Tracking *Legionella* in air generated from a biological treatment plant - A case study of the outbreak of legionellosis in Norway-

Janet Martha Blatny[1*], Jaran Strand Olsen[1], Øyvind Andreassen[1], Viggo Waagen[2] and Bjørn Anders Pettersson Reif[1]

[1]Norwegian Defence Research Establishment (FFI), P. O. Box 25, N-2027 Kjeller, Norway
[2]Borregaard Ind. Ltd., P. O. Box 162, N-1701 Sarpsborg, Norway

ABSTRACT

Two outbreaks of legionellosis occurred in the Sarpsborg/Fredrikstad region southeast of Norway in 2005 and 2008 where more than 60 exposed individuals were infected and 10 case patients died. The air scrubber at Borregaard, a wood-based chemical factory, was identified as the outbreak source. High concentration levels of *Legionella* species, including the etiological agent *L. pneumophila* SG1 was found in the aeration ponds, which belongs to Borregaard's biological treatment plant. Results showed that these ponds were able to generate *Legionella*-containing aerosols that were transported by the wind as such aerosols were measured up to 200 meters downwind of the pond. Our studies did not detect *L. pneumophila* SG1 isolates, only *L. pneumophila* SG4 during the air sampling measurement campaign. Furthermore, the operational conditions of the air scrubber proved to be harsh for *Legionella* growth as the outbreak *L. pneumophila* strains were not able to grow at 45ºC and pH8 (conditions during the outbreaks). These results, together, lead us to suggest that the aeration pond should be regarded as the primary amplifier and disseminator of *Legionella* and *L. pneumophila* and thereby most likely being the outbreak source.

Keywords: biological treatment plant, legionellosis, aerosol, air scrubber, Norway

1. INTRODUCTION

Borregaard, located in Sarpsborg, Norway (about 90 km south of Oslo) is the world's leading supplier of wood-based chemicals and selected niche products for the food ingredients and pharmaceutical industries. The company was established in 1889 with the construction of a cellulose and paper mill, and has today 1300 employees at production units and sales offices in 20 countries in Europe, America, Asia and Africa. Organic substances present in industrial wastewater originating from such paper mills must be degraded in order to reduce the impact these substances have on the ecosystem. Thus, all wastewater from Borregaard's wood refinement processing at Sarpsborg is biologically treated according to environmental requirements legislated by the The Climate and Pollution Agency before it is released into the river Glomma nearby the wastewater treatment plant. Glomma is the largest river in Norway with a water flow of 200 to more than 2000 m^3/sec.

The biological treatment facility at Borregaard, Sarpsborg, consists of two large aeration ponds containing 30 000 m^3 of liquid (Figure 1). The liquid is kept at approximately 37ºC to promote optimal growth of several types of microorganisms achieving efficient degradation of various organic substances. As about 30 000 m^3 of air is circulated through each pond per hour (20ºC), significant aerosolization takes place. Various *Legionella* species are present in these aeration ponds at concentration levels up to 10^{10} CFU/L and similar levels have also been detected at Swedish biological treatment plants (1, 2). *Legionella pneumophila* is the etiological agent of Legionnaires' disease (or legionellosis) and of the non-pneumonic Pontiac fever. In addition to *L. pneumophila*,

[1] *Corresponding author: Janet Martha Blatny, e-mail: janet-martha.blatny@ffi.no, tel: +47 63807827/+47 41615740

Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XII,
Edited by Augustus W. Fountain III, Patrick J. Gardner, Proc. of SPIE Vol. 8018, 801808
 · doi: 10.1117/12.886932

more than 49 different *Legionella* species have been described in which 19 species may cause infections in humans (3, 4). The species *L. pneumophila* contains at least 16 serogroups, in which serogroup 1 (SG1) is most frequently associated with legionellosis. Outbreaks of legionellosis caused by *L. pneumophila* SG1 have been traced back to various anthropogenic aerosol generating sources (5, 6 and 7), exemplified by industrial cooling towers, sanitary landfill sites, wastewater treatment plants, and evaporate condensers.

