



Seasonal Variations of the Growth of Filamentous Bacteria in Kuwait's Wastewater Treatment plants

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Overview

- Introduction
- Materials & Methods
- Results and Discussion
- Conclusions

Introduction


- ❑ Filamentous bacteria grow naturally in activated sludge systems.
- ❑ They provide the backbone for other types of bacteria to grow
- ❑ However, excessive filamentous sludge bulking and foaming are the most common operational problems of activated sludge systems.
- ❑ If not properly controlled, excessive sludge bulking and foaming can lead to a complete failure of the entire wastewater treatment process.
- ❑ Usually filamentous bacteria are identified through conventional microscopic method, which is laborious and unreliable.
- ❑ In this study, however, we used the German technology VIT, which is based on molecular biology techniques, to identify and quantify the filamentous bacteria dominating Kuwait's wastewater treatment plants.

Materials and Methods

- ❑ Grab samples of wastewater and sludge were collected weekly from four locations along Riqqa and Umm-Al-Haiman activated sludge systems: influents, aeration tank, secondary effluent and tertiary effluent streams.
- ❑ Routine wastewater quality parameters were also determined following APHA (2012) method. Accordingly, the system operating variables were then calculated.
- ❑ Filamentous bacteria were identified using the following six Vermicon Identification Technology (VIT) kits: VIT-1851, VIT-H. hydrossis, VIT-Nocardiaform, VIT-021N/Thiothrix, VIT-N. Limicola II and VIT-M. parvicella.
- ❑ Filaments identification method used was according to Vermicon's instructions
- ❑ Filaments quantification (scoring) was also according to Vermicon's instructions: 0: None, 1: few, 2: some, 3: many, 4: abundant and 5: excessive.

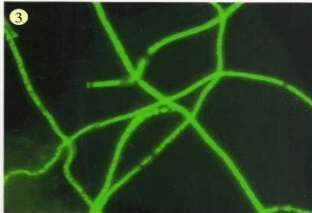
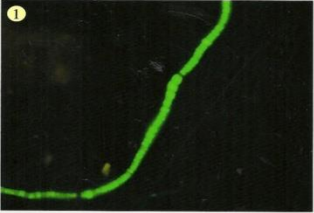
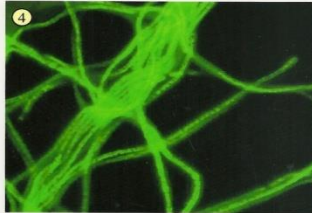
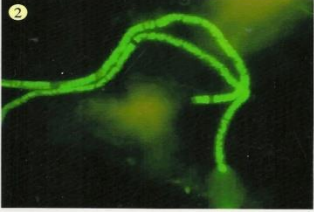
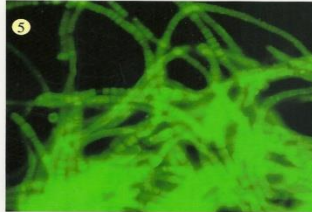
Cont. Materials and Methods

Fluorescence images



Blue light

All cells that light up **green** are viable Eikelboom Type 021N filaments.

Evaluation
Analyse 20 arbitrary visual fields using the VIT-key: each visual field must be assigned a value according to the amount of cells present (1-5). The numeric average is calculated by dividing the total sum of the values by the number of evaluated visual fields.

For example:
 $(6 \times \text{group 4}) + (5 \times \text{group 3}) + (6 \times \text{group 2}) + (3 \times \text{group 1}) = 24 + 15 + 12 + 3 = 54$;
 $54 / 20 = 2.7$
 According to the VIT-system, the level of Type 021N present in this sample has a value of 2.7.

Amount of cells:


- ① Few
- ② Some
- ③ Many
- ④ Abundant
- ⑤ Excessive

VIT-Key

VIT-1851

Microscopic Results


Positive control:
All viable bacteria light up red under green light.



Negative control:
No cells light up.

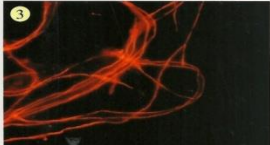



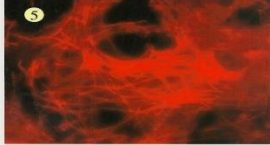
VIT-well with a positive result

Fluorescence images



Green light

All cells that light up **red** are viable Eikelboom Type 1851 filaments.

Evaluation
Analyse 20 arbitrary visual fields using the VIT-key: each visual field must be assigned a value according to the amount of cells present (1-5). The numeric average is calculated by dividing the total sum of the values by the number of evaluated visual fields.

For example:
 $(6 \times \text{group 4}) + (5 \times \text{group 3}) + (6 \times \text{group 2}) + (3 \times \text{group 1}) = 24 + 15 + 12 + 3 = 54$;
 $54 / 20 = 2.7$
 According to the VIT-system, the level of Type 1851 present in this sample has a value of 2.7.

