

Determining Growth of Streptococcus Mutans on Thermoplastic Orthodontic Aligners



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BACKGROUND

The purpose of this project was to test the hypothesis that *Streptococcus mutans*, a key cariogenic bacterium, is able to grow and form biofilms on thermoplastic orthodontic clear aligners. We quantified the colony forming units (CFUs) growing in Brain Heart Infusion (BHI) broth. Based on the results of this research, future objectives will include determining the ability of commercial products to eradicate *S. mutans* from binding and forming biofilms on aligners.

INTRODUCTION

Orthodontic treatment with clear aligners made of thermoplastic material has become more popular because of its attraction for ideal esthetics, ease of use, comfort, and simplicity of maintenance for teeth alignment.^{1,2,3} In addition, patients treated with the clear aligner technology have experienced improved periodontal health in comparison to those with fixed orthodontic appliances.⁴ Despite the advantages and preferable outcomes over orthodontic appliances, there are some disadvantages that should be highlighted: oral hygiene problems as they relate to dental plaque formation on these thermoplastic materials and increased risk for caries.⁵

S. mutans as a gram-positive coccoidal bacterium, is the principal cariogenic bacterium in enamel decay, as it possesses virulence factors that promote efficient fermentation of processed sugar resulting in lactic acid release and enamel destruction.⁶ Previous studies have shown that thermoplastic retainers may present similar microenvironments that *S. mutans* can bind to and form dental plaque, leading to dental caries.⁷ Additionally, some studies suggest that the use of thermoplastic materials that cover the teeth prevent proper salivary flow and buffering capacity to neutralize the acidic environment created by the cariogenic bacteria.⁷ Surface roughness and surface free energy of thermoplastic materials can contribute to the bacterial adhesion as well, providing suitable environments for colonization and attraction of more bacteria.⁸

This research will demonstrate that *S. mutans* readily binds to, colonizes and forms biofilms on aligner surfaces, as well as quantify the *S. mutans* CFUs on the aligners over time. This will be an important first step in formalizing strategies to eradicate this organism so that people using the aligners for orthodontic purposes are at lower risk of developing caries.

MATERIALS & METHODS

Stock solution was prepared by reconstituting *S. mutans*. The ideal solution was found by using serial dilutions of BHI broth (Research Products International, 5.5 mL) with stock solution (ATCC 25175, 0.5 mL) to achieve 12-fold dilutions. Ten dilutions were prepared and incubated and plated on sheep blood agar plates (Thermo Scientific Blood Agar, 37 °C, 24-48h) to see if there were acceptable CFUs (less than 300 CFUs). In order to determine the sterility of the aligners, the packaged pouch of aligners was opened and carefully massaged to directly submerge them into BHI broth (200 mL) and incubated (37 °C, 24-48h). These plated samples were sent to a laboratory (Charles River Research Animal Diagnostic Services) and used the Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) test to identify the organisms. After identification, aligners were treated with ultraviolet (UV) light and were autoclaved in attempt to sterilize them.

