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Research article

Microorganism adhesion using silicon dioxide: An experimental study

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ABSTRACT

In this study, spectrophotometry was used to measure changes in the absorbance properties of yeast, Gram-positive, and Gram-negative bacteria after their attachment to silicon dioxide microparticles (silica). The goal of this study was to determine whether spectrophotometry is an effective method to distinguish these microorganisms from one another and determine whether they have an affinity for silicon dioxide. The experiments were performed by examining the light absorption properties of yeast, Gram-positive and Gram-negative bacteria in a spectrophotometer, both with and without silicon dioxide microparticles. The experiments produced a number of promising results. First, the spectrophotometer graphs of yeast were noticeably different from those of both Gram-positive and Gram-negative bacteria. Second, the absorption of light in both Gram-positive and Gram-negative bacteria occurred at near infrared range (700–1500 nm) and, unlike yeast, the wavelengths increased when silicon dioxide microparticles were added to the suspension. When silicon dioxide microparticles were added to yeast, the absorption of light decreased during the entire wavelength interval of the spectrophotometer measurement. These results indicate that bacteria have an affinity for silicon dioxide, and that spectrophotometry may be used to distinguish yeast from bacteria and, possibly, different bacterial types from one another.

1. Introduction

A key factor in microorganism pathogenesis is its adhesion to surfaces. Binding to surfaces gives a microorganism an opportunity to grow and form biofilms, which are collections of microorganisms held together with a self-produced extracellular matrix [1, 2]. Hence, examining how adhesion occurs and determining the factors that affect adhesive ability may help in better understanding microorganism pathogenesis. Studies have suggested that silicon dioxide can be used to investigate the attracting forces between microorganisms and inorganic surfaces [3]. Therefore, in this study, spectrophotometry was used to examine the adhesion of microorganisms to inorganic surfaces. A spectrophotometer is a common device that has been used for research and diagnostic purposes since 1940 and measures the transmission and absorption of light at different wavelengths [4]. It is used in medicine to determine the concentration of substances in body fluids or following the addition of an enzyme-linked colouring agent [5, 6]

In one study in which densitometry was used to analyse the interactions between microorganisms and silica, it was found that decreases in turbidity occurred more rapidly when silicon dioxide was added to both bacteria and yeast in comparison to without silicon dioxide [7]. Another study demonstrated that silica is a more favourable surface for the development of biofilms than cellulose films, which are biotic surfaces [8].

An examination of microorganism adhesion is important given that adhesion plays a key role in pathogenesis. Furthermore, determining how microorganisms specifically interact with silicon dioxide is clinically important, as it is commonly used in the production of small joint prostheses, dental cements, and as a coating for other types of implantable prostheses [9, 10, 11]. While infection of a joint prosthesis is uncommon, it is the most severe complication that can occur after prosthetic implantation [12]. Additionally, finding new and more rapid methods for determining the type of microorganism causing an infection is important for the clinical management of infectious diseases.

Several mechanisms determine the adhesion properties of microorganisms. Adhesion pili play a vital role in allowing bacteria to stick to body surfaces. They are mostly seen on Gram-negative bacteria, although a few Gram-positive bacteria have them as well [13]. Van der Waals forces are another mechanism that can affect the surface-adhesion abilities of microorganisms. These are intermolecular forces that attract

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adjacent molecules to one another [14]. The Zeta potential can also affect microorganism adhesion. This is the potential of the stern plane of an electrical double layer that covers a cell in a solution. It is typically a negative charge for bacteria and provides a repulsive force from a negatively charged cell or particle [15]. Studies have indicated that silicon dioxide can increase the negative charge of a bacterial membrane when it is adsorbed onto a cell surface [16].

A number of studies have suggested that bacteria have an affinity for silicon dioxide [2, 16, 17]. Therefore, one aim of this study was to examine whether microorganisms have an affinity for silicon dioxide and, if so, what types of microorganisms exhibit this affinity. A further aim was to investigate the possibility of distinguishing one microorganism from another using spectrophotometry. Such a possibility has been studied previously using *Escherichia coli* and *Bacillus globigii*—with promising results [18]. This study included 4 yeasts, and 5 Gram-positive and 8 Gram-negative bacteria.

2. Materials and methods

2.1. Preparation of bacterial, yeast, and silicon dioxide suspensions

This study was conducted in the Microbiology laboratory of the Traumatology and Orthopaedics Hospital in Latvia. To prepare a 4 McFarland unit silicon dioxide suspension, Sigma-Aldrich silicon dioxide microparticles with a mean particle size of 9–13 μm and density of 1.05–1.15 g/ml were added to distilled water. Samples were taken from the suspension, placed in a test tube, and its turbidity was measured using a DEN-1 densitometer. If needed, additional silicon dioxide was added to the distilled water until the densitometer showed a turbidity of 4 ± 0.1 McFarland units. Samples were then taken from reference cultures of the microorganisms and placed in test tubes filled with 8 ml of distilled water. In total, 17 microorganisms were examined: 5 reference cultures of Gram-positive bacteria (*Enterococcus faecalis* ATCC (American Type Culture Collection) 29212, *Staphylococcus epidermidis* ATCC 12228, MSSA (Methicillin-sensitive *Staphylococcus aureus*) ATCC 25923, MRSA (Methicillin-resistant *Staphylococcus aureus*) ATCC 33591, and *Bacillus spizizenii* ATCC 66338); 8 reference cultures of Gram-negative bacteria (*Proteus mirabilis* ATCC 43071, *Enterobacter aerogenes* ATCC 13048, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundii* ATCC 438764, *Klebsiella pneumoniae* ATCC 700603, *Moraxella catarrhalis* ATCC 25238, and *Escherichia coli* ATCC 25922); and 4 pure cultures of yeasts: (*Kluyveromyces marxianus, Candida glabrata, Candida krusei, Candida albicans* ATCC 10231). The OD600 (optical density at 600 nm) of each microorganism at 4 McFarland units is shown in Table 1. The suspensions were placed inside the densitometer to measure their turbidity. If needed, additional microorganism samples from the reference cultures were added until the densitometer showed a turbidity of 4 ± 0.1 McFarland units.

Table 1. Each microorganism's OD600 at 4 McFarland units.

Microorganism	OD600
Kluyveromyces marxianus	1.306
Candida albicans	1.270
Candida glabrata	1.227
Candida krusei	1.299
Staphylococcus epidermidis	1.029
Enterococcus faecalis	1.001
MSSA	0.788
MRSA	0.825
Bacillus spizizenii	0.722
Proteus mirabilis	0.967
Enterobacter aerogenes	0.984
Salmonella enteritidis	0.978
Pseudomonas aeruginosa	0.890
Citrobacter freundii	0.985
Klebsiella pneumoniae	0.643
Moraxella catarrhalis	0.947
Escherichia coli	0.944

2.2. Microorganism samples with and without silicon dioxide suspensions

Seventeen cuvettes were filled with 4 ml of the 4 ± 0.1 McFarland unit suspensions of each microorganism. Another 17 cuvettes were filled with 3.5 ml of the 4 ± 0.1 McFarland unit microorganism suspensions. We added 0.5 ml of the 4 ± 0.1 McFarland unit silicon dioxide suspension to these cuvettes. One cuvette was filled with 4 ml of distilled water and another was filled with a 4 ml of 4 ± 0.1 McFarland unit silicon dioxide suspension.

2.3. Calibration of the spectrophotometer and measuring the absorbance of microorganisms

Samples were measured using a Shimadzu-1600 spectrophotometer (Kyoto, Japan: Shimadzu Corp.). The spectrophotometer's mode was set to 'spectrum'. A cuvette with distilled water was placed in the spectrophotometer and the 'base core' option was selected. This ensured that the light passing through the distilled water at all measured wavelengths was 100% of the light detected by the photodetector, i.e., 0 absorbance units. The spectrophotometer was set to measure absorbance at a wavelength interval of 285–1100 nm. The speed of the measurements was set to 'fast' and the distance between each measured wavelength was set to 1 nm. The initial wavelength of the measurements was 1100 nm and then lowered to 285 nm. To prevent settling, cuvettes were gently tilted several times before being placed in the spectrophotometer. Each sample at a 285–1100 nm wavelength was measured once. Measurements were repeated using a wavelength interval of 285–700 nm. The difference between each measured wavelength was set to 1 nm. Each sample was measured once, and each measurement for the wavelength interval of 285–1100 nm took 57 s. For the 285–700 nm wavelength interval, the measurement was completed in 30 s.