Amount of cells:

- ① Few
- ② Some
- ③ Many
- ④ Abundant
- ⑤ Excessive

Cont. Materials and Methods

VIT-Key

vermicon
solutions for microbiology

VIT-Haliscomenobacter

Microscopic Results

Positive control:
All viable bacteria light up red under green light.



Negative control:
No cells light up.

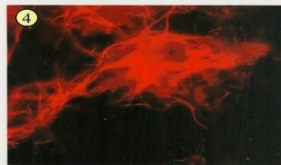
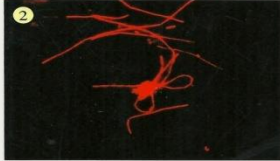
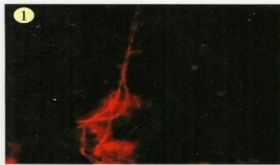
VIT-well with a positive result

Fluorescence images



Green light

All cells that light up red are viable *Haliscomenobacter hydrossis* filaments.



Evaluation

Analyse 20 arbitrary visual fields using the VIT-key: each visual field must be assigned a value according to the amount of cells present (1-5). The numeric average is calculated by dividing the total sum of the values by the number of evaluated visual fields.

For example:
(6 x group 4) + (5 x group 3) + (6 x group 2) + (3 x group 1) = 24+15+12+3 = 54;
54/20 = 2.7.

According to the VIT-system, the level of *Haliscomenobacter hydrossis* present in this sample has a value of 2.7.

Amount of cells:

- ① Few
- ② Some
- ③ Many
- ④ Abundant
- ⑤ Excessive

VIT-Key

vermicon
solutions for microbiology

VIT-Microthrix

Microscopic Results

Positive control:
All viable bacteria (especially Gram-positive cells not destroyed by the Breaker) light up red under green light.



Negative control:
No cells light up.

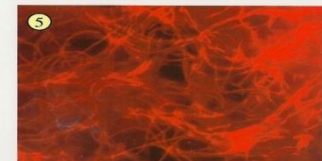
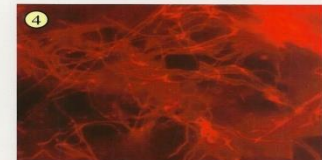
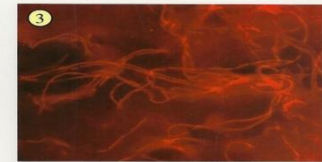
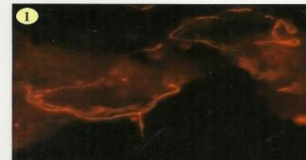
VIT-well with a positive result

Fluorescence images



Green light

All cells that light up red are viable *Microthrix parvicella* species.



Evaluation

Analyse 20 arbitrary visual fields using the VIT-key: each visual field must be assigned a value according to the amount of cells present (1-5). The numeric average is calculated by dividing the total sum of the values by the number of evaluated visual fields.

For example:
(6 x group 4) + (5 x group 3) + (6 x group 2) + (3 x group 1) = 24+15+12+3 = 54;
54/20 = 2.7.

According to the VIT-system, the level of *Microthrix parvicella* filaments present in this sample has a value of 2.7.

Amount of cells:

- ① Few
- ② Some
- ③ Many
- ④ Abundant
- ⑤ Excessive

Cont. Materials and Methods

VIT-Key

vermicon
solutions for microbiology

VIT-Nocardia

Microscopic Results

Positive control:
All viable bacteria (especially Gram-positive cells not destroyed by the Breaker) light up red under green light.



Negative control:
No cells light up.

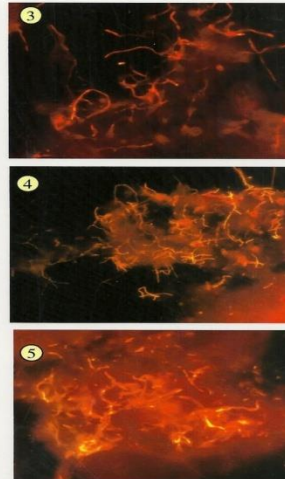
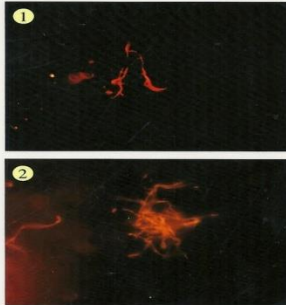
VIT-well with a positive result

Fluorescence images



Green light

All cells that light up **red** are viable **nocardioform actinomycetes**.



Evaluation

Analyse 20 arbitrary visual fields using the VIT-key: each visual field must be assigned a value according to the amount of cells present (1-5). The numeric average is calculated by dividing the total sum of the values by the number of evaluated visual fields.

