International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 04 Issue: 11 | Nov -2017 www.irjet.net p-ISSN: 2395-0072

A REVIEW OF OCCURRENCE, QUANTIFICATION AND ABATEMENT OF LEGIONELLA IN WASTEWATER TREATMENT PLANTS

N. Mani[1*], M. Usman[2], G. Andiego[3], M. Waseem[4]

[1]Jounior Scientist, Sahasrara Earth services & Resources Ltd, Coimbatore, India
[2]PhD Scholar Institute of water resources and water supply, TUHH Hamburg, Am Schwarzenberg-Campus 3 (E) 21073, Germany
[3]Director, Water resources and environmental services department, P.O. Box 35760 -00100, Nairobi, Kenya
[4]PhD Scholar, Faculty of agriculture and environmental sciences, Satower Straße 48, 18059, Universität Rostock, Germany

---

Abstract - *Legionella is a group of bacteria that is found all-over in artificial and natural environments. Certain species of the Legionella were identified as potential human pathogens. It is important to identify the different species of Legionella that can grow in virtual environments, their suitable growing conditions and methods to reduce their occurrence and to identify the preventive measures needed to lessen the occurrence of Legionella. Certain events forced for an investigation to detect the conditions in waste water treatment plants (WWTPs) of Taiwan, paper mills of Sweden with biological treatment plants (BTPs), and a Petrochemical plant in Pas-de-Calais, France. Investigations revealed that the conditions were positive for the growth of Legionella. In Taiwan and Sweden, Legionella was measured from waste water samples, where as in France the Legionella was analyzed from air samples. The dominating species of Legionella found in WWTPs of Taiwan were L. donaldsonii and uncultured L. spp (species). In the investigated BTPs of Sweden, Legionella concentration was found to be as high as 10^9 CFU/liter. In France, Legionella bacteria's airborne transmissions were extended over the distance of 6 km from the source. Certain measures like proper maintenance of waste water basin, cooling towers (CT) and installing Chlorination process in WWTPs reduce the concentration of Legionella.*

Key Words: Human health, Legionella, Wastewater treatment, Cooling Tower

1. INTRODUCTION

Legionella are known as the gram- negative bacteria which are etiological agents of Pontiac fever and Legionnaires disease (Nazarian et al. 2008). More than 50 species of Legionella are characterized today and human illness is associated with approximately half of Legionella species. Human infections occur only through inhaling contaminated aerosols. Inter human transmissions are yet to be found. Legionella have the capacity of reproducing between the temperatures 25^{0}C and 43^{0}C and they can survive up to 55-60^{0}C (Huang S. W. et al. 2009). Therefore, it is evident that they are all-over in artificial aquatic and natural environments such as lakes and ponds, rivers, swimming pools, water distribution systems and cooling towers (Hsu et al. 2006). In order to improve the monitoring of Legionella, several methods have been formulated. Recent molecular methods such as PCR help in identifying Legionella by specifically targeting regions within RNA or DNA (Morio et al. 2008). To understand Legionella's prevalence, correlations between water quality parameters and occurrence of Legionella needs to be investigated (Yu et al. 2008).

1.1 Source of Investigation

At present, only little considerations are given to the potential outbreak of large Legionella in Taiwan. Hence there was no data regarding Legionella's occurrence in WWTPs of Taiwan, an investigation was carried out to analyze the present situation in WWTPs (Huang S. W. et al. 2009). In Pas-de-Calais, France, a case of Legionnaire's disease was reported to the public-health authorities as a person had an infection with L. Pneumophila serogroup 1 on November 28, 2003. Local environmental authority, revealed the occurrence of Legionella bacteria in high levels in the cooling towers of a petrochemical plant located in Harnes was the possible source for the outbreak of Legionnaire's disease (Nguyen T.M.N et al. 2006) and incase of Sweden, a worker at a paper mill with a BTP was found to have developed Legionella disease (Allestam G et al. 2006).

