

## Application of Box-Behnken Response Surface Design for Optimization of Essential Oil Based Disinfectant on Mixed Species Biofilm

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**Abstract :** With the optimization of a new disinfectant the number of tests could be decreased and the cost of processing too. Good sanitizers are eco-friendly and allow no resistance evolvment of bacteria. The essential oils (EOs) are natural antimicrobials, and most of them have the Generally Recognized As Safe (GRAS) status. In our study, the effect of the EOs cinnamon, marjoram, and thyme was investigated against mixed species bacterial biofilms of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas putida*, and *Staphylococcus aureus*. The optimal concentration of EOs, disinfection time and level of pH were evaluated with the aid of Response Surface Box-Behnken Design (RSD) on 1 day and 7 days old biofilms on metal, plastic, and wood surfaces. The variable factors were in the range of 1-3 times of minimum bactericide concentration (MBC); 10-110 minutes acting time and 4.5- 7.5 pH. The optimized EO disinfectant was compared to industrial used chemicals (HC-DPE, Hypo). The natural based disinfectants were applicable; the acting time was below 30 minutes. EOs were able to eliminate the biofilm from the used surfaces except from wood. The disinfection effect of the EO based natural solutions was in most cases equivalent or better compared to chemical sanitizers used in food industry.

**Keywords :** biofilm, Box-Behnken design, disinfectant, essential oil

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