For example:
 $(6 \times \text{group 4}) + (5 \times \text{group 3}) + (6 \times \text{group 2}) + (3 \times \text{group 1}) = 24 + 15 + 12 + 3 = 54$
 $54/20 = 2.7$

According to the VIT-system, the level of nocardioform actinomycetes present in this sample has a value of 2.7.

Amount of cells:

- ① Few
- ② Some
- ③ Many
- ④ Abundant
- ⑤ Excessive

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VIT-Key

vermicon
solutions for microbiology

VIT-Nostocoida limicola II

Microscopic Results

Positive control:
All viable bacteria light up red under green light.



Negative control:
No cells light up.

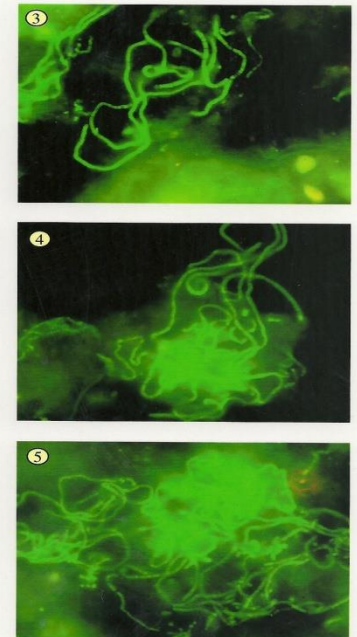
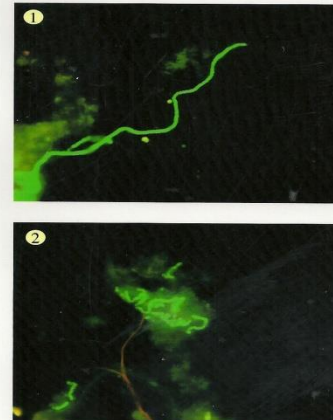
VIT-well with a positive result

Fluorescence images



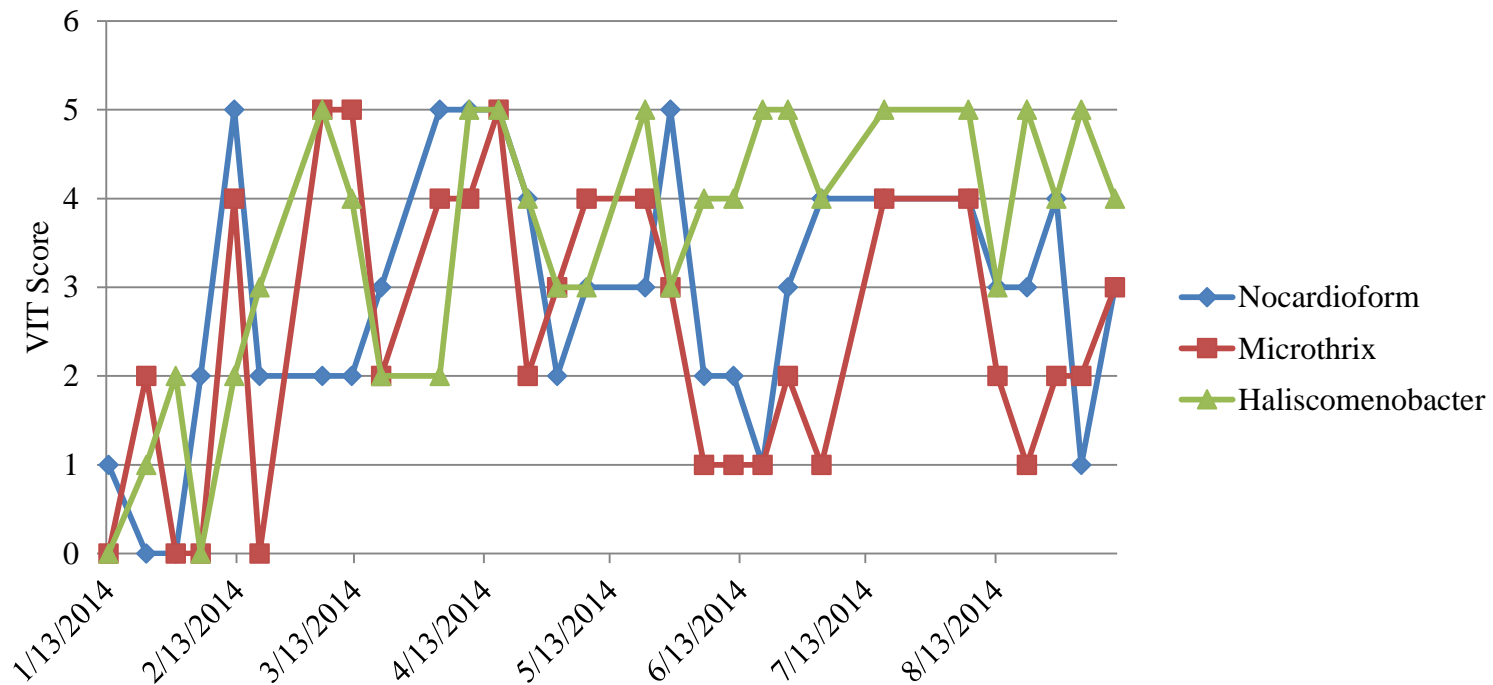
Blue light

All cells that light up **green** are viable **Nostocoida limicola II** filaments.