1.2 Case Identification

In Sweden, during December 2004 the BTP was sampled and this plant was found to be one of the several viable sources of infection. From the epidemiological investigation it was evident that there were no Legionella in the showers at worker's resident. But, the showers at work revealed the presence of Legionella pneumophila of serogroup 4 at fewer counts. But, the patient's isolate was found to be L. pneumophila of serogroup 1 subtype

Benidorm, which was different from the one found at work (Allestam G et al. 2006).

In France, Legionnaire's disease was detected from a person, who became ill during 1 November 2003 - 31 January 2004. From the laboratory report he was confirmed of having an infection with Legionella pneumophila serogroup 1. The investigation's objectives were to find the sources of transmission, identify the magnitude of outbreak, to determine the risk factors for Legionella disease acquisition, to prevent transmission by implementing control measures (Nguyen T.M.N et al. 2006).

1.3 Study Location and Sample Collection

In order to determine the current distribution of Legionella in WWTPs of Taiwan, 41 water samples were collected from 17 different WWTPs during September 2005 and 2006 March. There were 4 different waste water sources, including hospital wastewater, integrated wastewater, domestic wastewater and industrial wastewater. In order to find the potential outbreak of Legionella, the wastewater treatment processes cleaning frequency, surrounding environment, and parameters of physical, microbiological and chemical water quality were analyzed. The WWTPs samples included aeration tank, raw wastewater and effluent wastewater (Huang S. W. et al. 2009). All paper mills with BTP in Sweden were sampled for the presence of Legionella. Out of 66 paper mills, 43 have installed BTPs. During September 2005 and October 2005, each BTP in the 43 paper mills of Sweden were systematically sampled. Cooling towers, bio sludge and aeration pond outlets were the sample points (Allestam G et al. 2006).

In Pas-de-Calais, France a petrochemical plant with powerful industrial CTs was analyzed to detect the air borne spread and concentration of the Legionella bacteria. Sampling points were cooling towers, wastewater and aerosols (Nguyen T.M.N et al. 2006).

2. METHODS

In WWTPs of Taiwan, the samples which were taken from waste water sources, including hospital wastewater, integrated wastewater, domestic wastewater and industrial wastewater were tested along with WWTP samples from aeration tank, raw wastewater and effluent wastewater using polymerase chain reaction (PCR) method. PCR method amplifies DNA in several orders magnitude by generating millions of particular DNA sequence (Huang S. W. et al. 2009). In case of Sweden, the samples were taken from Cooling towers, bio sludge and aeration pond outlets and combined pre – treatment method was used to reduce the growth of Legionella (Allestam G et al. 2006). Where as in France, the sampling points were cooling towers, wastewater and aerosols (Nguyen T.M.N et al. 2006).

2.1 Microbial Investigation

In Taiwan, all the samples were measured to 1000ml and the samples were placed inside a sterile polypropylene bottle. The samples were first filtered, eluted and finally centrifuged to concentrate Legionella. The samples were then filtered through cellulose nitrate membranes of 45mm diameter with 0.45mm pore size in a filter holder made of stainless steel. The filter was then scraped and phosphate-buffered saline (eluting fluid) was used to wash the collected materials. It was then transferred into conical centrifuge tube of 50ml and centrifuged. Total genomic DNA was extracted according to kit manual and the suspension was then analyzed to detect the presence of specific genes of Legionella by semi-nested PCR (Huang S. W. et al. 2009). In case of France, from the environmental samples, Legionella bacteria were separated and isolated in accordance to the procedure of standard AFNOR NFT90-431. Using direct immune fluorescence and by random amplified polymorphic DNA PCR, the Legionella bacteria were identified to serogroup and to the species (Nguyen T.M.N et al. 2006).

2.2 Pre-treatment

In Sweden, the samples underwent combined pre-treatment to reduce the growth of heterotrophic/atypical micro flora along with the ISO 11731 standard method for isolating the Legionella bacteria. Pretreatment included heat treatment of 30 minutes at 50^0C. Then the samples were diluted for 5 minutes in acid buffer having pH 2.2 to reduce background growth (Allestam G et al. 2006).

