



BIOTEST RESULTS

ADERCO'S IMPACT ON BACTERIAL GROWTH

This document presents the outcomes of an investigation into the impact of bacterial growth, conducted by a reputable independent laboratory.

It is important to note that the Aderco technology uses a surfactant as the active molecule, at a high concentration*.

The objective of this test was to demonstrate the efficacy of the Aderco molecule in inhibiting bacterial growth. Biofuel samples were blended with bacteria-contaminated samples, and the resulting mixture was allowed to age, creating a conducive environment for the bacteria (TTC agar) to develop. Following this incubation period, the fuel underwent testing to assess the bacterial growth.

The Results

Two sets of tests were conducted —one involving the mixing of biofuel with uncontaminated water and another with water and contaminated MGO. Following the incubation period, the laboratory obtained the following results:

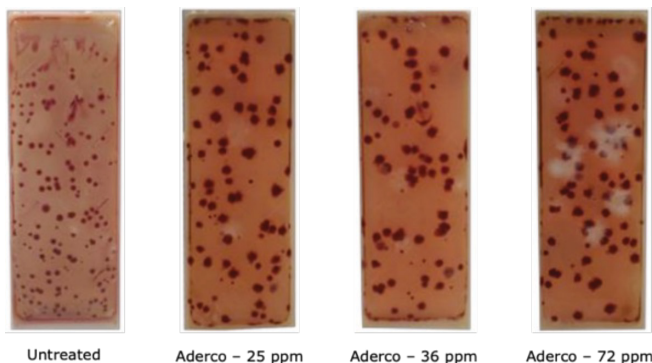


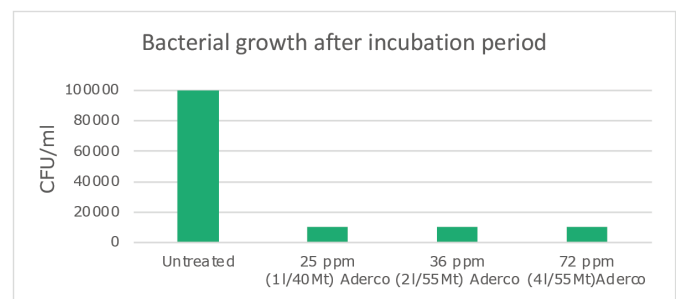
Figure 1: Testing samples after incubation period

To quantify the results from the testing sample (Figure 1), the slide containing the agar medium is compared to a reference chart. It is essential to note that, in this test, the focus is on colony density, not the size of the colonies.

After comparison with the reference chart, the lab obtained the following table:

| Biodiesel (B100) + DI Water | | Biodiesel (B100) + DI Water + contaminated MGO | |
|-----------------------------|-------------------|--|-------------------|
| | Bacteria (CFU/mL) | | Bacteria (CFU/mL) |
| Untreated | Negative | Untreated | Moderate - 10^5 |
| 25 ppm (1l/40Mt) | | 25 ppm (1l/40Mt) | Slight - 10^4 |
| 36 ppm (2l/55Mt) | | 36 ppm (2l/55Mt) | Slight - 10^4 |
| 72 ppm (4l/55Mt) | | 72 ppm (4l/55Mt) | Slight - 10^4 |

Which can be translated into a graph for easier visualisation:



Discussions and conclusions:

In this experiment, deionized (DI) water, completely free of any bacteria, was used. The blend of biofuel and DI water showed no signs of bacterial growth, indicating that clean, bacteria-free water does not support bacterial development. However, it is crucial to acknowledge that under real-world conditions, water is rarely free from microorganisms.

During the test, the **Aderco** active molecule effectively prevented the development of bacterial colonies by maintaining the number of forming units below the limit of 10^4 cfu/ml. As the test is qualitative, it analysed spots on the growing media, drawing comparisons with a reference. It's important to note that, under ideal conditions, some cases of bacterial growth can double every 20 minutes. Despite this, a reduction in the number of spots on the treated sample, compared to the untreated sample, was observed. This suggests that **Aderco** minimized bacterial development to a level where contamination poses no risk to the user.

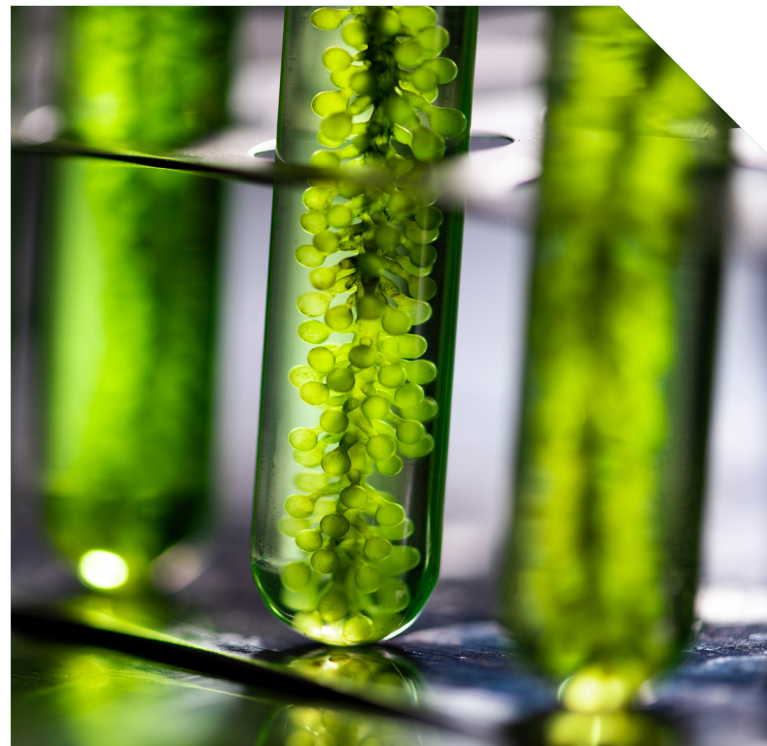
The **Aderco** molecule's physical action separates water from biofuel, trapping bacteria in the water without access to their sources of food, preventing their growth.

For this test, the standard concentration of 36 ppm (2l/55Mt) as initial dosage and 18 ppm (1l/55Mt) as the subsequent dosage significantly influenced the results compared to the untreated sample. This is attributed to the physical properties of **Aderco's** Technology. Therefore, a double dosage is recommended for the first treatment to ensure the fuel system's surface is covered by a layer

of **Aderco** surfactant molecules. The remaining molecules are then present in the fuel, capable of separating water.

This test emphasises the importance of continuous treatment for **Aderco** to fully showcase its potential.

*The **Aderco** molecule is the primary component in all our products, present at different concentrations, except for the LI050, which serves as a lubricity improver.



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