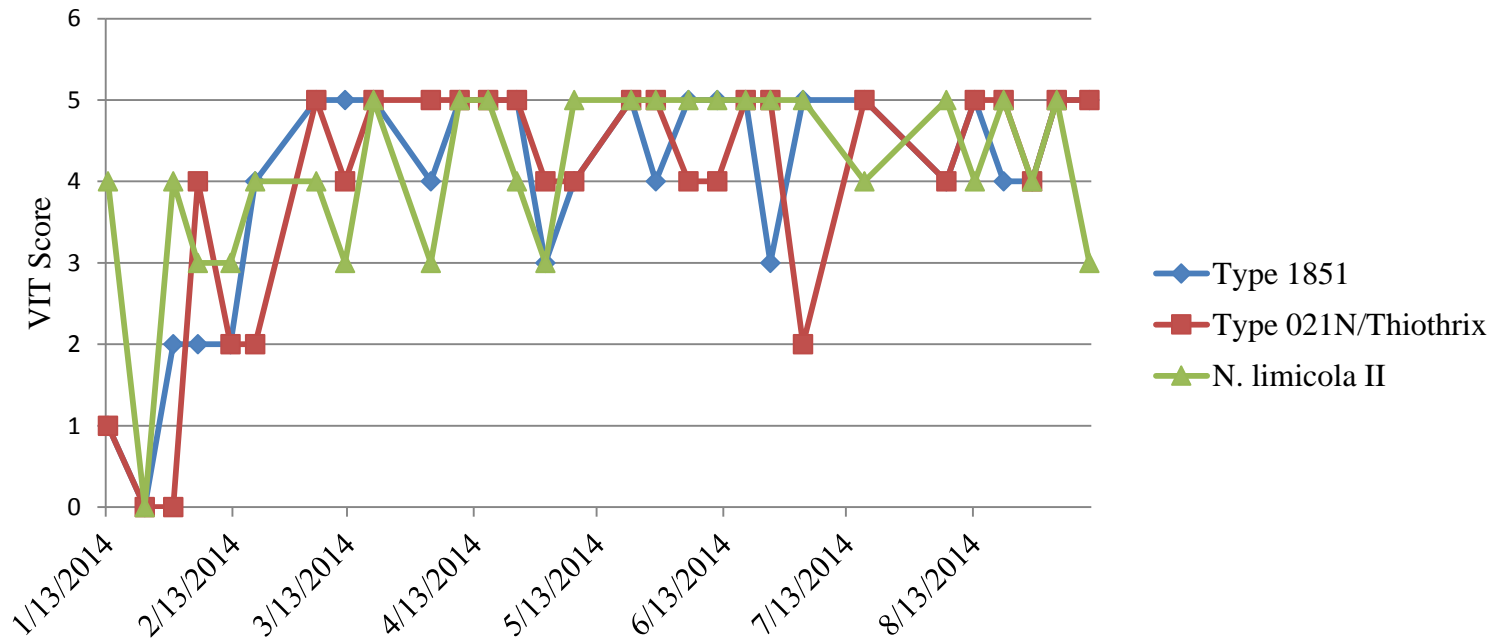
Results

Score of filamentous bacteria identified in Riqqa Aeration tank.