2.4. Comparison of microorganisms

All results were converted from an SPC (spectrum) format to a CSV (comma-separated values) format in the spectrophotometer. Each microorganism measurement contained the wavelengths and the absorbance units at the current wavelength (Supplementary content: Results with a wavelength of 285–700 nm, Results with a wavelength of 285–1100 nm, Description of classification in Excel files). All absorbance measurements of the specific microorganisms within the wavelength interval of 285–1100 nm, both with and without silicon dioxide microparticles, were compared to all other study microorganisms using a two-sample t-test assuming unequal variances. Alpha was set to 0.05 and a hypothesized mean difference set to 0. The comparisons, tables and graphs were done in Microsoft Excel (2016). Comparisons were repeated for measurements at 285–1100 nm with silicon dioxide microparticles, at 285–700 nm without silicon dioxide microparticles, and at 285–700 nm with silicon dioxide microparticles. A significant difference was assumed if $p < 0.05$.

3. Results

3.1. Microorganism comparisons at 285–1100 nm

Table 2 presents the results of comparisons between the microorganisms at 285–1100 nm without silicon dioxide. Assuming significance at $p < 0.05$, it was possible to distinguish one microorganism from another in 99/136 (72.8%) of all the comparisons. A significant difference was found in 52/52 (100%) of the yeast to bacteria comparisons,

Table 2. Comparison of microorganisms at 285–1100 nm without silicon dioxide using the two sample t-test.

Compared Microorganisms	*p* value
Kluyveromyces marxianus compared to *Candida albicans*	0.000128
Kluyveromyces marxianus compared to *Candida glabrata*	<0.000001
Kluyveromyces marxianus compared to *Candida krusei*	<0.000001
Kluyveromyces marxianus compared to *Staphylococcus epidermidis*	<0.000001
Kluyveromyces marxianus compared to *Enterococcus faecalis*	<0.000001
Kluyveromyces marxianus compared to MSSA	<0.000001
Kluyveromyces marxianus compared to MRSA	<0.000001
Kluyveromyces marxianus compared to *Bacillus spizizenii*	<0.000001
Kluyveromyces marxianus compared to *Proteus mirabilis*	<0.000001
Kluyveromyces marxianus compared to *Enterobacter aerogenes*	<0.000001
Kluyveromyces marxianus compared to *Salmonella enteritidis*	<0.000001
Kluyveromyces marxianus compared to *Pseudomonas aeruginosa*	<0.000001
Kluyveromyces marxianus compared to *Citrobacter freundii*	<0.000001
Kluyveromyces marxianus compared to *Klebsiella pneumoniae*	<0.000001
Kluyveromyces marxianus compared to *Moraxella catarrhalis*	<0.000001
Kluyveromyces marxianus compared to *Escherichia coli*	<0.000001
Candida albicans compared to *Candida glabrata*	<0.000001
Candida albicans compared to *Candida krusei*	0.000942
Candida albicans compared to *Staphylococcus epidermidis*	<0.000001
Candida albicans compared to *Enterococcus faecalis*	<0.000001
Candida albicans compared to MSSA	<0.000001
Candida albicans compared to MRSA	<0.000001
Candida albicans compared to *Bacillus spizizenii*	<0.000001
Candida albicans compared to *Proteus mirabilis*	<0.000001
Candida albicans compared to *Enterobacter aerogenes*	<0.000001
Candida albicans compared to *Salmonella enteritidis*	<0.000001
Candida albicans compared to *Pseudomonas aeruginosa*	<0.000001
Candida albicans compared to *Citrobacter freundii*	<0.000001
Candida albicans compared to *Klebsiella pneumoniae*	<0.000001
Candida albicans compared to *Moraxella catarrhalis*	<0.000001
Candida albicans compared to *Escherichia coli*	<0.000001
Candida glabrata compared to *Candida krusei*	0.131187
Candida glabrata compared to *Staphylococcus epidermidis*	<0.000001
Candida glabrata compared to *Enterococcus faecalis*	<0.000001
Candida glabrata compared to MSSA	<0.000001
Candida glabrata compared to MRSA	<0.000001
Candida glabrata compared to *Bacillus spizizenii*	<0.000001
Candida glabrata compared to *Proteus mirabilis*	<0.000001
Candida glabrata compared to *Enterobacter aerogenes*	<0.000001
Candida glabrata compared to *Salmonella enteritidis*	<0.000001
Candida glabrata compared to *Pseudomonas aeruginosa*	<0.000001
Candida glabrata compared to *Citrobacter freundii*	<0.000001
Candida glabrata compared to *Klebsiella pneumoniae*	<0.000001
Candida glabrata compared to *Moraxella catarrhalis*	<0.000001
Candida glabrata compared to *Escherichia coli*	<0.000001
Candida krusei compared to *Staphylococcus epidermidis*	<0.000001
Candida krusei compared to *Enterococcus faecalis*	<0.000001
Candida krusei compared to MSSA	<0.000001
Candida krusei compared to MRSA	<0.000001
Candida krusei compared to *Bacillus spizizenii*	<0.000001
Candida krusei compared to *Proteus mirabilis*	<0.000001
Candida krusei compared to *Enterobacter aerogenes*	<0.000001
Candida krusei compared to *Salmonella enteritidis*	<0.000001
Candida krusei compared to *Pseudomonas aeruginosa*	<0.000001
Candida krusei compared to *Citrobacter freundii*	<0.000001
Candida krusei compared to *Klebsiella pneumoniae*	<0.000001
Candida krusei compared to *Moraxella catarrhalis*	<0.000001
Candida krusei compared to *Escherichia coli*	<0.000001

Table 2 (*continued*)

Compared Microorganisms	*p* value
Staphylococcus epidermidis compared to *Enterococcus faecalis*	0.914399
Staphylococcus epidermidis compared to MSSA	<0.000001
Staphylococcus epidermidis compared to MRSA	0.000007
Staphylococcus epidermidis compared to *Bacillus spizizenii*	<0.000001
Staphylococcus epidermidis compared to *Proteus mirabilis*	0.660184
Staphylococcus epidermidis compared to *Enterobacter aerogenes*	0.770679
Staphylococcus epidermidis compared to *Salmonella enteritidis*	0.854942
Staphylococcus epidermidis compared to *Pseudomonas aeruginosa*	0.046329
Staphylococcus epidermidis compared to *Citrobacter freundii*	0.543804
Staphylococcus epidermidis compared to *Klebsiella pneumoniae*	<0.000001
Staphylococcus epidermidis compared to *Moraxella catarrhalis*	0.045225
Staphylococcus epidermidis compared to *Escherichia coli*	0.071304
Enterococcus faecalis compared to MSSA	<0.000001
Enterococcus faecalis compared to MRSA	0.000021
Enterococcus faecalis compared to *Bacillus spizizenii*	<0.000001
Enterococcus faecalis compared to *Proteus mirabilis*	0.611955
Enterococcus faecalis compared to *Enterobacter aerogenes*	0.711759
Enterococcus faecalis compared to *Salmonella enteritidis*	0.788713
Enterococcus faecalis compared to *Pseudomonas aeruginosa*	0.050461
Enterococcus faecalis compared to *Citrobacter freundii*	0.507301
Enterococcus faecalis compared to *Klebsiella pneumoniae*	<0.000001
Enterococcus faecalis compared to *Moraxella catarrhalis*	0.051130
Enterococcus faecalis compared to *Escherichia coli*	0.076582
MSSA compared to MRSA	0.182356
MSSA compared to *Bacillus spizizenii*	0.001273
MSSA compared to *Proteus mirabilis*	<0.000001
MSSA compared to *Enterobacter aerogenes*	<0.000001
MSSA compared to *Salmonella enteritidis*	<0.000001
MSSA compared to *Pseudomonas aeruginosa*	0.000462
MSSA compared to *Citrobacter freundii*	<0.000001
MSSA compared to *Klebsiella pneumoniae*	<0.000001
MSSA compared to *Moraxella catarrhalis*	0.000077
MSSA compared to *Escherichia coli*	0.000063
MRSA compared to *Bacillus spizizenii*	0.000005
MRSA compared to *Proteus mirabilis*	0.000213
MRSA compared to *Enterobacter aerogenes*	0.000125
MRSA compared to *Salmonella enteritidis*	0.000009
MRSA compared to *Pseudomonas aeruginosa*	0.031052
MRSA compared to *Citrobacter freundii*	0.000312
MRSA compared to *Klebsiella pneumoniae*	<0.000001
MRSA compared to *Moraxella catarrhalis*	0.013127
MRSA compared to *Escherichia coli*	0.010382
Bacillus spizizenii compared to *Proteus mirabilis*	<0.000001
Bacillus spizizenii compared to *Enterobacter aerogenes*	<0.000001
Bacillus spizizenii compared to *Salmonella enteritidis*	<0.000001
Bacillus spizizenii compared to *Pseudomonas aeruginosa*	<0.000001
Bacillus spizizenii compared to *Citrobacter freundii*	<0.000001
Bacillus spizizenii compared to *Klebsiella pneumoniae*	0.017308
Bacillus spizizenii compared to *Moraxella catarrhalis*	<0.000001
Bacillus spizizenii compared to *Escherichia coli*	<0.000001
Proteus mirabilis compared to *Enterobacter aerogenes*	0.891944
Proteus mirabilis compared to *Salmonella enteritidis*	0.815833
Proteus mirabilis compared to *Pseudomonas aeruginosa*	0.147890
Proteus mirabilis compared to *Citrobacter freundii*	0.883027
Proteus mirabilis compared to *Klebsiella pneumoniae*	<0.000001
Proteus mirabilis compared to *Moraxella catarrhalis*	0.161325
Proteus mirabilis compared to *Escherichia coli*	0.216673
Enterobacter aerogenes compared to *Salmonella enteritidis*	0.922032
Enterobacter aerogenes compared to *Pseudomonas aeruginosa*	0.114523