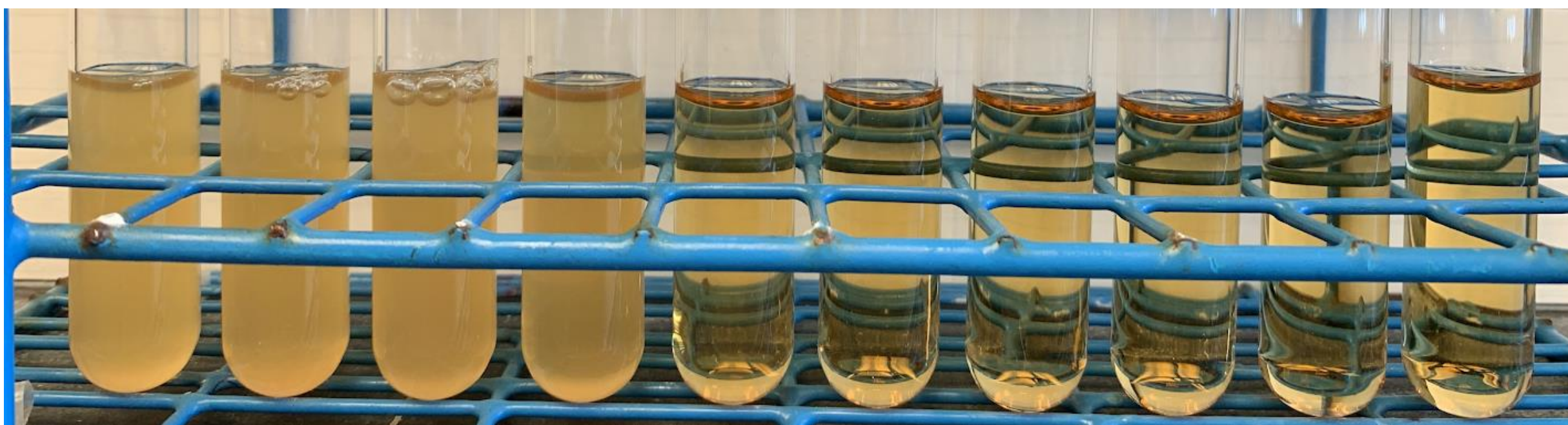


Figure 1—Serial dilutions. 5.5 mL of BHI broth and 0.5 mL of previous dilution. Incubated for 24 h. (From left to right) 1x12¹, 1x12², 1x12³, 1x12⁴, 1x12⁵, 1x12⁶, 1x12⁷, 1x12⁸, 1x12⁹, 1x12¹⁰ serial dilutions.