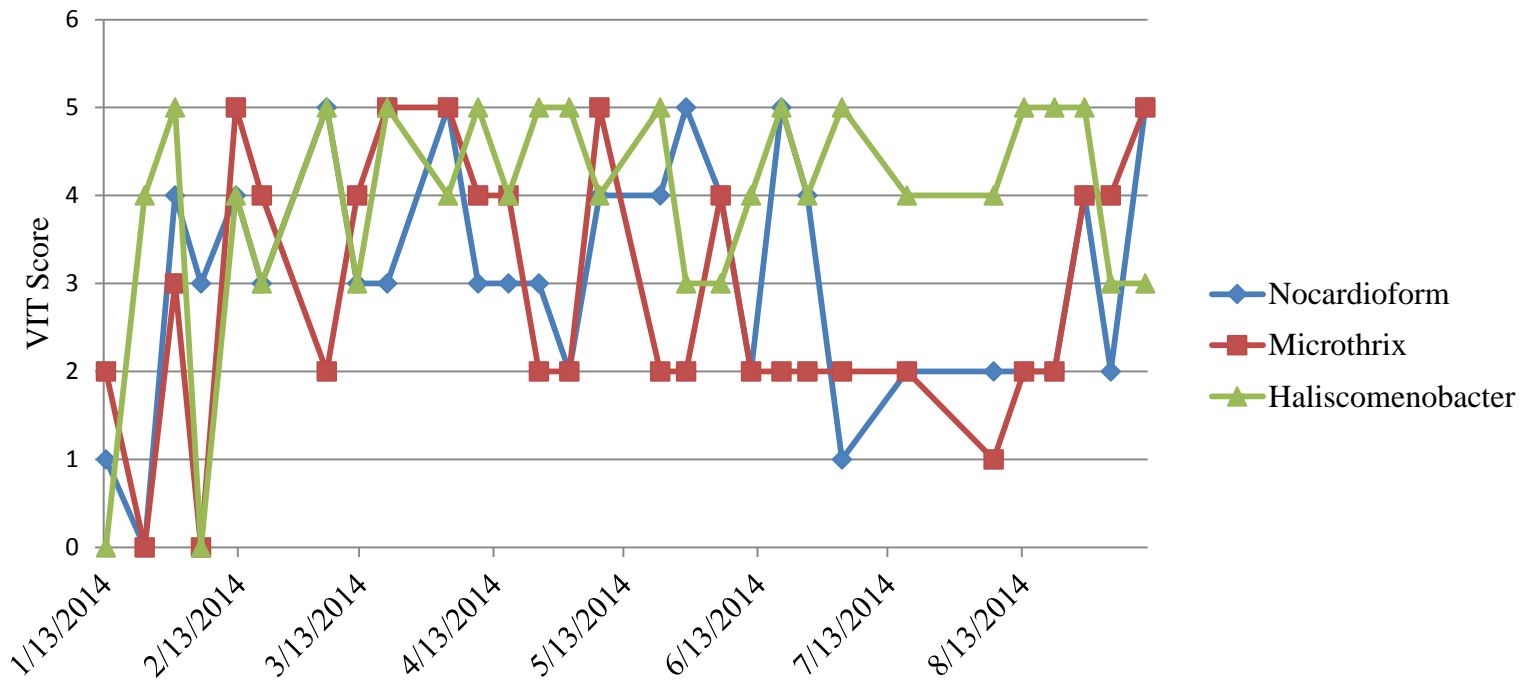
Cont. Results

Score of filamentous bacteria identified in Riqqa Aeration tank
Cont.



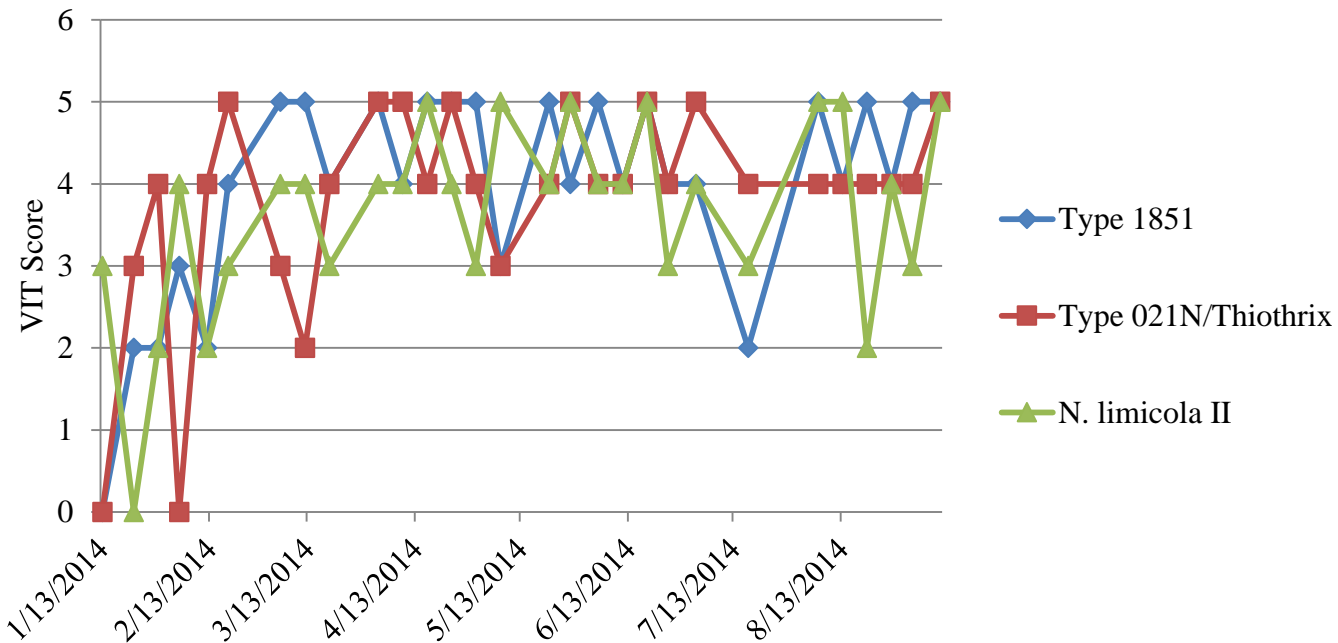
Cont. Results

Score of filamentous bacteria identified in Umm-Al-Haiman Aeration tank.



