EXECUTIVE SUMMARY

Activated sludge process (ASP) is an important biological stage in sewage treatment process. ASP comprises a consortium of macro and microorganisms known collectively as activated sludge that convert wastewater in-organics and organics into a flocculent microbial suspension that settles well in a conventional gravity clarifier. The species of microorganism that dominates a system depends on environmental conditions, process design, the mode of plant operation, and the characteristics of the secondary influent wastewater. The microorganisms that are of greatest numerical importance in activated sludge are aerobic and anaerobic bacteria which play a major role in the ASP. While both heterotrophic and autotrophic bacteria reside in activated sludge, the former predominate.

Filamentous bacteria are of major concern today and are the know indicators of sludge bulking and foaming which significantly influence the treatment efficiency. Filamentous bulking (excessive growth of filamentous bacteria give rise to a bulking sludge with SVI of >150 ml/g) is the number one cause of effluent noncompliance today. Filamentous bacteria are also known to cause activated sludge foaming which is most commonly due to organisms like Nocardia and Microthrix. Foam occurs as a thick stable "scum" on aeration basin and final clarifier surfaces. This foam consists of activated sludge solids containing large amounts of Nocardia filaments growing from their surface and is quite stable, compared to most other foams, due to the physical interlocking of the filaments. Severe foaming causes a number of operational problems. These include aesthetics, odors, and safety hazards when it overflows basins and cover walkways and handrails. Foam may also escape into the effluent and increase the suspended solids of the effluent.

The detection of bulking and foaming bacteria generally is performed by conventional
activated sludge microscopy, which is based on morphology description and different staining properties. Further, modern molecular technique called Fluorescent in-situ hybridization (FISH) is also successfully applied in the identification protocols. Once identified, filament types could be studied by cultivation dependent conventional microbiological methods. Knowledge on the causative filaments is extremely useful and is basic in charting out strategic control measures appropriate to each filament.

Dubai sewage treatment plant (DSTP) is the only wastewater treatment facility, serving above 1400000 inhabitants, with the capacity of treating about 260,000 m$^3$ of mostly domestic wastewater on the daily basis. Like several other activated sludge treatment plants in the world, DSTP too suffers sludge bulking and foaming. These problems are causing difficulty in further treatment by biological filters and also in removal of ammonia leading to effluent with unsettled flocs rendering it unfit for reuse. Till date, no information describing filamentous populations has been available on sewage treatment plants in UAE. It is therefore felt necessary that identification and characterization of DSTP specific filamentous bacteria is a necessary approach in understanding the problems and thus improvising the efficiency of the treatment plant.

The present study was focused on investigation of the filamentous bacterial community in bulking and foaming DSTP activated sludge system with the objective of determining and analyzing the dominant filament type occurring in DSTP activated sludge samples over a six months period by direct microscopy and Fluorescent In-Situ Hybridization (FISH) technique. Further more, isolation, cultivation, characterization of filamentous bacteria and identification of pure cultures isolates by FISH is also attempted.

Morphological characteristics analysis of foam and mixed liquor samples revealed occurrence of several filamentous bacteria identified as nocardioform species, Thiothrix,
Type 021N, *Sphaerotilus natans*, *Beggiaota*, *Nostocoida limicola* type I and occasional attached growth forms of *Eikelboom* type 0041/1851. A particular filament type belonging to nocardioform actinomycete group was found to be dominant and constantly present in all the samples irrespective of variable wastewater characteristics and seasonal variations. Culture independent approach based FISH analysis confirmed the identity of the gram variable, truly branched dominant filament type as *nocardia amarae* like organism (NALO). It was concluded that specific filamentous bacteria populations in mixed liquor and foaming activated sludge were constant and not dependent on. Further more, 16 filamentous isolates were obtained in pure cultures from the DSTP activated sludge samples and characterized with respect to morphology, physiology and biochemical activity. FISH technique was applied to all the pure cultures isolates to screen for NALOs.

Out of all the isolates, 12 were found to be gram positive rods forming filaments and remaining as gram negative filaments. At least 10 isolates were successfully identified as nocardioform actinomycetes by applying nocardioform specific probes in FISH technique. It was observed that nocardioform isolates exhibited variable morphological form (filamentous to non filamentous single celled appearance). The optimum growth of most of the isolates was found to be at 27°C and neutral pH. Most of the isolates were diverse in utilizing various sugars and sugar substrates as carbon source. The results indicated that NALO are quite diverse in their growth pattern, physiological and biochemical characteristics and could be of significance for further studies to investigate their possible role in bulking and foaming of activated sludge process at DSTP.

This study evaluated the microbial community structure in the activated sludge system of DSTP. The population changes of the major groups like proteobacteria (alpha,
beta and gamma). High GC, Low GC and other groups of microbes were analyzed using FISH. The samples tested were taken from foaming sludge and mixed liquor of activated sludge system and a nocardioform actinomycete group member was found to be dominating in the system. This group of suspected nocardioform actinomycete belonged to the High GC group of bacteria that was targeted by HGC69a and MNP1. However, this group of nocardioform actinomycete exhibited both branched and single cell morphotypes. The second largest dominating group belonged to Gamma sub-class of proteobacteria. Majority of the bacteria targeted by Gam42a probe were filamentous in their morphology indicating that they were probably Thiothrix or Type 021N or both. Specific probes such as SNA (Sphaerotilus natans), LDI (Leptothrix sp), LMU (Leucothrix sp), HHY (Haliscomenobacter sp), TNI (Thiothrix), 021N (Type 021N) and MPA60 (Microthrix parvicella) failed to hybridize in the sludge samples. However, a few of these filaments were observed in the samples. The sludge samples containing nocardioform populations detected by probe MNP1, Myc657 and HGC69a were further analyzed by hybridization with the Gordona amarae and genus Gordona specific probes. These two probes failed to detect bacterial populations in the activated-sludge sample from the Dubai sewage treatment plant indicating that Gordona amarae was not the dominant foam causing bacteria in Dubai STP. Out of 16 isolates, 10 were successfully hybridized by both HGC69a and MNP1 indicating that these isolates were nocardioform actinomycetes members. At least eight isolates hybridized with Myc657 probe indicating that these isolates were mycolic acid containing actinomycetes. However, all the isolates failed to hybridize with Gordona amarae and genus Gordona specific probe meaning that none of these isolates belonged to Gordona genus.