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Table 2 (*continued*)

Compared Microorganisms	*p* value
Enterobacter aerogenes compared to *Citrobacter freundii*	0.775902
Enterobacter aerogenes compared to *Klebsiella pneumoniae*	<0.000001
Enterobacter aerogenes compared to *Moraxella catarrhalis*	0.123126
Enterobacter aerogenes compared to *Escherichia coli*	0.169519
Salmonella enteritidis compared to *Pseudomonas aeruginosa*	0.096149
Salmonella enteritidis compared to *Citrobacter freundii*	0.702467
Salmonella enteritidis compared to *Klebsiella pneumoniae*	<0.000001
Salmonella enteritidis compared to *Moraxella catarrhalis*	0.102396
Salmonella enteritidis compared to *Escherichia coli*	0.142982
Pseudomonas aeruginosa compared to *Citrobacter freundii*	0.186131
Pseudomonas aeruginosa compared to *Klebsiella pneumoniae*	<0.000001
Pseudomonas aeruginosa compared to *Moraxella catarrhalis*	0.878623
Pseudomonas aeruginosa compared to *Escherichia coli*	0.778446
Citrobacter freundii compared to *Klebsiella pneumoniae*	<0.000001
Citrobacter freundii compared to *Moraxella catarrhalis*	0.205258
Citrobacter freundii compared to *Escherichia coli*	0.270597
Klebsiella pneumoniae compared to *Moraxella catarrhalis*	<0.000001
Klebsiella pneumoniae compared to *Escherichia coli*	<0.000001
Moraxella catarrhalis compared to *Escherichia coli*	0.886808

Table 3. Comparison of microorganisms at 285–1100 nm with silicon dioxide using the two-sample t-test.

Compared Microorganisms	*p* value
Kluyveromyces marxianus compared to *Candida albicans*	0.006617
Kluyveromyces marxianus compared to *Candida glabrata*	0.278350
Kluyveromyces marxianus compared to *Candida krusei*	0.014212
Kluyveromyces marxianus compared to *Staphylococcus epidermidis*	<0.000001
Kluyveromyces marxianus compared to *Enterococcus faecalis*	<0.000001
Kluyveromyces marxianus compared to MSSA	<0.000001
Kluyveromyces marxianus compared to MRSA	<0.000001
Kluyveromyces marxianus compared to *Bacillus spizizenii*	<0.000001
Kluyveromyces marxianus compared to *Proteus mirabilis*	<0.000001
Kluyveromyces marxianus compared to *Enterobacter aerogenes*	<0.000001
Kluyveromyces marxianus compared to *Salmonella enteritidis*	<0.000001
Kluyveromyces marxianus compared to *Pseudomonas aeruginosa*	<0.000001
Kluyveromyces marxianus compared to *Citrobacter freundii*	<0.000001
Kluyveromyces marxianus compared to *Klebsiella pneumoniae*	<0.000001
Kluyveromyces marxianus compared to *Moraxella catarrhalis*	<0.000001
Kluyveromyces marxianus compared to *Escherichia coli*	<0.000001
Candida albicans compared to *Candida glabrata*	<0.000001
Candida albicans compared to *Candida krusei*	0.889397
Candida albicans compared to *Staphylococcus epidermidis*	<0.000001
Candida albicans compared to *Enterococcus faecalis*	<0.000001
Candida albicans compared to MSSA	<0.000001
Candida albicans compared to MRSA	<0.000001
Candida albicans compared to *Bacillus spizizenii*	<0.000001
Candida albicans compared to *Proteus mirabilis*	<0.000001
Candida albicans compared to *Enterobacter aerogenes*	<0.000001
Candida albicans compared to *Salmonella enteritidis*	<0.000001
Candida albicans compared to *Pseudomonas aeruginosa*	<0.000001
Candida albicans compared to *Citrobacter freundii*	<0.000001
Candida albicans compared to *Klebsiella pneumoniae*	<0.000001
Candida albicans compared to *Moraxella catarrhalis*	<0.000001
Candida albicans compared to *Escherichia coli*	<0.000001
Candida glabrata compared to *Candida krusei*	0.000133
Candida glabrata compared to *Staphylococcus epidermidis*	<0.000001
Candida glabrata compared to *Enterococcus faecalis*	<0.000001
Candida glabrata compared to MSSA	<0.000001

Table 3 (*continued*)