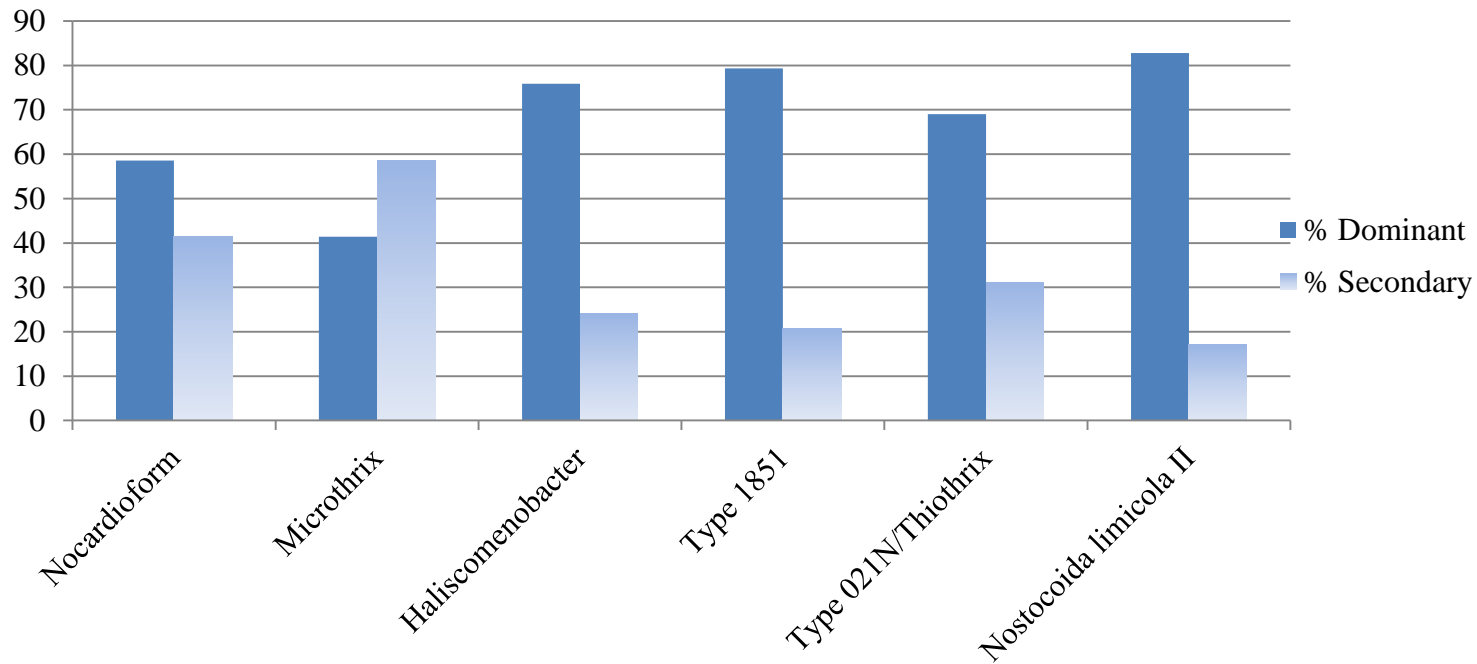
Cont. Results

Score of filamentous bacteria identified in Umm-Al-Haiman Aeration tank Cont.