Figure 1. One of two aeration ponds taking part in the biological degradation of organic substances in wastewater from Borregaard's production processes, Sarpsborg, Norway. (Photo: FFI, 2006)

In May 2005, an outbreak of legionellosis caused by *L. pneumophila* SG1 occurred in the Sarpsborg/Fredrikstad region in Norway, and the source was identified as an air scrubber at the wood-based chemical factory Borregaard (8). This was the first time an air scrubber had been identified as the dispersion source of *L. pneumophila*. During this outbreak, 56 people were infected over an area of approximately 1200 km^2 and ten died (8), in which the latter were elderly persons (68-94 years) with underlying medical conditions. 32 and 19 of the case patients had their residence in Sarpsborg and Fredrikstad, respectively, while five were visitors. It has later been stated that approximately additional 50 patients were infected by the 2005 outbreak strain (www.fhi.no). In November/December 2005, three new cases of legionellosis occurred in the same region, caused by the same *L. pneumophila* strain as in the outbreak in May 2005; ST15 (9). This strain was identified to be identical to the *L. pneumophila* Lens strain responsible for the outbreak in Pas-de-Calais, France, in 2003/2004 and where the cooling tower at a petrochemical factory was identified as outbreak source (5). Five additional cases of legionellosis were reported in Sarpsborg/Fredrikstad in June/July 2008, where the air scrubber, once again, was identified as being involved for disease transmission (10). These five patients were aged 51-84 years in which two died (underlying diseases and age > 80 years). Four of the five patients had been in the neighbouring area of Borregaard at distances 300 meters – 3 km. Based on the outbreak investigation studies both from Pas-de-Calais and Sarpsborg/Fredrikstad it was speculated that that infectious *Legionella* spp. could travel more than ten kilometres in air (5, 8). Furthermore, one year after the 2005 outbreak, a seroepidemiological study measuring levels of immunoglobulin G (IgG) and IgM antibodies to *L. pneumophila* was performed and increased levels of IgG and IgM antibodies were found in employees working proximal to the aeration ponds compared to those working more than 200 meters away (11). Interestingly, no employees at Borregaard and within a distance of 200 meters were diagnosed with legionellosis.

2. SAMPLING AND IDENTIFICATION OF *LEGIONELLA* IN AIR

To investigate whether *Legionella*-containing aerosols could be generated from Borregaard's aeration ponds and further transported and dispersed by wind, a comprehensive experiment using Computational Fluid Dynamics (CFD) as a planning tool was performed (Figure 2). This study was initiated in 2006 (2). The transport of water droplets, potentially containing *Legionella,* dispersed from the aeration ponds was estimated using CFD, and the results were used as a basis for selecting optimal sampling sites for air collection in the vicinity of the aeration ponds. The biological treatment plant at Borregaard and its surrounding cover an area of approximately 1 km x 1 km and 24 different wind directions were used in order to cover the full circle of 360 degrees. A steady state Reynolds Averaged Navier Stokes (RANS) approach was used and based on historical weather data a typically wind speed of 2 m/s was used in all 24 cases. The computational model was simplified by neglecting thermal (buoyancy) effects and the environmental fate of biological aerosols. The aerosols originating from the aeration ponds were treated as passive tracers following the local wind direction. The aerosol properties were assumed to remain constant during the transport. A map of optimal air sampling locations was thus obtained according to each of the 24 different wind directions.

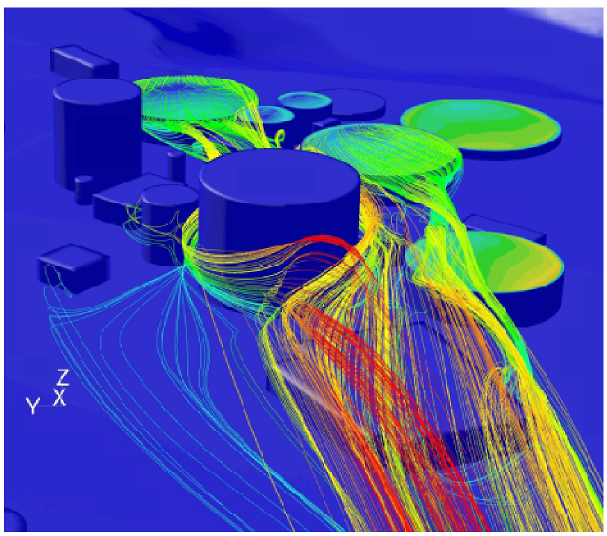


Figure 2. Computed paths of particles dispersed from the aeration ponds at Borregaard (2). The wind direction is from east to west (270 degrees) and wind speed at 2 m/s. Colors indicate altitudes relative to the aeration ponds; blue - low, red – high.

