

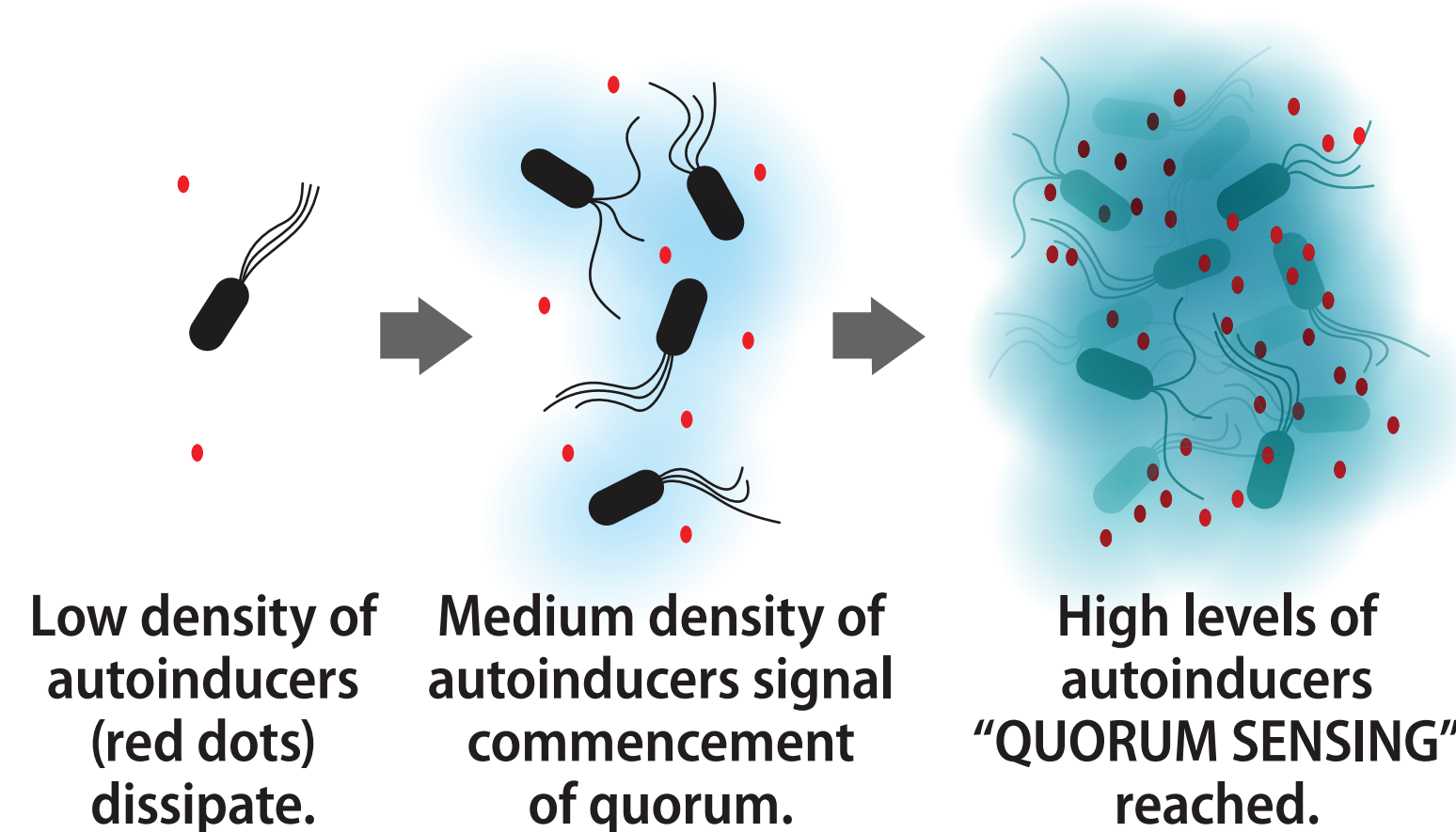
# Fentonite® Effectively Blocked the Release of AI-2 and Inhibited the Formation of New Biofilms Within 4 Hours