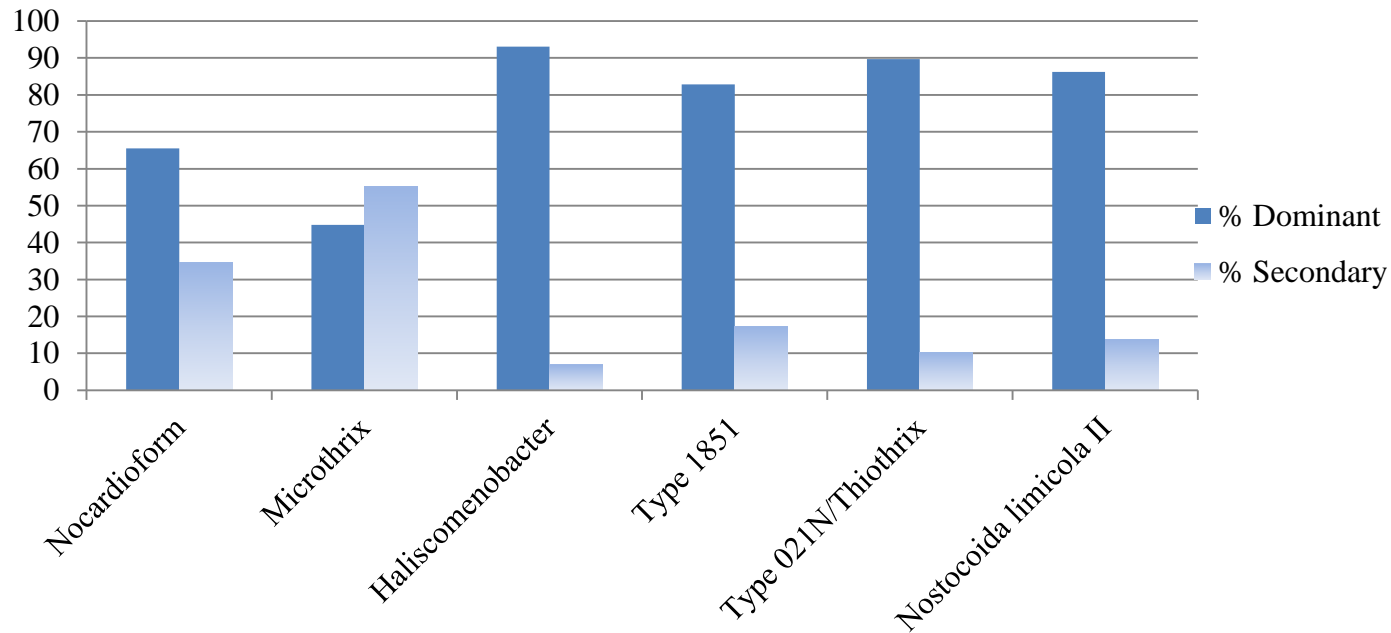
Cont. Results

Percent of time dominance of filaments in Riqqa system



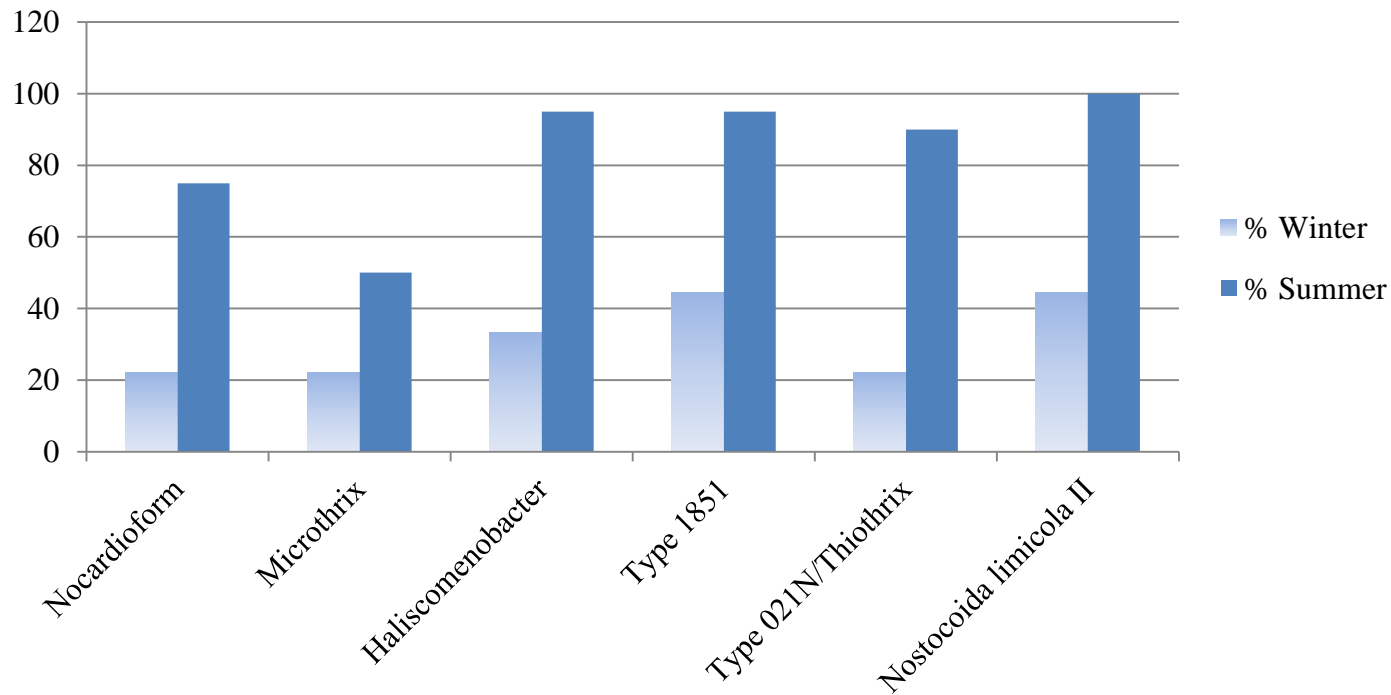
Cont. Results

Percent of time dominance of filaments in Umm-Al-Haiman system



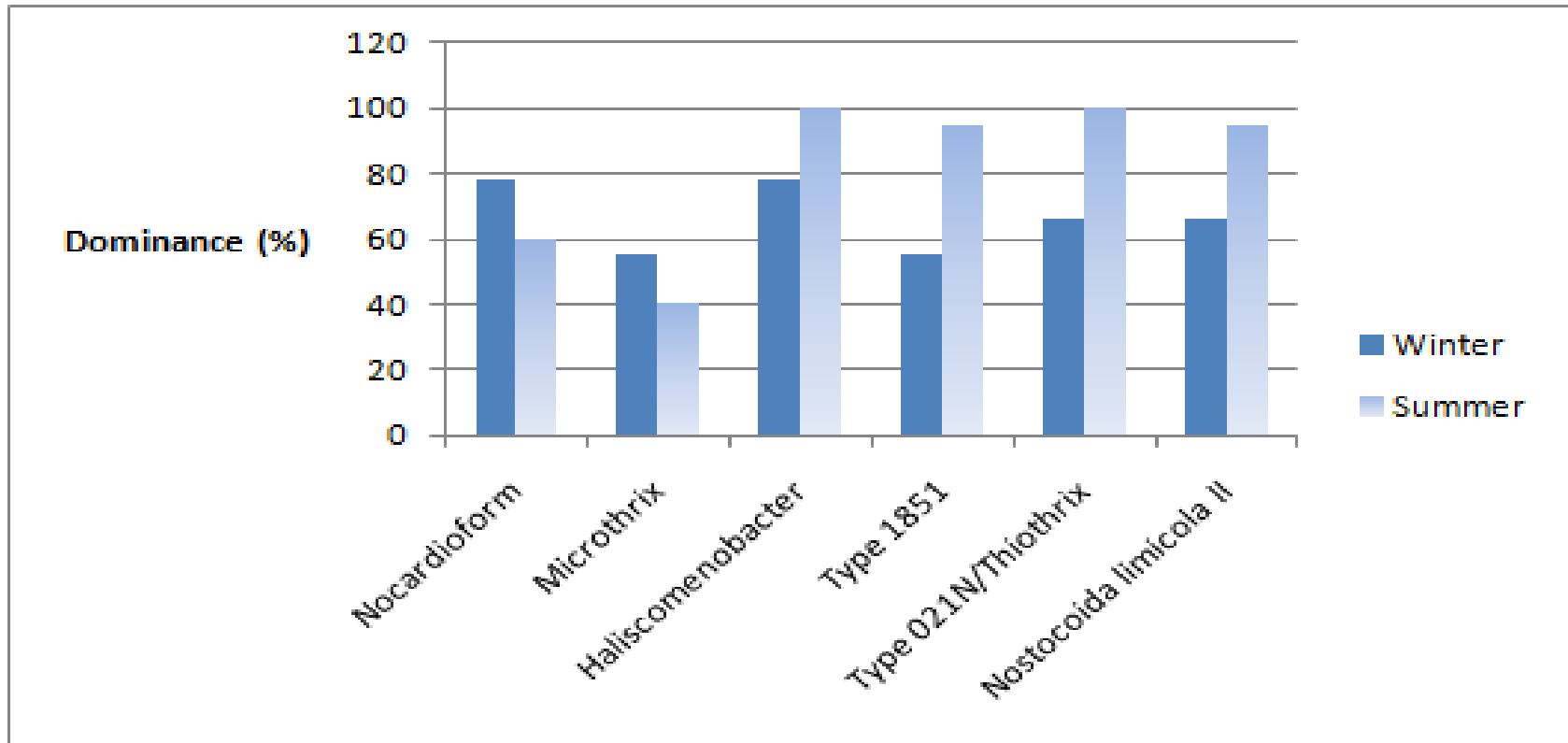
Cont. Results

Seasonal dominance of filaments in Riqqa system



Cont. Results

Seasonal dominance of filaments in Umm-Al-Haiman system



Conclusions

- ❑ The types of filamentous bacteria dominating in Riqqa and Umm Al-Haiman-activated sludge systems were identified using VIT kits (Vermicon Identification Technology, Munich, Germany).
- ❑ An analysis of the identification results obtained indicated that the following four types of filamentous bacteria were dominant (> 70% of observation time) in both systems: *N. limicola* II, Type 1851, *Haliscombacter* and *Nacordioform*.
- ❑ Dominance of these filaments was found to be higher during the summer season than the winter season

Acknowledgements

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