Air sampling was performed by using the wetted-wall cyclone SASS 2000^{PLUS} and the impactors MAS-100 and STA-204. Air was collected at 10 different dates during Sept 11 – Dec 5, 2006, at varying weather conditions. 26 sampling sites were selected using CFD modeling and sampling was performed at up to 300 meters from the aeration ponds and various altitudes (0-64 m). Air sampling was always performed upwind, directly above and downwind of the aeration ponds. Specific detection of airborne *Legionella* spp, including *L. pneumophila,* was obtained using microbiological (specific growth analysis) and molecular analysis. The *mip* gene was used as a target to specifically detected *L. pneumophila* by real-time PCR, while 16S rDNA sequencing was performed on non-*L. pneumophila* colonies to identify other *Legionella* spp sampled from air. Results showed that the SASS 2000^{PLUS} and the impactors collected concentration levels of airborne *Legionella* spp up to 3300 CFU/m^3 and 420 CFU/m^3, respectively, directly above the aeration ponds. *Legionella* was not detected in the upwind air samples. *Legionella* spp was detected in air up to 200 meters downwind of the aeration ponds and decreased as the distance increased from the ponds (Figure 3).

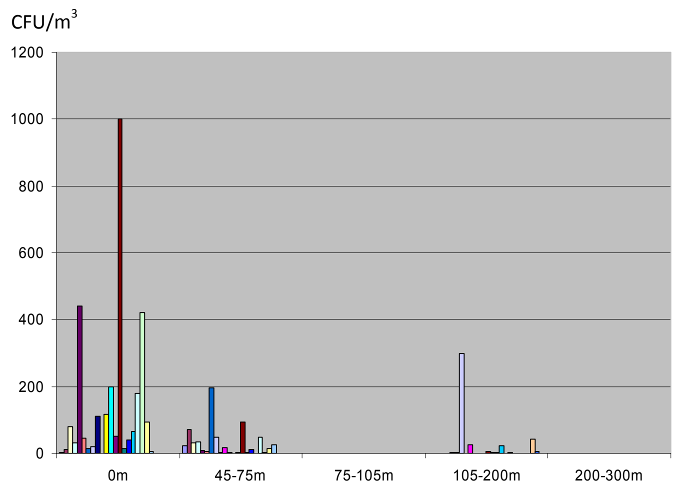


Figure 3. Concentration levels (CFU/m^3) of *Legionella*-containing aerosols sampled at Borregaard Sept-Dec 2006 as a function of distance from the aeration ponds.

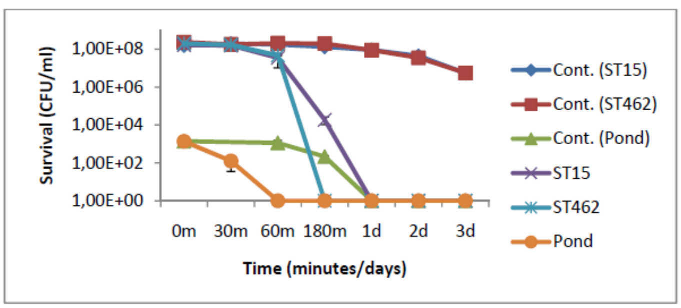
In addition to *L. pneumophila, L. bozemanii, L. dumoffi, L. oakridgensis, L. londiniensis* and/or *L. nautarum* were detected (16S rDNA). *L. bozemanii and L. dumoffi* are also classified as human pathogens. Only one sequence profile (6;23;3;28;19;14) of the *L. pneumophila* colonies was identified using sequence-based typing (MLST) and the identified *L. pneumophila* belonged to SG4. Interestingly, the highest and lowest concentrations of *Legionella* were observed during cloudy weather (3300 CFU/m^3) and rain (1.7 CFU/m^3), respectively, while the next lowest concentration was found at a combination of sunny and cloudy weather (4.6 CFU/m^3). This indicated that the relative humidity is important for the survival of airborne *Legionella* bacteria, which is consistent with other findings. The work concluded that the aeration ponds could generate *Legionella*-containing aerosols that could be further transported by the wind. However, the results raised further questions; whether *Legionella* could be transported over long distances and if *Legionella*-aerosols generated from the aeration ponds could contaminate the air scrubber in a 200 meter distance from the ponds. This work is currently being carried out at the Norwegian Defence Research Establishment. However, preventive actions were carried out at Borregaard after the air sampling measurement campaign; mandatory use of respiratory masks among employees within a 200 meter distance from the ponds. It should further be noted that the aeration ponds were shut downed in 2008 ordered by the Norwegian authorities (The Climate and Pollution Agency).

