



BIO CLEANING SOLUTIONS

GreenWorx

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*Formulation guidelines for Bio Tech GTX 10, 20 and 50
Non-formulated spore concentrates*

General guidelines:

Mix the non-formulated microbial spore product in its original container for a minimum of 30 minutes with an appropriately sized mixer. Ensure that mixer blades are close to the bottom of the container. Spores are biological materials and their colour may vary slightly from batch to batch. The presence of spores in the final formulated product should simply make the product homogeneously slightly turbid to turbid. Note that spores settle over time, depending on the viscosity of the final formulated product.

We recommend:

- Starting with a clean tank, free of all sanitisers or disinfectants from cleaning or a previous product
- Formulating by using the weight of all components where possible
- Formulating with soft or DI water (tap water is not recommended, but may be acceptable)
- Maintaining pH between 5 to 9.5
- Mixing all ingredients thoroughly and adjusting pH before adding microbial spores and re-adjusting pH after microbe addition if necessary
- Microbial spore counts are made on the final formulated product to ensure that it contains the desired count

Surfactant recommendations:

- Anionic surfactants, non-ionic surfactants, and blends of these two surfactants are generally acceptable for use in spore-containing formulations
- Mix all surfactants thoroughly before use
- Do not use cationic surfactants as these may cause spore instability and spore clumping
- The total surfactant content (anionic surfactant and non-ionic surfactant) may be up to approximately 15%
- It is important to use same grade water (soft, DI or tap) in formulation development as you will use in the full-scale process

Preservatives:

- Green Worx CS' non-formulated spore concentrates generally contain enough preservatives to preserve an end-use formulation when correctly diluted
- A preservative efficacy study should be completed
- Do not use reactive preservatives such as glutaraldehyde as these may kill spores and/or prevent germination
- Do not use any preservatives known to function by generating or releasing formaldehyde or that may be sporicidal
- In the US, preservatives can only be used if they have been registered and approved for the particular end use by the USEPA
- Consult the preservative label or manufacturer for approved usage
- In EU, a preservative shall only be used if it has been granted a Union authorization (EU commission) or a national authorisation (national competent authority)
- Any conditions or restrictions specified in the authorisation and on the preservative label shall be followed and respected by the user
- For other regions, please follow regional and national laws
- Do not formulate with chlorine, peroxide or peroxygen compounds

Dyes, fragrances and builders:

- Dyes and fragrance may be added if stable in the formulation as these generally do not impact spore stability
- Fragrance must be added with caution as it can cause phase instability with the surfactant system (a surfactant system that may be stable with one fragrance may be completely unstable with a different fragrance)
- Phase stability testing must be performed each time the fragrance or the amount of fragrance is changed
- Inorganic builders may be used but avoid high concentrations of divalent cations
- Inorganic builders can cause phase stability issues with the surfactant system, and testing the phase stability of the entire formulation without spores is recommended

Order of addition:

- Charge the mixing tank with the appropriate amount of water and begin mixing
- Two critical factors that insure a good formulation are adequate mixing and tight control of the amount (preferably weight) of ingredients added
- Surfactants are generally added after the water and before the other ingredients

- The exact order of addition of surfactants depends on the individual formulation and must be determined during formulation development
- Check and adjust pH at various points during the formulation, points to be determined during formulation development
- Caustic soda (40%) and phosphoric acid are generally used, although other acids may be used with proper safety precautions
- Organic acids may be used, but may place additional burden on the preservative system
- Add builders or other organic or inorganic materials and check/adjust pH as necessary
- Add any dyes or colorants
- Fragrances should be added as the next-to-last ingredient (before spores) resulting in a completely homogeneous formulation
- Ensure adequate mixing, check/adjust pH as necessary
- Spores should be added as the last ingredient in the formulation
- Allow adequate mixing time before sampling for QC
- Make sure the sampling port has been flushed so a representative sample of the formulation is obtained

Stability test recommendations:

- For new formulations, we recommend that the surfactant system be tested for phase stability from 5 ° to 45 °C, as well as freeze/thaw
- This testing should be done without spores, dyes, or pacifiers as these materials can potentially mask any phase instability of the surfactant system
- This testing can be done when choosing the surfactant system, when the entire formulation (surfactant system, fragrance, and inorganic builders) has been developed, or both
- Microbial spore count stabilities have to be conducted on the final formulated product to ensure that it contains the desired count and that this count is stable in time (not affected by any parameter of the formulation)
- We recommend testing at room temperature and at least 35 °C
- Physical and spore stabilities should be repeated in case of fragrance addition or change (concentration or supplier) as a different fragrance may affect physical and microbial stability)
- Inorganic builders can cause phase stability issues with the surfactant system so phase stabilities have to be conducted in the case of addition or change of such compounds

Materials	Microorganisms
Water	Soft, DI or tap water
Anionic surfactants	In general, good compatibility with anionic surfactants. Blends with non-ionic are generally acceptable.
Non-ionic surfactants	In general, good compatibility with non-ionic surfactants. Blend with anionic are generally acceptable.
Cationic surfactants	To be avoided as these may cause spore instability. Quaternary amine type compounds are not recommended.
Coupling agents/hydrotrope	MPG best. In general, good compatibility with hydrotrope agents. Buffers Keep pH between 5 to 9.5 for optimal stability in liquids.
Builders	Inorganic builders may be used. Avoid high concentrations of divalent cations. Keep pH at 5 – 9.5 for optimal stability.
Preservatives	<ul style="list-style-type: none"> • Green Worx CS non-formulated spore concentrates generally contain enough preservatives to preserve an end-use formulation when correctly diluted (check the PDS for formulation recommendations) • Do not use reactive preservatives such as glutaraldehyde as these may kill spores and/or prevent germination • Do not use any preservative known to function by generating or releasing formaldehyde or that may be sporicidal
Oxidisers	Do not formulate with percarbonate, perborate, peroxide or peroxygen compounds. Avoid chlorine, bleaches.
Thickener	In general, good compatibility with thickening agents. Cellulose based thickeners have to be avoided.
Dyes	In general, good compatibility with dyes, they do not impact spore stability.
Perfumes	Due to the complex mix of perfumes, check the compatibility with micro-organisms through stability test in time.

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