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Assessment of workers' exposure to microorganisms when using biological degreasing stations

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ABSTRACT

Biological degreasing stations (BDSs) are used by mechanics. These BDSs use a water-based solution with a microbial degradation process. Occupational exposure during the use of BDSs has not been reported and few studies have identified the bacteria present. The objectives were to measure the concentration of microorganisms during BDSs' use and monitor the bacterial community in the liquid over time.

Five mechanical workshops were studied. Six 30-min samples were taken at each workshop over one year. Bioaerosols in the ambient air samples were collected with Andersen impactors near the BDS Bioaerosols in the workers' breathing zone (WBZ) were collected on filters. Fresh bio-degreasing fluids were collected from unopened containers, and used bio-degreasing fluids were collected in the BDS.

The results show that the use of BDSs does not seem to increase bioaerosols concentrations in the WBZ (concentrations lower than 480 CFU/m^3) and that the bacterial communities (mainly yeasts, *Bacillus subtilis* and *Pseudomonas aeruginosa*) in the bio-degreasing fluids change through time and differ from the original community (*B. subtilis)*. This study established that workers using BDSs were exposed to low levels of bioaerosols. No respiratory protection is recommended based on bioaerosols concentrations, but gloves and strict personal hygiene practices are essential.

1. Introduction

Solvent-based cleaning stations are used by mechanics for manually degreasing dirty vehicle parts (Guillemin and Lupin, 2008). The solvents used in those cleaning stations are generally petroleum-based such as Stoddard solvent. Most solvents are composed of high flash aliphatic petroleum cuts known to cause potential ocular, cutaneous and respiratory irritations, as well as light neurological effects like headaches, nausea, dizzy spells and tiredness (Guillemin and Lupin, 2008; McKee et al., 2015). To protect human health, aqueous solutions are favoured over solvent cleaners for degreasing mechanical parts (Lavoué et al., 2003). Progress in biotechnology has brought water-based degreasing stations, or biological degreasing stations (BDS), which use a microbial degradation process instead of solvent for degreasing (Bégin et al., 2014a). Indeed, some bacteria can use hydrocarbons as carbon and energy sources to produce biosurfactants. These biosurfactants act as emulsifying agents by decreasing the surface tension and forming micelles, thus making the oil available for the bacteria. Bacteria can then transform hydrocarbonated materials into simple water, carbon dioxide and other organic compounds (Boucher et al., 2011). Since BDS use microorganisms as cleaning ingredients, they can be categorized as microbial-based cleaning products (MBSP).

Manufacturers of bio-degreasing fluids for BDSs claim that the microorganisms used in their preparations are harmless since they are classified in the biosafety risk group 1 (McNally, 2011). According to the Public Health Agency of Canada, risk group 1 microorganisms are unlikely to cause disease in healthy workers or animals and therefore are considered a low infectious risk for humans and communities (PHAC, 2015). However, these microorganisms may present other health risks to workers under certain exposure conditions. For instance, the multiplication of certain toxin-producing Gram-negative bacteria increases the likelihood of finding endotoxins in the ambient air of the workplace (Marchand, 1996). These substances are known to cause respiratory problems in exposed workers (Rylander, 2006).

Few studies have been done to identify the types of bacteria present in bio-degreasing fluids. David et al. studied the bacterial flora of seven biological degreasing stations in France (David et al., 2009). In addition to risk group 1 bacteria, the authors identified several risk group 2

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bacteria in the bio-degreasing fluids of the BDSs, e.g. *Pseudomonas aeruginosa* (PHAC, 2012) and *Klebsellia pneumoniae* (PHAC, 2011). Biosafety risk group 2 microorganisms present a moderate infectious risk for humans, but low for communities (PHAC, 2015). The authors suggested that the contamination of the liquid was not only coming from the environment but from the workers as well. David et al. and Boucher et al. demonstrated that there is an important temporal variation of the bacterial community between and within washing stations (Boucher et al., 2011; David et al., 2009). The authors hypothesized that workers using BDSs might get exposed to bioaerosols generated from the brush from which the bio-degreasing fluid is projected onto the parts to be cleaned (Boucher et al., 2011). Workers' exposure to bioaerosols, which can be measured by sampling the air within 30 cm of the worker's breathing zone (near the worker's lapel), was not assessed in either of those studies.

Bodin and Larivé visited five Swiss workplaces that use biological degreasing stations. They found bioaerosols from bio-degreasing fluids in four out of the five workplaces. These bioaerosols contained both the original microorganisms of the bio-degreasing fluids and other microorganisms arising from contamination (Bodin and Larivé, 2013). Bégin et al. visited four Québec workplaces where they observed the use of blow guns for drying out parts that had been cleaned with the BDSs. They hypothesized that this technique might generate bioaerosols and therefore enhance the inhalation of microorganisms by the workers (Bégin et al., 2014a).

The main objectives of this study were 1) to assess workers' exposure to bioaerosols by verifying whether the use of biological degreasing stations causes a significant increase in bioaerosols in the worker's breathing zone, 2) to check whether bioaerosols come from the liquid of the biological degreasing stations, 3) to monitor the bacterial community composition of BDSs over time and 4) to make recommendations regarding the safe use of BDSs.