3. GROWTH ANALYSIS OF *LEGIONELLA* IN AIR SCRUBBER

As a result of that the air scrubber Borregaard had been identified as the outbreak source for the legionellosis outbreaks in Sarpsborg/Fredrikstad both in 2005 and 2008, further analysis was performed to investigate the growth of *Legionella* in environments resembling an air scrubber, i.e. the operational conditions of the air scrubber. An air scrubber is an air pollution control device that used to remove particulates/gases from industrial exhaust streams. Until 2005, cooling towers have been frequently identified as installations able to disseminate *Legionella* which often is linked to poorly and ineffective decontamination processes. All industries in possession of cooling towers, and/or similar installations, are obliged to follow defined decontamination/cleaning procedures according to national regulations. Today, these regulations are being revised and to raise awareness regarding the potential dissemination of airborne *Legionella* from such plants.

The 2005 and 2008 outbreak strains, *L. pneumophila* ST15 and ST462 (identified by MLST (12)), respectively, were used for growth studies in two different air scrubber media (AS1 and 3). The conditions for analysis (45°C, pH 8) were chosen according to the operating conditions of the scrubbers during the outbreak in 2005 and 2008. The hypothesis was that if *L. pneumophila* is not able to multiply and/or survive in the harsh conditions provided by the scrubbers at Borregaard, it is less likely that these devices would be the main disseminators of *L. pneumophila*-containing aerosols during the outbreaks. Survival of *L. pneumophila* ST15 and ST462 was not observed after one day and three hour incubation, respectively, at 45°C, pH 8, in AS1 media (Figure 4). In AS3 media, survival of *L. pneumophila* ST15 and ST462 was not observed after two and one day incubation, respectively (12). These data show that the air scrubber media have an impact on the survival of the outbreak strains. These findings supported our hypothesis that the air scrubbers' operational conditions during the outbreaks did not promote survival of *L. pneumophila.*

A



B

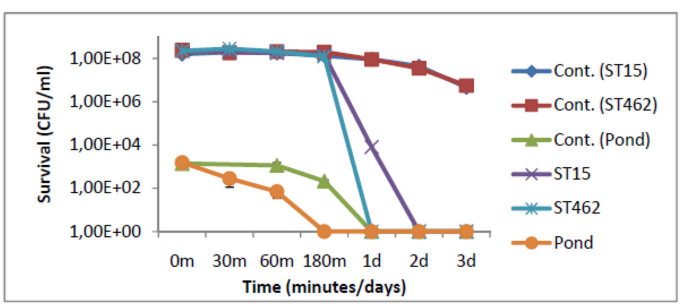


Figure 4. Survival of *L. pneumophila* in two various air scrubber media at 45°C, pH 8 in; a) AS1 and b) AS3 (12). Bars indicate standard deviation of three parallels.

4. IDENTIFICATION OF *LEGIONELLA* IN RIVER

The wastewater from the biological treatment plant/aeration ponds was released into the neighbouring river Glomma and, thus, investigations after the 2005 outbreak were carried out to analyze the presence of *L. pneumophila* in this aquatic environment (12). Samples were harvested from the river at various locations up- and downstream of the wastewater outlet. *L. pneumophila* was not detected upstream of the outlet. The highest concentration level of *L. pneumophila* SG2-14 was detected at the outlet (1.9×10^6 CFU/L (2005) and 2.1×10^5 CFU/L (2008)), the lowest level (4.0×10^4 CFU/L) was identified approximately 1.6 km downstream of the outlet in 2005 and no *L. pneumophila* was detected at 18.5 km downstream of the outlet in 2008. *L. pneumophila* SG1 was detected in the river in 2005 > 300 meters downstream of the outlet. These results clearly showed that the river Glomma was contaminated with *L. pneumophila* SG1 originating from Borregaard's biological treatment plant both in 2005 and 2005.

5. CONCLUSION

Non-closed aeration ponds taking part in the biologically degradation of organic substances in wastewater are able to generate *Legionella*-containing aerosols which are further transported by the wind. The highest concentration level of airborne *Legionella* was measured to 3300 CFU/m^3 directly over the aeration ponds at Borregaard and which decreased as a function of distance from the ponds. Even though one air scrubber at Borregaard was identified as the main source for the 2005 and 2008 outbreaks in Sarpsborg/Fredrikstad, Norway, our retrospective study suggests that the operational conditions in the air scrubber do not promote growth of *Legionella* and that the air scrubber should therefore not be regarded as the primary disseminator of *L. pneumophila*. Furthermore, we suggest that the aeration ponds are to be considered as the main effective amplifiers and primary disseminators of the outbreak *L. pneumophila* strains. Also, the river Glomma should not be neglected as a potential secondary source for dissemination as *L. pneumophila* was detected up to 18,5 km downstream of the outlet.

