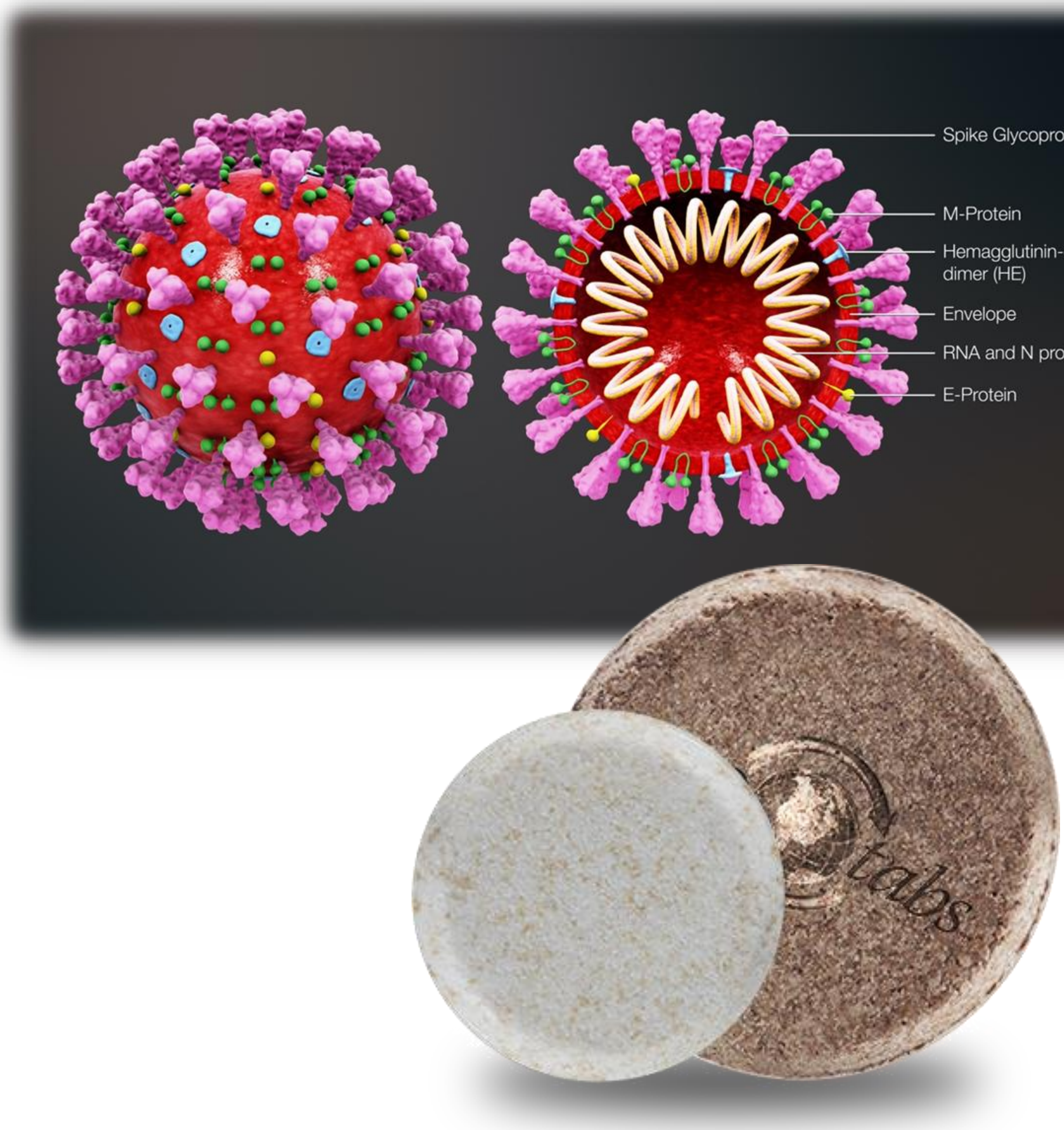
Technical proposal for use of Eco-Tabs™ Tablets to bio-remediate waste water that may be contaminated with SARS-CoV-2 Viral RNA from COVID-19-positive patients



Prepared By: Sherrie Bain, MS
Microbiology Consultant
Current, relevant certifications include: Bloodborne Pathogen Handling, Hazardous Communication, Biohazard Safety, Biological Safety & BioSecurity Training
04.04.2020

