 33 (2009) 969–998.

[6] S. Posavac, T.L. Dragicevic M.Z. Hren, The improvement of dairy wastewater efficiency by the addition of bio-activator, Mljekarstvo, 60 (2010) 198–206.

[7] Z. Li, T. Zhang, N. Li, X. Wang, Granulation of filamentous microorganisms in sequencing batch reactor with saline wastewater, J. Env. Sci., 22 (2010), 62–67.

[8] D. Soltysik, I. Bednarek, S. Galka, D. Sypniewski, Repetitive extragenic palindromic PCR (REP-PCR) as a method used for bulking process detection in activated sludge, Env. Mon. Assess., 176 (2011) 343–354.

[9] A.M.P. Martins, C. Picioreanu, J.J. Heijnen, Three-dimensional dual-morphotype species modeling of activated sludge flocs, Env. Sci. Technol., 38 (2004) 5632–5641.

[10] T. Mino, Survey on filamentous microorganisms in activated sludge processes in Bangkok, Thailand, Wat. Sci. Technol., 31 (1995) 193–202.

[11] S.M. Faheem, M.A. Khan, A study on filamentous bacteria in activated sludge process of sewage treatment plant in Dubai, United Arab Emirates. Wat. Pract. Technol., 4 (2009) 1–8.

[12] A.T. Miekczrek, C. Kragelund, P.S. Eriksen, H.P. Nielsen, Population dynamics of filamentous bacteria in Danish wastewater treatment plants with nutrient removals, Wat. Res., 46 (2012) 3781–3795.

[13] E. Fourest, D. Craperi, C. Deschamps-Roupert, J.L. Pisicchio, G. Lenon, Occurrence and control of filamentous bulking in aerated wastewater treatment plants of the French paper industry, Water Sci. Technol., 50 (2004) 29–37.

[14] J.L. Sanz, T. Kochling, Molecular biology techniques used in wastewater treatment - an overview. Proc. Biochem., 42 (2007) 119–113.

[15] V. Tandoi, D. Jenkin, J. Wanner, Activated Sludge Separation Problems. IWA Publishing, London, 2006.

[16] M.S. Switzenbaum, T.R. Plante, B.K. Woodworth, Filamentous bulking in Massachusetts: Extent of the problem and case studies, Wat. Sci. Technol., 25 (1992) 265–271.

[17] R. Noori, M.S. Sabahi, A.R. Karbassi, A. Baghvand, H.T. Zadel, Multivariate statistical analysis of surface water quality based on correlations and variations in the dataset. Desalination, 260 (2010) 129–136.

[18] L.A. Santos, V.D. Ferreira, M.M. Neto, M.A. Perreira, M. Mota, A. Nicolau, Study of 16 Portuguese activated sludge systems based on filamentous bacteria populations and their relationships with environmental parameters, Appl. Microbiol. Biotechnol., 99 (2015) 5307–5316.

[19] C. Cao, L. Lou, Analysis of environmental variables on population dynamic change of Haliscomenobacter hydrossis – the bulking causative filament in Macau wastewater treatment plant. Desal. Wat. Treat., 57 (2016) 7182–7195.

[20] A. Abusam, F. Al-Salameen, A. Mydlarczyk, M.I. Ahmed, Filamentous bacteria identification by VIT method, I.J. Env. Sci. Develop., 7 (2016) 177–180.

[21] Standard Methods for the Examination of Water and Wastewater. 21st edition, American Public Health Association, Baltimore, Maryland, USA, 2012.

[22] P.K. Hope, Receptor Modeling in Environmental Chemistry, Wiley, USA, 1985.

[23] J.F. Hair, R.E. Anderson, W.C. Lack, Multivariate data analysis, N.J., US, Prentice Hall, Englewood Cliffs, 1995.

[24] J. Wanner, I. Ruzicková, P. Jetmarová, O. Krhutková, J. Paraniaková, A national survey of activated sludge separation problems in the Czech Republic: Filaments, floc characteristics and activated sludge metabolic properties, Wat. Sci. Technol., 37 (1998) 271–279.

[25] O. Krhutková, I. Ruzicková, J. Wanner, Microbial evaluation of activated sludge and filamentous population at eight Czech nutrient removal activated sludge plants during year 2000, Wat. Sci. Technol., 46 (2002) 471–478.

[26] L. Graveleau, E. Cotteux, P. Dochene, Bulking and foaming in France – the 1999–2001 survey, Acta Hydrochim. Hydrobiol., 33 (2005) 223–231.

[27] D.H. Eikelboom, A. Andreadakis, K. Andreasen, Survey of filamentous populations in nutrient removal plants in four European countries, Wat. Sci. Technol., 37 (1998) 281–289.

[28] M.J. Donkin, Statistical analysis to associate specific microorganism populations with operating conditions and treatment performance in laboratory-scale systems treating diary processing wastewater. Env. Technol., 20 (1999) 1085–1093.

[29] W.D. Marizo, First results from a screening of filamentous organisms present in Buenos Aires's activated sludge plants, Wat. Sci. Technol., 46 (2002) 119–122.

[30] C. Pelletier, M.A. Fitzsimmons, S. Deschenes, M. Paice, Impact of septic compounds and operational conditions on the microbiology of an activated sludge system. Wat. Sci. Technol., 55 (2007) 135–142.