6. ACKNOWLEDGEMENTS

The authors would like to thank Gunnar Skogan, Tone Aarskaug and Ingjerd Thrane for excellent technical assistance.

7. REFERENCES

[1] Allestam, G. and Långmark, J. "Legionella i bioreningsanlaggningar, Kartlaggning och riskbedømning 2005-2007;" Swedish institute for infectious disease control, (2007).
[2] Blatny, J. M., Reif, B. A. P., Skogan, G., Andreassen, Ø., Høiby, E. A., Ask, E., Waagen, V., Aanonsen, D., Aaberge, I. S. and Caugant D. A. "Tracking Airborne *Legionella* spp. and *Legionella pneumophila* at a biological treatment plant." Environ. Sci. Technol. 42, 7360-73687 (2008).
[3] Edelstein, P. H. "Clinical features of Legionnaires' disease: A selective review." In *Legionella: State of the art 30 years after its recognition*; Cianciotto, N. P., Kwaik, Y. A., Edelstein, P. H., Fields, B. S., Geary, D. F., Harrison, T. G., Joseph, C. A., Ratcliff, R. M., Stout, J. E., Swanson, M. S.; Eds.; ASM press: Washington DC, 2006.
[4] Dideren, B. M. W. "*Legionella* spp. and Legionnaires' disease." J. Infect. 56, 1-12 (2008)
[5] Nguyen, T. M. N., Ilef, D., Jarraud, S., Rouil, L., Campese, C., Che, D., Haeghebaert, S., Ganiayre, F., Marcel, F., Etienne, J. and Desenclos. J. "A community-wide outbreak of Legionnaires disease linked to industrial cooling towers – How far can contaminated aerosols spread?" J. Infect. Dis. 193, 102-111 (2006)
[6] Palmore, T. N., Stock, F., White, M., Bordner, M., Michelin, A., Bennett, J. E., Murray, P. R. and Henderson, D. K. "A cluster of cases of nosocomial legionnaires disease linked to a contaminated hospital decorative water fountain." Infect. Control. Hosp. Epidemiol. 30, 764-768 (2009).
[7] Jernigan, D. B., Hofmann, J., Cetron, M. S., Genese, C. A., Nuorti, J. P., Fields, B. S., Benson, R. F., Carter, R. J., Edelstein, P. H., Guerrero, I. C., Paul, S. M., Lipman, H. B. and Breiman, R. "Outbreak of Legionnaires' disease among cruise ship passengers exposed to a contaminated whirlpool spa." Lancet. 347, 494-499 (1996).
[8] Nygård, K. Werner-Johansen, Ø., Rønsen, S., Caugant, D. A., Simonsen, Ø., Kanestrøm, A., Ask, E., Ringstad, J., Ødegård, R., Jensen, T., Krogh, T., Høiby, E. A., Ragnildstveit, E., Aaberge, I. S. and Aavidtsland, P. "An outbreak of Legionnaires' disease caused by long-distance spread from an air scrubber in Sarpsborg, Norway." Clin. Infect. Dis. 46, 61-69 (2008).
[9] "http://www.fhi.no/dav/ce069fc583.pdf"
[10] Borgen, K., Aaberge, I., Werner-Johansen, Ø., Gjøsund, K., Størsrud, B., Haugsten, S., Nygard, K., Krogh, T., Høiby, E. A., Caugant, D. A., Kanestrøm, A., Simonsen, Ø. and Blystad, H. "A cluster of Legionellaires' disease linked to an industrial plant in southeast Norway, June-July 2008." Eurosurveillance, 38 (13) (2008). www.eurosurveillance.org
[1]1 Wedege, E., Bergdal, T., Bolstad, K., Caugant, D. A., Efskind, J., Heier, H. E., Kanestrøm, A., Strand, B. H. and Aaberge, I. S. "Seroepidemiological study after a long-distance industrial outbreak of Legionnnaires' disease." Clin. Vac. Immunol. 16, 528-534 (2009).
[12] Olsen, J. S., Aarskaug, T., Thrane, I., Pourcel, C., Ask, E., Johansen, G., Waagen, V. and Blatny, J. M. **"**Tracking the Environmental Source Causing Three Legionnaires' disease Outbreaks in Norway." Environ. Sci. Technol. 44, 8712–8717 (2010).