Overview

The novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently responsible for a global pandemic with alarming morbidity and mortality rates. SARS-CoV-2 is the etiological agent of Coronavirus Disease 2019 (COVID-19). COVID-19 is a newly emerging infectious disease that was initially identified in Wuhan, China in December 2019. Since its initial appearance COVID-19 has rapidly spread around the world. As of April 5th, 2020 there have been more than 1.3 million confirmed cases, and over 74,000 deaths from this disease (*COVID-19 Map*, n.d.). Several case studies from early infections in China reveal that even after COVID-19 patients have recovered and test negative for the presence of the viral nucleic via throat swabs, there is evidence that fecal samples from these patients still tested positive for SARS-CoV-2 nucleic acid more than 10. Another study also found fecal shedding of later (Tian et al., 2020; Zhang T, 2020). A third, larger study involving 95 COVID-19 patients from Wuhan, China found that approximately 33% of those patients also tested positive for SARS-CoV-2 in their fecal samples (Xiao et al.). There was also a documented case of in Italian tourist who returned from Wuhan, China and who tested negative for COVID-19 via pharyngeal swabs, but whose stools continually tested positive for the presence of the virus (Nicastri et al. 2020).

Previous outbreaks of other closely-related coronaviruses including SARS-CoV, the etiological agent of the 2002-2003 SARS outbreak, and MERS-CoV, the etiological agent of the deadly 2013 MERS outbreak, led to the discovery that these viruses could remain viable in fecal samples for days. Tests also showed that the contaminated feces could potentially seed/contaminate the environment in a way that might lead to widespread fecal-oral transmission of the infectious agents (Xiao et al. ; Yeo et al. 2020). Therefore, the possibility of fecal-oral transmission of SARS-CoV-2 infection, could result via the wastewater stream and other biological waste.

Problems and issues

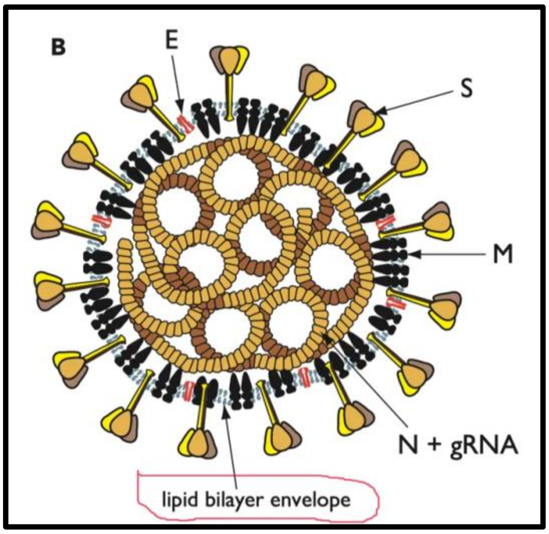
1. The waste stream from COVID-19 patients may contain SARS-CoV-2, the etiological agent of the disease. This may potentially represent a serious and imminent environmental hazard that facilitates the spread of the disease, if the wastewater is not properly treated at/near facilities such as hospitals, nursing homes, or cruise ships that have multiple patients, prior to entering the municipal wastewater collection system.

2. There is also concern among some scientists, about the potential impact of stormwater run-off from areas surrounding hospitals, and other facilities that may house many COVID-19 patients, or wastewater treatment plants that service these facilities. The concern is that fecal contamination may end up at the beaches after heavy rainstorms, and further expose individuals to COVID-19 infections, even if they have not interacted directly with COVID-19-positive individuals (Cahill 2020).

Concepts of Proposed Technology

The use of biological control/bioremediation agents has a well-established track record with respect to the successful remediation of wastewater containing raw sewage (US Environmental Protection Agency, 1999). Bacterial biological control agents provide a safer alternative to harmful chemicals that may have a lasting detrimental impact on the environment and people living in and around the treated areas. The organisms used in the sewage bioremediation process typically include a variety of bacterial strains. These strains are usually found within the natural environment, and do not cause adverse harm when used for the bioremediation process. The bacteria function synergistically to effectively degrade the flocculants and other organic and inorganic components of the raw sewage. Some examples of bacteria that are commonly used in the process include species of *Bacillus* and *Pseudomonas* bacteria. These microbes are not only able to utilize oxygen to break down organic material, but they are also able to use other substances such as nitrogen as electron acceptors. This increases the versatility and efficiency of the sewage remediation process (U.S. Environmental Protection Agency, 2010). The specific formulation of the Eco-Tabs® Tablet provides a highly selective mixture of bacterial strains that are formulated to effectively target organic waste such as raw sewage. The Eco-Tabs® tablet proven record of success provides some measure of expectations with regards to the potential outcome of using the tabs to assist with the bioremediation of wastewater that contains SARS-CoV-2. The tables below outline scientific evidence of the efficacy of the strains included in the Eco-Tabs® Tablet as well as their designations with regards to potential human and environmental hazards.