## STUDY TO ELIMINATE AI-2 WITH FENTONITE®

This study is proposed to unveil a potential breakthrough- the ability of Fentonite to eliminate the release of Autoinducer-2 (AI-2). AI-2, a universal signaling molecule, plays a crucial role in bacterial communication, enabling them to assess population density and coordinate collective behaviors, such as biofilm formation, virulence factor expression, and symbiotic interactions.

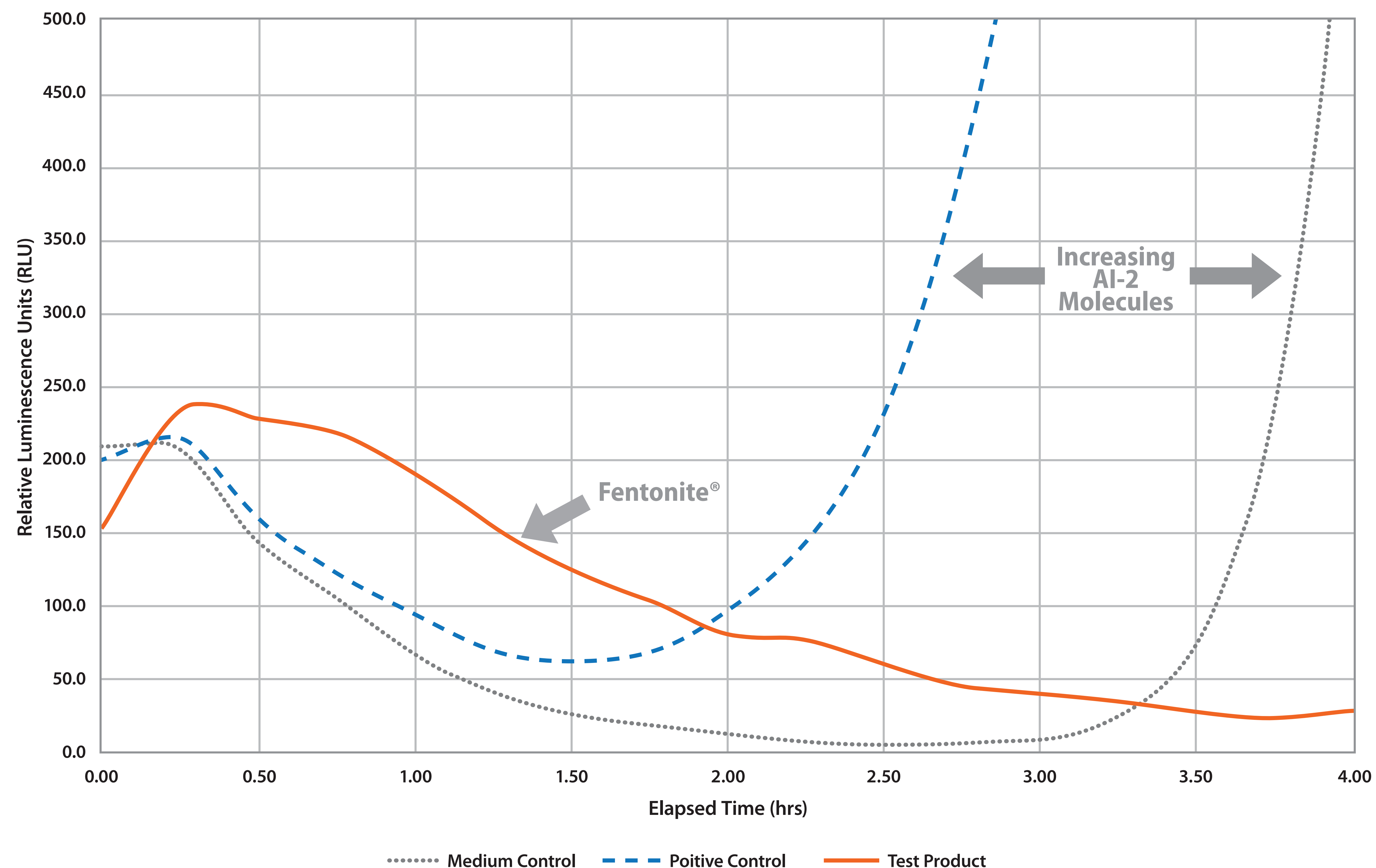
AI-2, a pivotal signaling molecule deeply embedded in the intricate quorum sensing process, is a cornerstone of bacterial communication. It orchestrates group behaviors based on population density. Initially discovered in the marine bacterium *Vibrio harveyi*, AI-2 has since been found in a diverse array of bacterial species, underscoring its universal importance and the relevance of our research.