Compared Microorganisms	*p* value
Candida glabrata compared to MRSA	<0.000001
Candida glabrata compared to *Bacillus spizizenii*	<0.000001
Candida glabrata compared to *Proteus mirabilis*	<0.000001
Candida glabrata compared to *Enterobacter aerogenes*	<0.000001
Candida glabrata compared to *Salmonella enteritidis*	<0.000001
Candida glabrata compared to *Pseudomonas aeruginosa*	<0.000001
Candida glabrata compared to *Citrobacter freundii*	<0.000001
Candida glabrata compared to *Klebsiella pneumoniae*	<0.000001
Candida glabrata compared to *Moraxella catarrhalis*	<0.000001
Candida glabrata compared to *Escherichia coli*	<0.000001
Candida krusei compared to *Staphylococcus epidermidis*	<0.000001
Candida krusei compared to *Enterococcus faecalis*	<0.000001
Candida krusei compared to MSSA	<0.000001
Candida krusei compared to MRSA	<0.000001
Candida krusei compared to *Bacillus spizizenii*	<0.000001
Candida krusei compared to *Proteus mirabilis*	<0.000001
Candida krusei compared to *Enterobacter aerogenes*	<0.000001
Candida krusei compared to *Salmonella enteritidis*	<0.000001
Candida krusei compared to *Pseudomonas aeruginosa*	<0.000001
Candida krusei compared to *Citrobacter freundii*	<0.000001
Candida krusei compared to *Klebsiella pneumoniae*	<0.000001
Candida krusei compared to *Moraxella catarrhalis*	<0.000001
Candida krusei compared to *Escherichia coli*	<0.000001
Staphylococcus epidermidis compared to *Enterococcus faecalis*	0.037622
Staphylococcus epidermidis compared to MSSA	0.000184
Staphylococcus epidermidis compared to MRSA	0.722643
Staphylococcus epidermidis compared to *Bacillus spizizenii*	<0.000001
Staphylococcus epidermidis compared to *Proteus mirabilis*	0.007244
Staphylococcus epidermidis compared to *Enterobacter aerogenes*	0.000257
Staphylococcus epidermidis compared to *Salmonella enteritidis*	0.000004
Staphylococcus epidermidis compared to *Pseudomonas aeruginosa*	0.140340
Staphylococcus epidermidis compared to *Citrobacter freundii*	0.000026
Staphylococcus epidermidis compared to *Klebsiella pneumoniae*	0.00552
Staphylococcus epidermidis compared to *Moraxella catarrhalis*	0.071354
Staphylococcus epidermidis compared to *Escherichia coli*	0.654009
Enterococcus faecalis compared to MSSA	<0.000001
Enterococcus faecalis compared to MRSA	0.027201
Enterococcus faecalis compared to *Bacillus spizizenii*	<0.000001
Enterococcus faecalis compared to *Proteus mirabilis*	0.535533
Enterococcus faecalis compared to *Enterobacter aerogenes*	0.116586
Enterococcus faecalis compared to *Salmonella enteritidis*	0.012177
Enterococcus faecalis compared to *Pseudomonas aeruginosa*	0.649447
Enterococcus faecalis compared to *Citrobacter freundii*	0.043448
Enterococcus faecalis compared to *Klebsiella pneumoniae*	0.000002
Enterococcus faecalis compared to *Moraxella catarrhalis*	0.772104
Enterococcus faecalis compared to *Escherichia coli*	0.131859
MSSA compared to MRSA	0.002679
MSSA compared to *Bacillus spizizenii*	0.001238
MSSA compared to *Proteus mirabilis*	<0.000001
MSSA compared to *Enterobacter aerogenes*	<0.000001
MSSA compared to *Salmonella enteritidis*	<0.000001
MSSA compared to *Pseudomonas aeruginosa*	0.000002
MSSA compared to *Citrobacter freundii*	<0.000001
MSSA compared to *Klebsiella pneumoniae*	0.237971
MSSA compared to *Moraxella catarrhalis*	<0.000001
MSSA compared to *Escherichia coli*	0.000103
MRSA compared to *Bacillus spizizenii*	<0.000001
MRSA compared to *Proteus mirabilis*	0.005793
MRSA compared to *Enterobacter aerogenes*	0.000271
MRSA compared to *Salmonella enteritidis*	0.000006

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Table 3 (*continued*)

Compared Microorganisms	*p* value
MRSA compared to *Pseudomonas aeruginosa*	0.096462
MRSA compared to *Citrobacter freundii*	0.000036
MRSA compared to *Klebsiella pneumoniae*	0.036991
MRSA compared to *Moraxella catarrhalis*	0.050037
MRSA compared to *Escherichia coli*	0.461519
Bacillus spizizenii compared to *Proteus mirabilis*	<0.000001
Bacillus spizizenii compared to *Enterobacter aerogenes*	<0.000001
Bacillus spizizenii compared to *Salmonella enteritidis*	<0.000001
Bacillus spizizenii compared to *Pseudomonas aeruginosa*	<0.000001
Bacillus spizizenii compared to *Citrobacter freundii*	<0.000001
Bacillus spizizenii compared to *Klebsiella pneumoniae*	0.000001
Bacillus spizizenii compared to *Moraxella catarrhalis*	<0.000001
Bacillus spizizenii compared to *Escherichia coli*	<0.000001
Proteus mirabilis compared to *Enterobacter aerogenes*	0.347364
Proteus mirabilis compared to *Salmonella enteritidis*	0.061169
Proteus mirabilis compared to *Pseudomonas aeruginosa*	0.301413
Proteus mirabilis compared to *Citrobacter freundii*	0.173284
Proteus mirabilis compared to *Klebsiella pneumoniae*	<0.000001
Proteus mirabilis compared to *Moraxella catarrhalis*	0.362275
Proteus mirabilis compared to *Escherichia coli*	0.036913
Enterobacter aerogenes compared to *Salmonella enteritidis*	0.348114
Enterobacter aerogenes compared to *Pseudomonas aeruginosa*	0.053585
Enterobacter aerogenes compared to *Citrobacter freundii*	0.696695
Enterobacter aerogenes compared to *Klebsiella pneumoniae*	<0.000001
Enterobacter aerogenes compared to *Moraxella catarrhalis*	0.061709
Enterobacter aerogenes compared to *Escherichia coli*	0.002674
Salmonella enteritidis compared to *Pseudomonas aeruginosa*	0.004847
Salmonella enteritidis compared to *Citrobacter freundii*	0.561159
Salmonella enteritidis compared to *Klebsiella pneumoniae*	<0.000001
Salmonella enteritidis compared to *Moraxella catarrhalis*	0.004938
Salmonella enteritidis compared to *Escherichia coli*	0.000095
Pseudomonas aeruginosa compared to *Citrobacter freundii*	<0.000001
Pseudomonas aeruginosa compared to *Klebsiella pneumoniae*	<0.000001
Pseudomonas aeruginosa compared to *Moraxella catarrhalis*	0.852092
Pseudomonas aeruginosa compared to *Escherichia coli*	0.327486
Citrobacter freundii compared to *Klebsiella pneumoniae*	<0.000001
Citrobacter freundii compared to *Moraxella catarrhalis*	0.019583
Citrobacter freundii compared to *Escherichia coli*	0.000489
Klebsiella pneumoniae compared to *Moraxella catarrhalis*	0.000008
Klebsiella pneumoniae compared to *Escherichia coli*	0.002762
Moraxella catarrhalis compared to *Escherichia coli*	<0.000001

41/78 (52.6%) of the comparisons among the bacteria, and in 5/6 (83.3%) of the comparisons among the yeasts.

In Table 3, comparisons at 285–1100 nm with silicon dioxide are presented. It was possible to distinguish one microorganism from another in 109/136 (81.1%) of all the comparisons. There was a significant difference in 52/52 (100%) of the comparisons between yeasts and bacteria, 54/78 (69.2%) of the comparisons among bacteria, and in 3/6 (50%) of the comparisons among the yeasts.

3.2. Microorganisms compared at 285–700 nm

In Table 4, comparisons at 285–700 nm without silicon dioxide are presented. Distinguishing one microorganism from another was possible in 106/136 (77.9%) of all the comparisons. There was a significant difference in 44/52 (84.6%) of the comparisons between yeast and bacteria, in 56/78 (71.8%) of the comparisons among the bacteria, and in 6/6 (100%) of the comparisons among the yeasts.

In Table 5, comparisons at 285–700 nm with silicon dioxide are presented. Distinguishing one microorganism from another was possible

Table 4. Comparison of microorganisms at 285–700 nm without silicon dioxide using the two-sample t-test.