Mode of action of the product



The SARS-CoV-2 viral particles are made up of a lipid envelope/coat. The bacteria as well as the hydrogen peroxide initiate the breakdown of the lipid bilayer envelope allowing the COVID protein to become vulnerable to degradation. In addition to the bacterial strains that are present in the Eco-Tabs, its main chemical components--hydrogen peroxide--have been recommended by the EPA to be effective at destroying the SARS-CoV-2 viral particles (Epa and OCSPP 2020). The tabs are engineered to slowly dissolve over time to release the microbes and other active ingredients. This effervescence is facilitated by the slow decomposition of the oxidizing agent sodium carbonate peroxyhydrate (Sodium Percarbonate). Sodium carbonate peroxyhydrate is a solid form of hydrogen peroxide. Water acts as a catalyst to drive the decomposition reaction. Sodium carbonate peroxyhydrate decomposes to water and oxygen in solution. The oxygen molecules are released as millions of tiny effervescent bubbles that can then stimulate the activity of aerobic microbes. The microflora in the tabs provide a non-toxic, environmentally-safe means of bioremediation that will reduce the harmful raw sewage wastes without causing additional adverse impact on the inhabitants. The tabs can be added directly to the sewage and wastewater lines or they can be placed in a special container and allowed to settle at the bottom of a treatment tank/tanker.

Potential Advantages of Eco-Tabs Product

The bacteria used to facilitate bioremediation are natural inhabitants of the environment, that have been used for the treatment of wastewater and sewage for decades. The Eco-Tabs™ tablets contain an optimized blend of more than eleven bacterial strains that are commonly present and sewage bioremediation (U.S. Environmental Protection Agency, 2010). In addition, the design of the tablet includes the patent-pending Microdot Separation Technique (MST) that ensures that the core components of the tablets are released at a constant rate. The micro oxygen bubbles enhance the aerobic efficiency of bacterial strains. This allows for effective organic

waste decomposition even in environments that have become oxygen-starved because of heavy pollution. In addition to the micro-oxygen bubbles that infuse the tablets, there are non-hazardous/non-toxic, chemical components. The activated sludge degradation process uses mixtures of bacteria and other microbes. Given that individual microbes may utilize different substrates as food sources, it is further predicted that any non-organic, non-toxic chemical components of the Eco-Tabs™ tablet would be quickly consumed by the bacteria and other microbes present. Thus, the use of the tablets for wastewater remediation is not expected to result in any enhanced toxicity. Eco-Tabs have been used successfully in a wide variety of treatment environments including wastewater treatment ponds and containment facilities.

Table 1. Microbiological Strain Description

Genus & Species	ATCC# (American Type Culture Collection)	Description Hazard risk per OSHA 29CFR 1910.1200
Bacillus megaterium	14581	Biosafety Level (BSL) 1. Non-hazardous material ATCC MSDS reviewed
Pseudomonas flourescens	13525	Biosafety Level (BSL) 1. Non-hazardous material ATCC MSDS reviewed
Pseudomonas putida	12633	Biosafety Level (BSL) 1. Non-hazardous material ATCC MSDS reviewed
Bacillus licheniformis	14580	Biosafety Level (BSL) 1. Non-hazardous material ATCC MSDS reviewed
Brevibacillus parabrevis (Bacillus brevis)	10027	Biosafety Level (BSL) 1. Non-hazardous Material ATCC MSDS Reviewed MSDS Evaluation indicates non-pathogenic strains of *Bacillus.* Strains have not been genetically modified. Product does not produce hazardous by-product on decomposition

Bacillus subtilis	**202137 202138 202139 6051**	**Biosafety Level (BSL) 1. Non-hazardous Material ATCC MSDS Reviewed MSDS Evaluation indicates non-pathogenic strains of *Bacillus.* Strains have not been genetically modified. Product does not produce hazardous by-product on decomposition**
Bacillus pumilus	202136	Biosafety Level (BSL) 1. Non-hazardous Material ATCC MSDS Reviewed MSDS Evaluation indicates non-pathogenic strains of *Bacillus.* Strains have not been genetically modified. Product does not produce hazardous
Bacillus macerans	202132 202135	Biosafety Level (BSL) 1. Non-hazardous Material ATCC MSDS Reviewed MSDS Evaluation indicates non-pathogenic strains of *Bacillus.* Strains have not been genetically modified. Product does not produce hazardous
Bacillus amyloliquefaciens	202133 202134	Biosafety Level (BSL) 1. Non-hazardous Material ATCC MSDS Reviewed MSDS Evaluation indicates non-pathogenic strains of *Bacillus.* Strains have not been genetically modified. Product does not produce hazardous

As indicated in Table 1, all of the microbiological information for the strains listed has been verified and reviewed. This reflects MSDS information independently obtained from ATCC and MSDS review directly from the supplier's website, independent research and various investigative sources.