2.3 Case Control Study

In Pas-de-Calais, France, to identify the risk factors associated to activities, personal characteristics and potential exposure towards contaminated aerosols, a case-control study had been initiated. From municipal electoral list the controls were selected randomly. Controls and cases were interviewed after the illness of case. Standardized questionnaire was made with 150 variables needed 3 important information: personal characteristics and medical history, living and housing conditions and exposure in 10 days before illness of case and daily outdoor activities (Nguyen T.M.N et al. 2006).

2.4 Cultivation Method

Legionella Cultivation is an ISO approved method for determination of water quality (Horng et al. 2006). In France, to reduce the growth of micro flora, combined pre-treatment was added to standard method (Allestam G et al. 2006).

2.5 Environmental Investigations

In France, Cooling towers and industrial cooling systems in epidemic zone was derived from census done by local environmental authority. Record reviews, water sampling and site inspections were done on these installations, also on additional potential contamination sources. These included wastewater treatment plants, wells, decorative fountains, canals, car-wash stations, air-conditioning systems in the public buildings and other installations which are capable of dissemination of aerosols. Preventive decontamination was performed on the installations that had Legionella bacteria of the levels ≥ 1000 cfu/L. Air samples were taken with a microbial air sampler prototype (Nguyen T.M.N et al. 2006).

2.6 Modeling of Aerosol Dispersion

Presumably infectious aerosols dispersion was simulated from cooling towers of petrochemical plant by ADMS 3, a Gaussian dispersion model (Nguyen T.M.N et al. 2006).

3. RESULTS

In Taiwan, out of 17 WWTPs which was investigated, the Legionella were discovered in 10 WWTPs (58.8%). Out of the collected samples 41, the Legionella were identified in 25 samples (61%). In the hospital, integrated, domestic and industrial wastewater systems: 7/12 (58.3%), 13/18 (72.2%), 3/4 (75.0%) and 2/7 (28.6%) of samples were found to be positive for Legionella. The pH and average temperature was in the range of 7.6 to 8.4 and 22.18C to 24.98C respectively. The average pH values in the industrial waste water systems were found to be 8.4 which were much higher compared to other waste water systems (Huang S. W. et al. 2009). Most of the pathogenic species of Legionella were found in the hospital waste water samples. L. pneumophila were identified from the hospital and integrated waste water samples.

From the survey, five species of Legionella were identified. The dominating species of Legionella found in WWTPs were L. donaldsonii and uncultured L. spp. Both the species of Legionella were detected in 10 samples (i.e. 24.4% of total samples). L. lytica and L. pneumophila were found in 4.9% of total samples. The scarcely found species of Legionella was L. anisa which was detected in only one sample (2.4%). Except the uncultured L. spp., all the identified Legionella species causes Legionellosis. The most pathogenic species are the L. pneumophila, which are responsible for more than 80% of legionellosis outbreaks around the world (Huang S. W. et al. 2009).

3.1 Correlation between Occurrence of Legionella and Physical-Chemical Parameters

Legionella species were discovered in water with the temperature range of 22˚c to 45˚c. Legionella's highest incidence was detected in samples with the temperatures spanning from 15 - 20˚C (3/4, 75.0%), 20–25˚C (8/12, 66.7%), followed by 25–30˚C (14/25, 56.0%). Positive Legionella samples were taken from weakly alkaline (pH 7–9, 61.3%) and weakly acidic water (pH 5–7, 71.4%). L. pneumophila, which is the most pathogenic species was found in water samples which had the temperature of 25–30˚C with pH of 7–9 (Huang S. W. et al. 2009).

3.2 Correlation of the Occurrence of Legionella with Microbiological Parameters

In the water samples of dental units and cooling towers, total amount of bacteria in Legionella-positive samples were lower than Legionella-negative samples. In Legionella-positive samples, the average heterotrophic (3.2 £ 106 CFU/ml) plate counts were lower than Legionella-negative samples (4.3 £ 106 CFU/ml) (Huang S. W. et al. 2009). In Sweden, Legionella were isolated at sampling points from 65% of paper mills which reveal the frequent occurrence of Legionella bacteria in the BTPs. However, only 3 cooling towers out of 12 had the presence of Legionella. Extremely high concentrations of Legionella were found in more than 50 % of aeration ponds. In 14% of aeration ponds, Legionella concentration higher than 10^8/liter was determined. The dominating type found in the BTP was L. pneumophila sero group (sg) 2-14 and L. pneumophila sg 1 was found in five BTPs. In several BTPs, mixed Legionella population was found. Bio sludge samples that had undergone the process of high temperature composting over a period of 6 months showed Legionella concentration of high counts up to $10^5/g_{ww}$ (Allestam G et al. 2006).