Compared Microorganisms	*p* value
Kluyveromyces marxianus compared to *Candida albicans*	0.000034
Kluyveromyces marxianus compared to *Candida glabrata*	<0.000001
Kluyveromyces marxianus compared to *Candida krusei*	<0.000001
Kluyveromyces marxianus compared to *Staphylococcus epidermidis*	0.001229
Kluyveromyces marxianus compared to *Enterococcus faecalis*	<0.000001
Kluyveromyces marxianus compared to MSSA	0.503270
Kluyveromyces marxianus compared to MRSA	0.925838
Kluyveromyces marxianus compared to *Bacillus spizizenii*	<0.000001
Kluyveromyces marxianus compared to *Proteus mirabilis*	<0.000001
Kluyveromyces marxianus compared to *Enterobacter aerogenes*	<0.000001
Kluyveromyces marxianus compared to *Salmonella enteritidis*	<0.000001
Kluyveromyces marxianus compared to *Pseudomonas aeruginosa*	0.000004
Kluyveromyces marxianus compared to *Citrobacter freundii*	<0.000001
Kluyveromyces marxianus compared to *Klebsiella pneumoniae*	<0.000001
Kluyveromyces marxianus compared to *Moraxella catarrhalis*	0.000002
Kluyveromyces marxianus compared to *Escherichia coli*	<0.000001
Candida albicans compared to *Candida glabrata*	<0.000001
Candida albicans compared to *Candida krusei*	<0.000001
Candida albicans compared to *Staphylococcus epidermidis*	<0.000001
Candida albicans compared to *Enterococcus faecalis*	<0.000001
Candida albicans compared to MSSA	0.426952
Candida albicans compared to MRSA	0.135974
Candida albicans compared to *Bacillus spizizenii*	<0.000001
Candida albicans compared to *Proteus mirabilis*	<0.000001
Candida albicans compared to *Enterobacter aerogenes*	<0.000001
Candida albicans compared to *Salmonella enteritidis*	<0.000001
Candida albicans compared to *Pseudomonas aeruginosa*	<0.000001
Candida albicans compared to *Citrobacter freundii*	<0.000001
Candida albicans compared to *Klebsiella pneumoniae*	<0.000001
Candida albicans compared to *Moraxella catarrhalis*	<0.000001
Candida albicans compared to *Escherichia coli*	<0.000001
Candida glabrata compared to *Candida krusei*	<0.000001
Candida glabrata compared to *Staphylococcus epidermidis*	<0.000001
Candida glabrata compared to *Enterococcus faecalis*	<0.000001
Candida glabrata compared to MSSA	0.234312
Candida glabrata compared to MRSA	0.062722
Candida glabrata compared to *Bacillus spizizenii*	<0.000001
Candida glabrata compared to *Proteus mirabilis*	<0.000001
Candida glabrata compared to *Enterobacter aerogenes*	<0.000001
Candida glabrata compared to *Salmonella enteritidis*	<0.000001
Candida glabrata compared to *Pseudomonas aeruginosa*	<0.000001
Candida glabrata compared to *Citrobacter freundii*	<0.000001
Candida glabrata compared to *Klebsiella pneumoniae*	<0.000001
Candida glabrata compared to *Moraxella catarrhalis*	<0.000001
Candida glabrata compared to *Escherichia coli*	<0.000001
Candida krusei compared to *Staphylococcus epidermidis*	0.000004
Candida krusei compared to *Enterococcus faecalis*	0.006735
Candida krusei compared to MSSA	<0.000001
Candida krusei compared to MRSA	<0.000001
Candida krusei compared to *Bacillus spizizenii*	<0.000001
Candida krusei compared to *Proteus mirabilis*	0.039461
Candida krusei compared to *Enterobacter aerogenes*	0.001872
Candida krusei compared to *Salmonella enteritidis*	0.003174
Candida krusei compared to *Pseudomonas aeruginosa*	0.519551
Candida krusei compared to *Citrobacter freundii*	0.031600
Candida krusei compared to *Klebsiella pneumoniae*	<0.000001
Candida krusei compared to *Moraxella catarrhalis*	0.030641
Candida krusei compared to *Escherichia coli*	0.810323

(*continued on next page*)

Table 4 (*continued*)

Compared Microorganisms	*p* value
Staphylococcus epidermidis compared to *Enterococcus faecalis*	<0.000001
Staphylococcus epidermidis compared to MSSA	0.007855
Staphylococcus epidermidis compared to MRSA	0.052664
Staphylococcus epidermidis compared to *Bacillus spizizenii*	<0.000001
Staphylococcus epidermidis compared to *Proteus mirabilis*	0.000006
Staphylococcus epidermidis compared to *Enterobacter aerogenes*	<0.000001
Staphylococcus epidermidis compared to *Salmonella enteritidis*	<0.000001
Staphylococcus epidermidis compared to *Pseudomonas aeruginosa*	0.036588
Staphylococcus epidermidis compared to *Citrobacter freundii*	0.000002
Staphylococcus epidermidis compared to *Klebsiella pneumoniae*	<0.000001
Staphylococcus epidermidis compared to *Moraxella catarrhalis*	0.139104
Staphylococcus epidermidis compared to *Escherichia coli*	0.001456
Enterococcus faecalis compared to MSSA	<0.000001
Enterococcus faecalis compared to MRSA	<0.000001
Enterococcus faecalis compared to *Bacillus spizizenii*	<0.000001
Enterococcus faecalis compared to *Proteus mirabilis*	0.708334
Enterococcus faecalis compared to *Enterobacter aerogenes*	0.737493
Enterococcus faecalis compared to *Salmonella enteritidis*	0.807431
Enterococcus faecalis compared to *Pseudomonas aeruginosa*	0.021208
Enterococcus faecalis compared to *Citrobacter freundii*	0.667577
Enterococcus faecalis compared to *Klebsiella pneumoniae*	<0.000001
Enterococcus faecalis compared to *Moraxella catarrhalis*	0.000360
Enterococcus faecalis compared to *Escherichia coli*	0.067634
MSSA compared to MRSA	0.581649
MSSA compared to *Bacillus spizizenii*	<0.000001
MSSA compared to *Proteus mirabilis*	<0.000001
MSSA compared to *Enterobacter aerogenes*	<0.000001
MSSA compared to *Salmonella enteritidis*	<0.000001
MSSA compared to *Pseudomonas aeruginosa*	0.000051
MSSA compared to *Citrobacter freundii*	<0.000001
MSSA compared to *Klebsiella pneumoniae*	<0.000001
MSSA compared to *Moraxella catarrhalis*	0.000154
MSSA compared to *Escherichia coli*	<0.000001
MRSA compared to *Bacillus spizizenii*	<0.000001
MRSA compared to *Proteus mirabilis*	<0.000001
MRSA compared to *Enterobacter aerogenes*	<0.000001
MRSA compared to *Salmonella enteritidis*	<0.000001
MRSA compared to *Pseudomonas aeruginosa*	0.000597
MRSA compared to *Citrobacter freundii*	<0.000001
MRSA compared to *Klebsiella pneumoniae*	<0.000001
MRSA compared to *Moraxella catarrhalis*	0.002160
MRSA compared to *Escherichia coli*	0.000008
Bacillus spizizenii compared to *Proteus mirabilis*	<0.000001
Bacillus spizizenii compared to *Enterobacter aerogenes*	<0.000001
Bacillus spizizenii compared to *Salmonella enteritidis*	<0.000001
Bacillus spizizenii compared to *Pseudomonas aeruginosa*	<0.000001
Bacillus spizizenii compared to *Citrobacter freundii*	<0.000001
Bacillus spizizenii compared to *Klebsiella pneumoniae*	0.396335
Bacillus spizizenii compared to *Moraxella catarrhalis*	<0.000001
Bacillus spizizenii compared to *Escherichia coli*	<0.000001
Proteus mirabilis compared to *Enterobacter aerogenes*	0.486847
Proteus mirabilis compared to *Salmonella enteritidis*	0.545609
Proteus mirabilis compared to *Pseudomonas aeruginosa*	0.057226
Proteus mirabilis compared to *Citrobacter freundii*	0.968203
Proteus mirabilis compared to *Klebsiella pneumoniae*	<0.000001
Proteus mirabilis compared to *Moraxella catarrhalis*	0.002265
Proteus mirabilis compared to *Escherichia coli*	0.164145
Enterobacter aerogenes compared to *Salmonella enteritidis*	0.929737
Enterobacter aerogenes compared to *Pseudomonas aeruginosa*	0.009600
Enterobacter aerogenes compared to *Citrobacter freundii*	0.446085
Enterobacter aerogenes compared to *Klebsiella pneumoniae*	<0.000001
Enterobacter aerogenes compared to *Moraxella catarrhalis*	0.000106
Enterobacter aerogenes compared to *Escherichia coli*	0.031823
Salmonella enteritidis compared to *Pseudomonas aeruginosa*	0.012653
Salmonella enteritidis compared to *Citrobacter freundii*	0.504783
Salmonella enteritidis compared to *Klebsiella pneumoniae*	<0.000001
Salmonella enteritidis compared to *Moraxella catarrhalis*	0.000174
Salmonella enteritidis compared to *Escherichia coli*	0.041231
Pseudomonas aeruginosa compared to *Citrobacter freundii*	0.054315
Pseudomonas aeruginosa compared to *Klebsiella pneumoniae*	<0.000001
Pseudomonas aeruginosa compared to *Moraxella catarrhalis*	0.411301
Pseudomonas aeruginosa compared to *Escherichia coli*	0.506630
Citrobacter freundii compared to *Klebsiella pneumoniae*	<0.000001
Citrobacter freundii compared to *Moraxella catarrhalis*	0.001695
Citrobacter freundii compared to *Escherichia coli*	0.160385
Klebsiella pneumoniae compared to *Moraxella catarrhalis*	<0.000001
Klebsiella pneumoniae compared to *Escherichia coli*	<0.000001
Moraxella catarrhalis compared to *Escherichia coli*	0.090763

in 107/136 (78.7%) of all the comparisons. There was a significant difference in 44/52 (84.6%) of the comparisons between yeast and bacteria, in 58/78 (74.6%) of the comparisons among the bacteria, and in 5/6 (83.3%) of the comparisons among the yeasts.