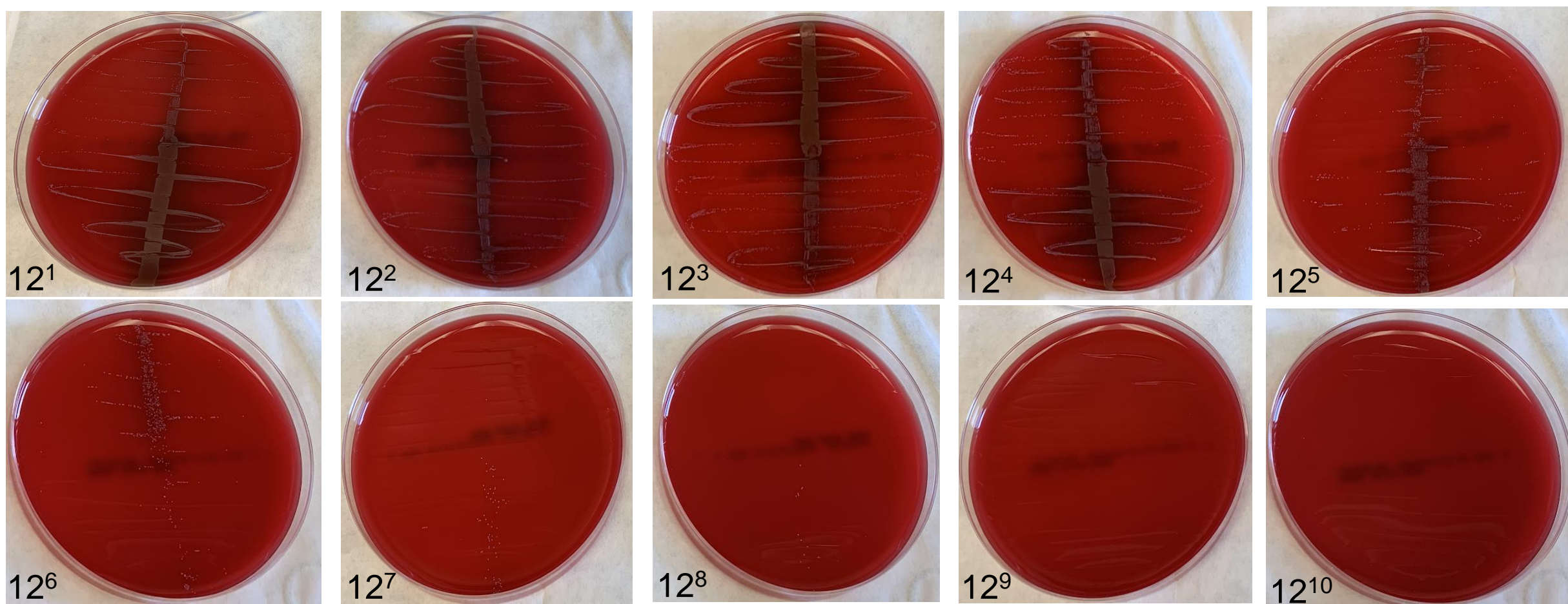


Figure 2—Serial dilutions plated on blood agar plates. Inoculated serial dilution on sheep blood agar plates. Each plate was incubated for 24-48 h. Colony counts were too great for dilutions 1x12¹ through 1x12⁶ (>300 CFUs). Dilution 1x 12⁷ was ideal with 82 CFUs, 1x12⁸ with 15, and 1x12⁹ and 1x12¹⁰ with 0.

RESULTS SUMMARY

The ideal serial dilution of *S. mutans* was determined to be 1x12⁷, with 82 CFUs. The qualitative observations suggested bacterial growth. The Charles River Research Animal Diagnostic Services identified the microorganisms to be of the *Bacillus* genus. Possibly one of the following: *B. altitudinis*, *B. pumilus*, or *B. safensis*.

After UV treating the aligners, there were observations suggesting contamination. Autoclaving the aligners did not maintain the integrity of their shape.

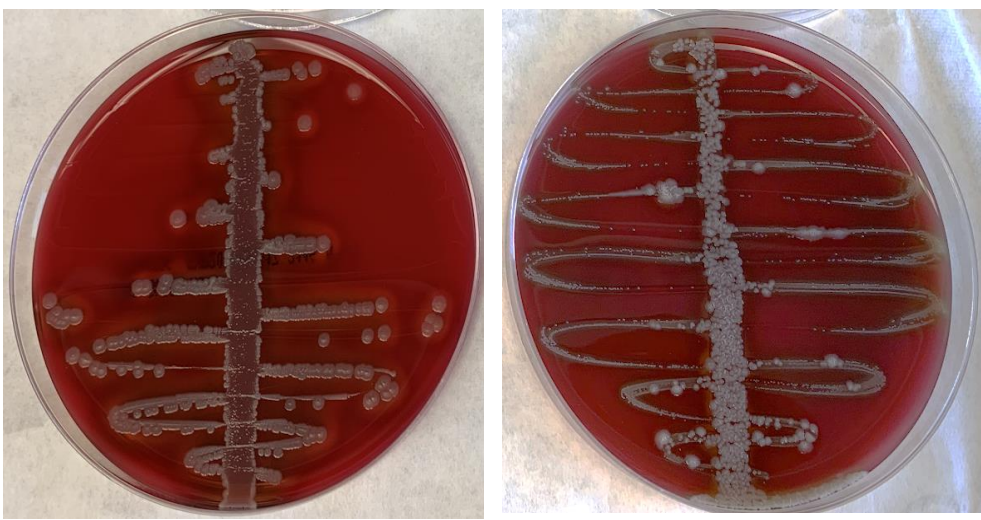


Figure 3—*B. altitudinis*, *B. pumilus*, or *B. safensis* possible contamination from aligners. Plated the liquid from the submerged aligner in the BHI broth. These blood agar plates were sent for identification.