Common factor in all BTPs which promoted the heavy growth of Legionella was the temperature of 38±4˚c which was given as daily mean of inlet water. Legionella was not found when the temperature of the inlet water was lower than 30^0C. Apart from BTPs, growth of Legionella was discovered in wastewater basin which was located in a petrochemical industry in France. Cooling towers and wastewater basins were found to be the main cause of outbreak and aerosols were spread over long distance (Allestam G et al. 2006).

3.3 Descriptive Epidemiology

In France, out of 104 clinical cases, 86 were diagnosed with Legionella Disease. The center of outbreak was found to be Harnes (16.7/10,000 population) where the highest epidemic was observed. In Harnes or in adjacent 3 communes 40 cases lived (47%), in 15 communes that are within radius of 6km of Harnes, 70 cases lived (81%) and in 22 communes that are within the radius of 12 km of Harnes 83 cases lived (97%). From the case-control study carried out at France, 177 controls and 59 confirmed cases were included. Response rate among the controls and cases are 72% (177/246) and 91% (59/ 65) respectively (Nguyen T.M.N et al. 2006).

3.4 Environmental and Microbiological Investigation

In France, the numbers of tested/positive samples in installations and sites were as follows: 43/97 in wastewater treatment plants, 44/610 in CTs, 6/68 in case's homes, 5/165 in car wash stations respectively. 238 out of 260 identified environmental isolates were L. pneumophila. From the conclusion of environmental risk assessment in France;

1) For the bio film formation, configuration of cooling circuit was highly prone; thereby the cleaning operations were made inadequate for eliminating Legionella bacteria,

2) The contact surface of entire cooling tower was not reached by high-pressure cleaning and contaminated aerosols might have generated in large amounts,

3) The restarting conditions of cooling towers were favorable for airborne release and for Legionella contaminated bio film detaching,

4) Considerable number of Legionella bacteria present in the waste water basin of plant could have become airborne in the aerosols which were created by surface ventilators,

5) Legionella bacteria's airborne transmissions were extended over the distance of 6 km from the source (Nguyen T.M.N et al. 2006).

4 DISCUSSIONS

In Taiwan, the samples were analyzed to identify the Legionella using PCR instead of culturing methods. PCR method was used since the culture method was inappropriate for finding Legionella in the samples of waste water because the indigenous bacteria in the culture helps Legionella to overgrow rapidly. PCR only detects the dominant Legionella species in every water samples. Due to this limitation of sequencing/PCR method, the Legionella species at low concentrations were not been able to detect. In aquatic environments the species of Legionella were detected frequently, but in the extreme conditions of WWTPs their occurrence was not evaluated to date. Therefore, long term investigation should be conducted to find the Legionella occurrence in extreme conditions. Chlorination process is not employed in WWTPs; hence the final effluents still have pathogenic Legionella species. Therefore, chlorination process should be installed compulsorily in WWTPs (Huang S. W. et al. 2009).

In Sweden, the researchers didn't identify any linear correlation with factors such as retention time in aeration pond, treatment plant type, age and amount of sludge, iron sulphate used for flocculation of return sludge and conductivity. It is necessary to do more research in order to find: risk level for visitors and workers and the people who live nearby, how far the Legionella can be spread through air, Legionella bacteria's environmental origin in paper mills with BTPs and to make efficient BTP's at lower temperature (Allestam G et al. 2006).