3.3. Results summary

Results from the previous tables are summarized in Table 6. We found that silicon dioxide improved the probability of distinguishing one bacteria from another at wavelengths between 285-1100 nm. Silicon dioxide also decreased the probability of distinguishing one yeast from another. At 285–700 nm silicon dioxide did not have a significant effect on the results, but the probability of distinguishing one bacteria from another increased at this wavelength interval in comparison with the 285–1100 nm wavelength interval. Furthermore, the addition of silicon dioxide increased the ability to distinguish between Gram-positive and Gram-negative bacteria.

3.4. Silicon dioxide effects on average absorbance

Silicon dioxide decreased the average absorbance of most yeasts in the 285–1100 nm measurement interval, assuming a significant change of 0.03 absorbance units; and, it also increased the average absorbance of most bacteria in the same measurement interval (Table 7). For *Candida glabrata*, the average absorbance did not exhibit a significant decrease. For *Staphylococcus epidermidis*, the average absorbance after the addition of silicon dioxide decreased, but for *Enterococcus faecalis*, MSSA, *Proteus mirabilis*, and *Escherichia coli* silicon dioxide produced no significant changes.

3.5. Distinctive qualities of microorganism graphs

The average absorbance graphs of yeast, Gram-positive, and Gram-negative bacteria (Figure 1) showed distinctive characteristics corresponding to the type of microorganism the spectrophotometer was analysing. The following graphs are shown from the longest wavelength to the shortest. This order was maintained as the spectrophotometer presented measured absorbance units starting at longer wavelengths. All of the yeasts exhibited higher absorbance than the Gram-positive and Gram-negative bacteria at near infrared wavelengths. Absorbance for bacteria increased at a higher rate than the yeasts, and, at some point, the absorbance units of bacteria surpassed the absorbance units of the yeasts.

Table 5. Comparison of microorganisms at 285–700 nm with silicon dioxide using the two-sample t-test.

Compared Microorganisms	*p* value
Kluyveromyces marxianus compared to *Candida albicans*	0.601437
Kluyveromyces marxianus compared to *Candida glabrata*	<0.000001
Kluyveromyces marxianus compared to *Candida krusei*	<0.000001
Kluyveromyces marxianus compared to *Staphylococcus epidermidis*	0.993349
Kluyveromyces marxianus compared to *Enterococcus faecalis*	<0.000001
Kluyveromyces marxianus compared to MSSA	0.518039
Kluyveromyces marxianus compared to MRSA	<0.000001
Kluyveromyces marxianus compared to *Bacillus spizizenii*	<0.000001
Kluyveromyces marxianus compared to *Proteus mirabilis*	<0.000001
Kluyveromyces marxianus compared to *Enterobacter aerogenes*	<0.000001
Kluyveromyces marxianus compared to *Salmonella enteritidis*	<0.000001
Kluyveromyces marxianus compared to *Pseudomonas aeruginosa*	<0.000001
Kluyveromyces marxianus compared to *Citrobacter freundii*	<0.000001
Kluyveromyces marxianus compared to *Klebsiella pneumoniae*	0.170752
Kluyveromyces marxianus compared to *Moraxella catarrhalis*	<0.000001
Kluyveromyces marxianus compared to *Escherichia coli*	<0.000001
Candida albicans compared to *Candida glabrata*	<0.000001
Candida albicans compared to *Candida krusei*	<0.000001
Candida albicans compared to *Staphylococcus epidermidis*	0.808098
Candida albicans compared to *Enterococcus faecalis*	<0.000001
Candida albicans compared to MSSA	0.399741
Candida albicans compared to MRSA	<0.000001
Candida albicans compared to *Bacillus spizizenii*	<0.000001
Candida albicans compared to *Proteus mirabilis*	<0.000001
Candida albicans compared to *Enterobacter aerogenes*	<0.000001
Candida albicans compared to *Salmonella enteritidis*	<0.000001
Candida albicans compared to *Pseudomonas aeruginosa*	<0.000001
Candida albicans compared to *Citrobacter freundii*	<0.000001
Candida albicans compared to *Klebsiella pneumoniae*	0.223873
Candida albicans compared to *Moraxella catarrhalis*	<0.000001
Candida albicans compared to *Escherichia coli*	<0.000001
Candida glabrata compared to *Candida krusei*	<0.000001
Candida glabrata compared to *Staphylococcus epidermidis*	0.000956
Candida glabrata compared to *Enterococcus faecalis*	<0.000001
Candida glabrata compared to MSSA	0.059364
Candida glabrata compared to MRSA	0.000168
Candida glabrata compared to *Bacillus spizizenii*	<0.000001
Candida glabrata compared to *Proteus mirabilis*	<0.000001
Candida glabrata compared to *Enterobacter aerogenes*	<0.000001
Candida glabrata compared to *Salmonella enteritidis*	<0.000001
Candida glabrata compared to *Pseudomonas aeruginosa*	<0.000001
Candida glabrata compared to *Citrobacter freundii*	<0.000001
Candida glabrata compared to *Klebsiella pneumoniae*	0.000031
Candida glabrata compared to *Moraxella catarrhalis*	<0.000001
Candida glabrata compared to *Escherichia coli*	<0.000001
Candida krusei compared to *Staphylococcus epidermidis*	<0.000001
Candida krusei compared to *Enterococcus faecalis*	<0.000001
Candida krusei compared to MSSA	<0.000001
Candida krusei compared to MRSA	0.169116
Candida krusei compared to *Bacillus spizizenii*	<0.000001
Candida krusei compared to *Proteus mirabilis*	<0.000001
Candida krusei compared to *Enterobacter aerogenes*	<0.000001
Candida krusei compared to *Salmonella enteritidis*	<0.000001
Candida krusei compared to *Pseudomonas aeruginosa*	0.000310
Candida krusei compared to *Citrobacter freundii*	<0.000001
Candida krusei compared to *Klebsiella pneumoniae*	<0.000001
Candida krusei compared to *Moraxella catarrhalis*	0.000062
Candida krusei compared to *Escherichia coli*	<0.000001
Staphylococcus epidermidis compared to *Enterococcus faecalis*	<0.000001
Staphylococcus epidermidis compared to MSSA	0.594318
Staphylococcus epidermidis compared to MRSA	<0.000001
Staphylococcus epidermidis compared to *Bacillus spizizenii*	<0.000001
Staphylococcus epidermidis compared to *Proteus mirabilis*	<0.000001
Staphylococcus epidermidis compared to *Enterobacter aerogenes*	<0.000001
Staphylococcus epidermidis compared to *Salmonella enteritidis*	<0.000001
Staphylococcus epidermidis compared to *Pseudomonas aeruginosa*	<0.000001
Staphylococcus epidermidis compared to *Citrobacter freundii*	<0.000001
Staphylococcus epidermidis compared to *Klebsiella pneumoniae*	0.264942
Staphylococcus epidermidis compared to *Moraxella catarrhalis*	<0.000001
Staphylococcus epidermidis compared to *Escherichia coli*	<0.000001
Enterococcus faecalis compared to MSSA	<0.000001
Enterococcus faecalis compared to MRSA	0.029654
Enterococcus faecalis compared to *Bacillus spizizenii*	<0.000001
Enterococcus faecalis compared to *Proteus mirabilis*	0.273961
Enterococcus faecalis compared to *Enterobacter aerogenes*	0.005731
Enterococcus faecalis compared to *Salmonella enteritidis*	0.000023
Enterococcus faecalis compared to *Pseudomonas aeruginosa*	0.753577
Enterococcus faecalis compared to *Citrobacter freundii*	0.024527
Enterococcus faecalis compared to *Klebsiella pneumoniae*	<0.000001
Enterococcus faecalis compared to *Moraxella catarrhalis*	0.440614
Enterococcus faecalis compared to *Escherichia coli*	0.686266
MSSA compared to MRSA	0.000037
MSSA compared to *Bacillus spizizenii*	<0.000001
MSSA compared to *Proteus mirabilis*	<0.000001
MSSA compared to *Enterobacter aerogenes*	<0.000001
MSSA compared to *Salmonella enteritidis*	<0.000001
MSSA compared to *Pseudomonas aeruginosa*	<0.000001
MSSA compared to *Citrobacter freundii*	<0.000001
MSSA compared to *Klebsiella pneumoniae*	0.141919
MSSA compared to *Moraxella catarrhalis*	<0.000001
MSSA compared to *Escherichia coli*	<0.000001
MRSA compared to *Bacillus spizizenii*	<0.000001
MRSA compared to *Proteus mirabilis*	0.001639
MRSA compared to *Enterobacter aerogenes*	0.000002
MRSA compared to *Salmonella enteritidis*	<0.000001
MRSA compared to *Pseudomonas aeruginosa*	0.085947
MRSA compared to *Citrobacter freundii*	0.000017
MRSA compared to *Klebsiella pneumoniae*	<0.000001
MRSA compared to *Moraxella catarrhalis*	0.122789
MRSA compared to *Escherichia coli*	0.010245
Bacillus spizizenii compared to *Proteus mirabilis*	<0.000001
Bacillus spizizenii compared to *Enterobacter aerogenes*	<0.000001
Bacillus spizizenii compared to *Salmonella enteritidis*	<0.000001
Bacillus spizizenii compared to *Pseudomonas aeruginosa*	<0.000001
Bacillus spizizenii compared to *Citrobacter freundii*	<0.000001
Bacillus spizizenii compared to *Klebsiella pneumoniae*	0.000001
Bacillus spizizenii compared to *Moraxella catarrhalis*	<0.000001
Bacillus spizizenii compared to *Escherichia coli*	<0.000001
Proteus mirabilis compared to *Enterobacter aerogenes*	0.107392
Proteus mirabilis compared to *Salmonella enteritidis*	0.002348
Proteus mirabilis compared to *Pseudomonas aeruginosa*	0.188112
Proteus mirabilis compared to *Citrobacter freundii*	0.280240
Proteus mirabilis compared to *Klebsiella pneumoniae*	<0.000001
Proteus mirabilis compared to *Moraxella catarrhalis*	0.059122
Proteus mirabilis compared to *Escherichia coli*	0.478179
Enterobacter aerogenes compared to *Salmonella enteritidis*	0.145333
Enterobacter aerogenes compared to *Pseudomonas aeruginosa*	0.004296