The presence of AI-2 enables bacteria to sense the density of their population. Once the concentration of AI-2 reaches a threshold level, it indicates a sufficiently large community of bacteria. This triggers a coordinated response or behavior, such as the expression of specific genes, the secretion of virulence factors, biofilm formation, or other collective actions that are advantageous only when performed by a large group of bacteria.



By Katie Acken – NC DNA Day Blog

Effect of 25% Test Article / 75% AB Mixture,  
Verticle Axis Reduced to Highlight Changes < 500 RLU;  
Horizontal Axis Reduced for Focused Look at Just First 4 Hours



## GRAPH LINES INTERPRETATION

- The gray line is the control assay containing only the AB medium and the *Vibrio harveyi* BB170 reporter strain. These results are consistent with literature data, showing a gradual reduction in luminescence for several hours and a self-induced increase in luminescence around 3 hours.
- The blue line is the positive control assay, which shows a response to AI-2 produced from the BB152 *Vibrio harveyi* strain. These results are consistent with the literature data, showing that the reporter strain responds to the additional external AI-2 at about 2 hours (i.e., earlier than the natural self-induced increase that the reporter strain exhibits later).
- The orange line is the assay containing a diluted version of the product [25% test product [itself a 60:40 mixture of the clay/hydrogel] / 75% AB medium] and the reporter strain. This control tests whether the product's addition is compatible with the assay. It should resemble the gray line (medium only) if it does not interfere with the assay. As can be seen, the luminescence initially increases and then decreases for the remainder of the duration, indicating effectiveness against AI-2.

## PRESENTERS

Darlene McCord, PhD – Robert G. Frykberg, DPM

## STUDY PROTOCOL

The first step of the protocol involves the collection of cell-free culture fluids. In the second step, the cell-free culture fluids are added to the *V. harveyi* reporter strain, and the resulting light production is measured using a luminometer or scintillation counter. AI-2 activity is calculated as the induction of luminescence of *V. harveyi* BB170. This protocol focuses on techniques for determining the growth conditions under which a bacterial strain of interest produces AI-2.

The procedure was used to compare the relative concentrations of AI-2 produced by various bacterial mutants and AI-2 produced in vitro; however, the alternate protocol described below is better suited for quantification purposes. The *V. harveyi* assay is a method for quantifying AI-2.

## MATERIALS

- The bacterial strain to be tested is *V. harveyi*.
- Growth medium specific for bacterial strain
- V. harveyi* strain in BB152 (luxM::Tn5), frozen glycerol stock
- V. harveyi* strain in BB170 (luxN::Tn5), frozen glycerol stock
- AB medium
- Sterile syringe filters, 0.2-µm pore size
- 1-ml syringes
- Sterile 1.5-ml microcentrifuge tubes
- Luminometer, scintillation counter, or equivalent device for measuring light.

## CONCLUSION

Fentonite effectively blocked the release of AI-2 and inhibited the formation of new biofilms within four hours.