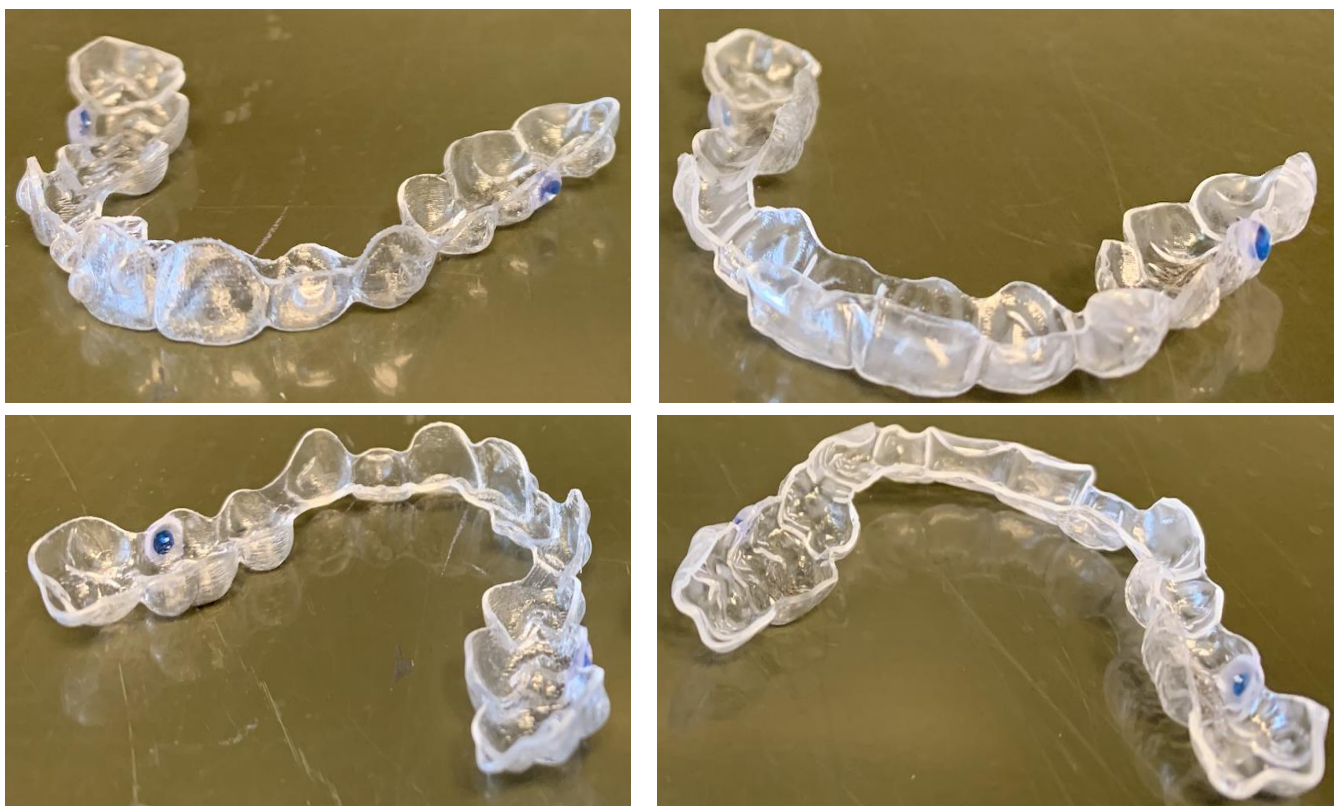


Figure 4 Non-autoclaved and autoclaved aligners comparison—Before (left) and after (right) autoclaving. Edges of the aligners were warped and width