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Table 5 (*continued*)

Compared Microorganisms	*p* value
Enterobacter aerogenes compared to *Citrobacter freundii*	0.563406
Enterobacter aerogenes compared to *Klebsiella pneumoniae*	<0.000001
Enterobacter aerogenes compared to *Moraxella catarrhalis*	0.000273
Enterobacter aerogenes compared to *Escherichia coli*	0.017078
Salmonella enteritidis compared to *Pseudomonas aeruginosa*	0.000025
Salmonella enteritidis compared to *Citrobacter freundii*	0.038555
Salmonella enteritidis compared to *Klebsiella pneumoniae*	<0.000001
Salmonella enteritidis compared to *Moraxella catarrhalis*	<0.000001
Salmonella enteritidis compared to *Escherichia coli*	0.000111
Pseudomonas aeruginosa compared to *Citrobacter freundii*	0.017290
Pseudomonas aeruginosa compared to *Klebsiella pneumoniae*	<0.000001
Pseudomonas aeruginosa compared to *Moraxella catarrhalis*	0.705969
Pseudomonas aeruginosa compared to *Escherichia coli*	0.492334
Citrobacter freundii compared to *Klebsiella pneumoniae*	<0.000001
Citrobacter freundii compared to *Moraxella catarrhalis*	0.001741
Citrobacter freundii compared to *Escherichia coli*	0.063245
Klebsiella pneumoniae compared to *Moraxella catarrhalis*	<0.000001
Klebsiella pneumoniae compared to *Escherichia coli*	<0.000001
Moraxella catarrhalis compared to *Escherichia coli*	0.230218

The increased rate of the bacteria was observed when examining the range of absorbance units for bacteria and yeast (Figure 2, Figure 3). However, at approximately 305 nm, all of the microorganisms displayed a dramatic spike in the increase of absorbance. This increase was more noticeable in the bacteria than the yeasts and may have been caused by the proteins and nucleic acids of the microorganisms [19]. Taken together, these criteria could be used to distinguish bacteria from yeast. For Gram-negative bacteria, the spike occurring at the end of the measurement interval was, in comparison to Gram-positive bacteria, larger by an average of 0.303 absorbance units without silicon dioxide and by 0.345 absorbance units with silicon dioxide. This criterion might be used to distinguish Gram-negative bacteria from Gram-positive bacteria when using spectrophotometry, with the exception of the Gram-negative bacteria *Klebsiella pneumonia* and the Gram-positive bacteria *Enterococcus faecalis*. *Klebsiella pneumoniae* appeared to have a lower spike, similar to Gram-positive bacteria, occurring at the end of the graph, while *Enterococcus faecalis* appeared to have a higher spike, similar to Gram-negative bacteria, also occurring at the end of the graph.

After the addition of silicon dioxide, the specific characteristics used to distinguish bacteria from yeasts without silicon dioxide remained the same (Figure 4). However, silicon dioxide did produce alterations in the microorganism graphs. Adding silicon dioxide decreased the average absorbance of all yeasts (Figure 5, Figure 6, Figure 7, Figure 8), with the exception *Candida glabrata*, which remained nearly the same (Figure 9, Figure 10). For all bacteria, absorbance increased at near infrared wavelengths. For several bacteria, absorbance increased in the visible light spectrum (380–700 nm) and for some, the increase occurred at ultraviolet (<380 nm) wavelengths (Figures 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26, Table 8). *Staphylococcus epidermidis* exhibited the smallest increase in absorbance at near infrared wavelengths, which was barely noticeable from 1100 nm and decreased until 900 nm (Figure 12). *Klebsiella pneumoniae* had the most noticeable increase in absorbance after the addition of silicon dioxide, and its absorbance increased during the entire measurement interval (Figure 23). For *Salmonella enteritidis*, the increase in absorbance occurred during most of the measurement interval following the addition of silicon dioxide (Figure 20).

Table 6. Summary of all comparisons.

Types of cultures compared	Comparisons withou t silicon dioxide at 285–1100 nm	Comparisons with silicon dioxide at 285–1100 nm	Comparisons without silicon dioxide at 285–700 nm	Comparisons with silicon dioxide at 285–700 nm
Overall	99/136 (72.8%)	109/136 (81.1%)	106/136 (77.9%)	107/136 (78.7%)
Yeast compared to bacteria	52/52 (100%)	52/52 (100%)	44/52 (84.6%)	44/52 (84.6%)
Bacteria compared to bacteria (overall)	41/78 (52.6%)	54/78 (69.2%)	56/78 (71.8%)	58/78 (74.6%)
Gram-positive bacteria compared to Gram-negative bacteria	28/40 (70.0%)	29/40 (72.5%)	32/40 (80.0%)	32/40 (80.0%)
Yeast compared to yeast	5/6 (83.3%)	3/6 (50%)	6/6 (100%)	5/6 (83.3%)

Table 7. Effects of silicon dioxide on the average absorbance of different microorganisms.