Works Cited

Aus-e-Tute. (n.d.). *Solvay Process for the Production of Sodium Carbonate*. Retrieved December 13, 2013, from Aus-e-Tute: http://www.ausetute.com.au/solvay.html

Dwidar, M., Kim, S., Jeong, B., Um, Y., & Mitchell, R. (2013). Co-culturing a novel Bacillus strain with Clostridium tyrobutyricum ATCC 25755 to produce butyric acid from sucrose. *Biotechnology for Biofuels*, 35-45.

Inoue, H., Takimura, O., Kawaguchi, K., Nitoda, T., Fuse, H., & Murakami, K. Y. (2003). in-Carbon Cleavage of Organotin Compounds by Pyoverdine from Pseudomonas chlororaphis. *Applied and Environmental Microbiology*, 878-883.

Lawler, D., & Smith, S. (2000, December 19). *Enzyme-producing strain of Bacillus bacteria.* Retrieved December 14, 2013, from Google Patents: http://www.google.com/patents?hl=en&lr=&vid=USPAT6162635&id=5dIFAAAAEBAJ&oi=fnd&dq=Enzyme+producing+strain+of+bacillus+bacteria+us+patent+office+lawler&printsec=abstract#v=onepage&q&f=false

Lee, N., Neilsen, P. H., Andreasen, K. H., Juretschko, S., Neilsen, J., Schleife, K.-H., & Wagner, M. (1999). Combination of Fluorescent In Situ Hybridization and Microautoradiography—a New Tool for Structure-Function Analyses in Microbial Ecology. *Applied and Environmental Microbiology*, 1289 - 1297.

Mass.Gov Energy and Environmental Affairs. (2010). Retrieved December 13, 2013, from Massachusetts Department of Environmental Protection: http://www.mass.gov/eea/docs/agr/pesticides/aquatic/sodium-carbonate-peroxyhydrate-and-hydrogen-peroxide.pdf

Moller, S., Pedersen, A., Poulsan, L., Arvin, E., & Molin, S. (1996). Activity and Three-Dimensional Distribution of Toluene-Degrading Pseudomonas putida in a Multispecies Biofilm Assessed by Quantitative In Situ Hybridization and Scanning Confocal Laser Microscopy. *Applied and Environmental Microbiology*, 4632-4640.

Shangyu Jiehua Chemical Co., Ltd. (n.d.). *Sodium Percarbonate Material Safety Data Sheet.* Retrieved December 13, 2013, from Shangyu Jiehua Chemical Co., Ltd: http://www.shangyuchem.com/en/upLoad/product/month_1302/20130227115950147.pdf

Strom, P. (1995). Identification of Thermophilic Bacteria in Solid-Waste Composting. *Applied & Environmental Microbiology*, 906-913.

Taber, D. T. (2011). Organic Synthesis: State of the Art 2007 - 2009. In D. T. Faber, *Organic Synthesis: State of the Art 2007 - 2009.* New York: Oxford Press.

Tata Chemicals Europe. (2013). *Tata Chemicals Europe Detergent Industry.* Retrieved December 13, 2013, from Tata Chemicals Europe: http://tatachemicals.com/Europe/touching_lives/pdf/detergent.pdf

Torcris Bioscience. (2013, August 2). *Sodium Bicarbonate Safety Data Sheet.* Retrieved December 13, 2013, from Torcris Bioscience: http://www.tocris.com/literature/3152_sds.pdf?1386965906

U.S. Environmental Protection Agency. (2010, August). Retrieved December 12, 2013, from National Center for Environmental Protection: http://nepis.epa.gov/Adobe/PDF/P1008KTD.pdf

US Environmental Protection Agency. (1998, October 1). *Bacillus thuringiensis subspecies israelensis strain EG2215 (006476) Fact Sheet .* Retrieved April 2014, from Environmental Protection Agency: http://www.epa.gov/opp00001/chem_search/reg_actions/registration/fs_PC-006476_01-Oct-98.pdf

US Environmental Protection Agency. (1999, November 1). *Bacillus sphaericus serotype H5a5b strain 2362 (128128) Fact Sheet .* Retrieved April 2014, from EPA: http://www.epa.gov/opp00001/chem_search/reg_actions/registration/fs_PC-119801_01-Nov-99.pdf

COVID-19 Map. (n.d.). Johns Hopkins Coronavirus Resource Center. Retrieved April 6, 2020, from https://coronavirus.jhu.edu/map.html

Tian, Y., Rong, L., Nian, W., & He, Y. (2020). Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Alimentary Pharmacology & Therapeutics*. https://doi.org/10.1111/apt.15731

Zhang T, E. al. (2020, March 29). *Detectable SARS-CoV-2 Viral RNA in Feces of Three Children during Recovery Period of COVID-19 Pneumonia. - PubMed - NCBI*. https://www.ncbi.nlm.nih.gov/pubmed/32222992