2. Methods

2.1. Work sites and BDSs

A total of 5 mechanical workshops were visited over the course of a year. Each workshop was visited every 2 months for a 30-min period, for a total of 6 sampling periods per workshop. The participating employees were trained mechanics accustomed to the use of BDSs. During air sampling, they were asked to use the BDS in a manner that was representative of their normal working habits.

Two models of BDSs provided by different manufacturers were studied (M1 and M2). Both models include a sink in which the worker cleans the dirty pieces with a brush from which springs the bio-degreasing fluids. The contaminated fluid goes through a tank where the dirt is allegedly gradually degraded by bacteria. The fluid is heated slightly to maintain bacterial growth. The circulation of the bio-degreasing fluid is ensured by an electric pump. The circuit has a filter in which insoluble dirt and particles are retained. The difference between the two models lies in the way that bacteria are added in the BDS. In the M1 model, the degreaser fluid already contains the degrading bacteria in suspension and a non-ionic surfactant, while in the M2 model, the bacteria are enclosed in a cloth filter and are released into the degreasing fluids as it passes through the filter.

2.2. Sampling

2.2.1. Ambiant air sampling

Bioaerosols were collected within a meter of the BDS using an Andersen impactor in the N-6 configuration (Thermo Fisher Scientific, Franklin, MA). The Andersen Impactor was placed on a trolley at the height of the wash basin of the BDS. It was set back 30 cm to the left, between the worker and the BDS. Five consecutive 5-min samples were collected on trypticase soy agar (TSA) (Oxoid, Ottawa, ON, Canada) culture media. The impactor was operated using a Gast 1531 pump (Gast, Benton Harbor, MI) calibrated at 28.3 L/minute with the TSI 4043 mass flowmeter (Shoreview, MN).

2.2.2. Worker breathing zone air sampling

Bioaerosols were collected in the WBZ using a three-section 37 mm plastic cassette with a polycarbonate filter (SKC, Eighty Four, PA) placed directly on the worker. The cassette was operated using a GilAir-3 sampling pump (Sensidyne FL) calibrated at 2 L/minute with the Defender 510 flow calibrator (Mesa Laboratories, NJ). The WBZ samples were taken in duplicates for 30 min.

2.2.3. Liquid sampling

At the end of each sampling period, the bio-degreasing fluid in the BDS was collected in a sterile 50 ml falcon tube (Thermo Fisher Scientific, Franklin, MA). The temperature and pH of the bio-degreasing fluids were measured directly from the tube. As a control for the initial bacterial flora in the bio-degreasing fluids, a sample was also collected directly from an unopened storage container. For the M2 model, a new cloth filter was also collected. The microbial content was extracted and then treated as a fresh bio-degreasing fluid to evaluate its microbial content.

2.3. Sample analysis

2.3.1. Inoculation and incubation

5 ml of phosphate-buffered saline was added directly in the cassette. The cassette was then shaken for 5 min at 1200 RMP. The cassette was opened and the liquid was collected in a sterile 15 ml falcon tube (Thermo Fisher Scientific, Franklin, MA). 100 µl of the liquid was spread on TSA triplicates and incubated at 37 °C for 24 h. Counting was performed under a stereomicroscope and no bacterial colony was analyzed.

Since the sampling with the Andersen Impactor was done directly on culture media, the air samples were directly incubated at 37 °C for 24 h within a few hours of sampling. Counting was performed under a stereomicroscope and 100% of the Petri dishes surface was analyzed.

Bio-degreasing fluid samples were inoculated on culture media. To do so, 200 µl were spread on TSA in triplicates. All samples were incubated at 37 °C for 24 h and counting was performed under a stereomicroscope and 100% of the Petri dishes surface was analyzed.

2.3.2. Identification

On each Petri dish, the different colonies (bacteria and yeasts) were isolated in pure culture prior to their identification. Since the performance of each identification method depends on the strain analyzed, four different methods were used in a complementary way to identify all the strains. Half of the bacterial strains were identified by the comparison of the fatty acid methyl ester (FAME) profile using the Sherlock method (MIDI, Newark, DE). The other half was identified by biochemical-based Microscan identification plates (Beckman Coulter, Brea, CA), MALDI-TOF mass spectrophotometer (VITEK® MS, bioMérieux, Marcy-l'étoile, France), and genomic 16S rRNA sequencing. The 16S rRNA amplifications were performed with the 27F (5′ AGAGTTTGATMCTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) primers from Genome Quebec (Montreal, Canada). A consensus sequence for each strain was created and the species identification was obtained by sequence comparison with the Ribosomal Database Project (Maidak et al., 1994). The yeasts were isolated but not specified.

2.4. Statistical analysis

Analyses of variance were performed on the log-transformed data in order to compare the effect of the cleaning process on the airborne microbial concentrations. Morisita-Horn indices were calculated to compare the flora composition. EstimateS (Colwell, 2013) and NCSS (Hintze, 2013) were used.