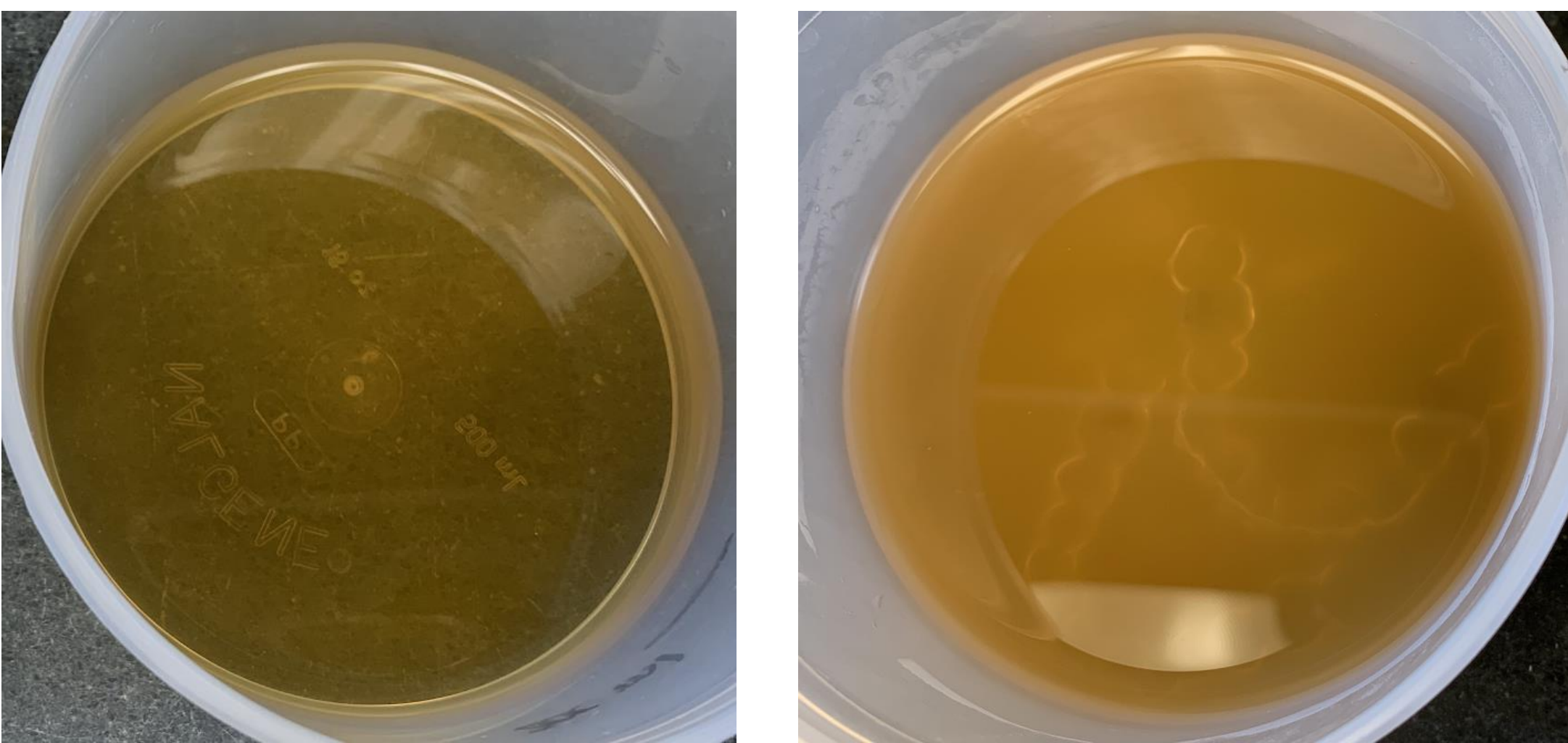


Figure 5 Control and UV-treated aligners—Control (left) and UV-treated aligners (right) in 200 mL of BHI broth and incubation for 24-48 h.

CONCLUSIONS

- The results of the ideal serial dilution suggest a factor for the colonies that grew on the aligners suggesting that the aligners do not come completely sterilized.
- UV treatment is not an effective way to eradicate microorganisms and autoclave sterilization fails to keep the integrity of the aligner shape.
- *S. mutans* does bind to the aligners.

DISCUSSION

- Possible further study can evaluate ways to ensure that the thermoplastic aligners are sterilized prior to usage.
- We can evaluate ways to collect data from aligners from patients who use them to better understand the growth behavior of *S. mutans*.
- What are some possible ways to halt or prevent the growth of *S. mutans* on the aligners?

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