In France, ineffective and hazardous control measures, powerful industrial cooling towers and environment contamination in extensive level of petrochemical plant resulted together in Legionnaire's disease community-wide outbreak. In the cooling circuit of CT, it is very likeable to be created a biofilm formation, making cleaning Legionella bacteria out of the system very difficult. The operating conditions which favored the Legionella growth in biological treatment were:

1) Uninterrupted contamination of Legionella from its reservoir in waste water basin,

2) Massive airborne Legionella-laden aerosols release by cooling towers,

3) Inappropriate control measures were used, which includes the use of high pressure hazardous cleaning of cooling system,

4) Meteorological conditions with flat terrain favored the airborne dissemination of respirable droplets of Legionella-laden over a large area.

After the outbreak, new regulations regarding the maintenance and installation of cooling towers were issued by authorities, also the guidelines for management and investigation of the risk related with Legionella bacteria. Intervention and operation of cooling tower at the petrochemical plant were only temporally associated with outbreak (Nguyen T.M.N et al. 2006).

5 CONCLUSIONS

Chlorination process should be installed in WWTPs, so that the final effluents won't contain pathogenic Legionella species in it. Respiratory protective masks should be worn mandatorily by workers when they are working in cooling towers, WTP's and BTP's to minimize the spread of the Legionnaire's disease. Maintenance and regular cleaning of cooling towers and waste water basin is recommended. Effective control measures should be taken in powerful industrial cooling towers to produce fewer aerosols in order to reduce the occurrence of Legionella. Long-term investigations should be conducted to find the linear correlation with factors such as retention time in aeration pond, treatment plant type, age and amount of sludge, iron sulphate used for flocculation of return sludge and conductivity.

ACKNOWLEDGEMENT

We would like to thank ever body who has helped and guided us towards improvement of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Allestam G., de Jong B., Långmark J.:Biological treatment of industrial wastewater: a possible source of Legionella infection. In Legionella State of Art 30 Years after Its Recognition. Edited by: Cianciotto NP, Abu Kwaik Y, Edelstein PH, Fields BS, Geary DF, Harrison TG, Joseph CA, Ratcliff RM, Stout JE, Swanson MS. Washington (DC): ASM Press; 2006, 493-496.

2. Horng, Y.T., Soo, P.C., Shen, B.J., Hung, Y.L., Lo, K.Y., Su, H.P., Wei, J.R., Hsieh, S.C., Hsueh, P.R. and Lai, H.C., (2006). Development of an improved PCR-ICT hybrid assay for direct detection of Legionellae and Legionella pneumophila from cooling tower water specimens Water research, 40 (11). 2221-2229.

3. Huang S. W., Hsu B. M., Ma P. H., Chien K.T. (2009). Legionella prevalence in wastewater treatment plants of Taiwan. Water Science and Technology 60(5), 1303-1310.

4. Hsu, B. M., Chen, C. H., Wan, M. T., & Cheng, H. W. (2006). Legionella prevalence in hot spring recreation areas of Taiwan. Water research, 40(17), 3267-3273.

5. Nazarian, E. J., Bopp, D. J., Saylors, A., Limberger, R. J., & Musser, K. A. (2008). Design and implementation of a protocol for the detection of Legionella in clinical and environmental samples. Diagnostic microbiology and infectious disease, 62(2), 125-132.

6. Morio, F., Corvec, S., Caroff, N., Le Gallou, F., Drugeon, H., & Reynaud, A. (2008). Real-time PCR assay for the detection and quantification of Legionella pneumophila in environmental water samples: utility for daily practice. International journal of hygiene and environmental health, 211(3), 403-411.

7. Nguyen T.M.N., Ilef D., Jarraud S., Rouil L., Campese C., Che D., Haeghebaert S., Ganiayre F., Marcel F., Etienne J., Desenclos J.-C. (2006). A community-wide outbreak of legionnaires disease linked to industrial cooling towers - How far can contaminated aerosols spread? The Journal of Infectious Diseases 193, 102-111.

8. Yu, P.Y., Lin, Y.E., Lin, W.R., Shih, H.Y., Chuang, Y.C., Ben, R.J., Huang, W.K., Chen, Y.S., Liu, Y.C., Chang, F.Y. and Yen, M.Y., (2008). The high prevalence of Legionella pneumophila contamination in hospital potable water systems in Taiwan: implications for hospital infection control in Asia. International Journal of Infectious Diseases, 12(4), 416-420.