Microorganisms	Average absorbance without silicon dioxide	Average absorbance with silicon dioxide	Silicon dioxide's effect on average absorbance
Kluyveromyces marxianus	1.262251	1.158944	Decrease
Candida albicans	1.227039	1.135811	Decrease
Candida glabrata	1.180000	1.168283	Decrease
Candida krusei	1.194647	1.134603	Decrease
Staphylococcus epidermidis	0.946494	0.895001	Decrease
Enterococcus faecalis	0.949259	0.942527	Did not alter
MSSA	0.793623	0.812563	Increase
MRSA	0.830097	0.886495	Increase
Bacillus spizizenii	0.715224	0.745946	Increase
Proteus mirabilis	0.935026	0.957902	Increase
Enterobacter aerogenes	0.938885	0.982013	Increase
Salmonella enteritidis	0.941685	1.006821	Increase
Pseudomonas aeruginosa	0.892804	0.930857	Increase
Citrobacter freundii	0.930903	0.991864	Increase
Klebsiella pneumoniae	0.661763	0.837743	Increase
Moraxella catarrhalis	0.897066	0.935586	Increase
Escherichia coli	0.900817	0.905401	Did not alter

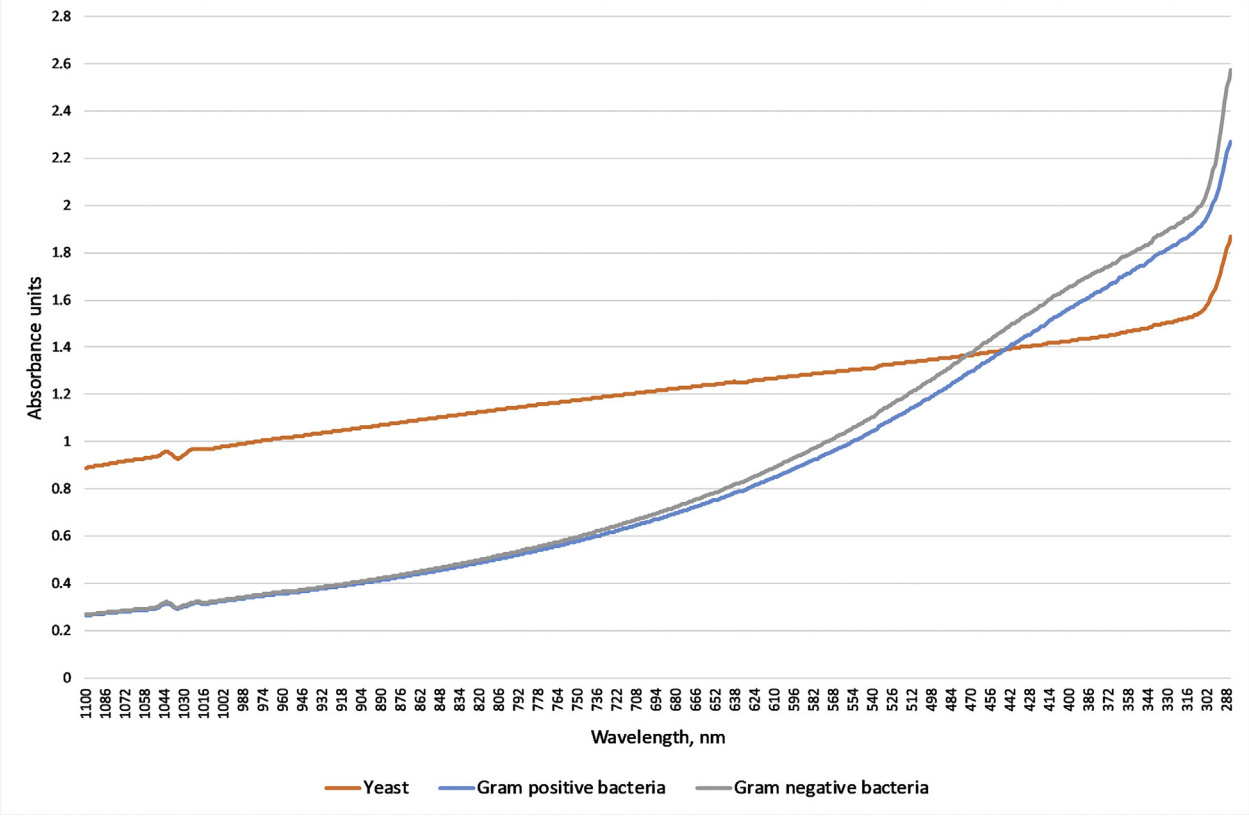

Figure 1. Average absorbance of yeast, Gram-positive and Gram-negative bacteria at 285–1100 nm without silicon dioxide.

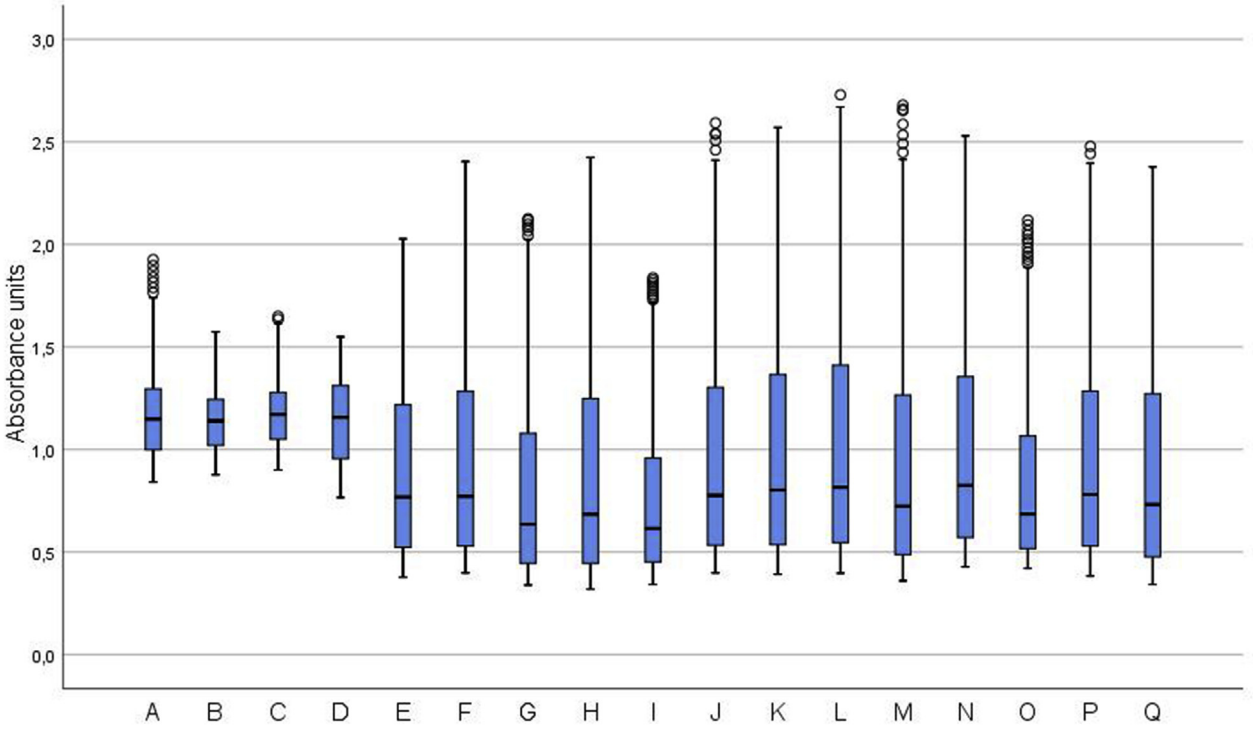

Figure 2. Microorganism absorbance unit ranges without silicon dioxide. *Kluyveromyces marxianus* (A), *Candida albicans* (B), *Candida glabrata* (C), *Candida krusei* (D), *Staphylococcus epidermidis* (E), *Enterococcus faecalis* (F), MSSA (G), MRSA (H), *Bacillus spizizenii* (I), *Proteus mirabilis* (J), *Enterobacter aerogenes* (K), *Salmonella enteritidis* (L), *Pseudomonas aeruginosa* (M), *Citrobacter freundii* (N), *Klebsiella pneumoniae* (O), *Moraxella catarrhalis* (P), *Escherichia coli* (Q).

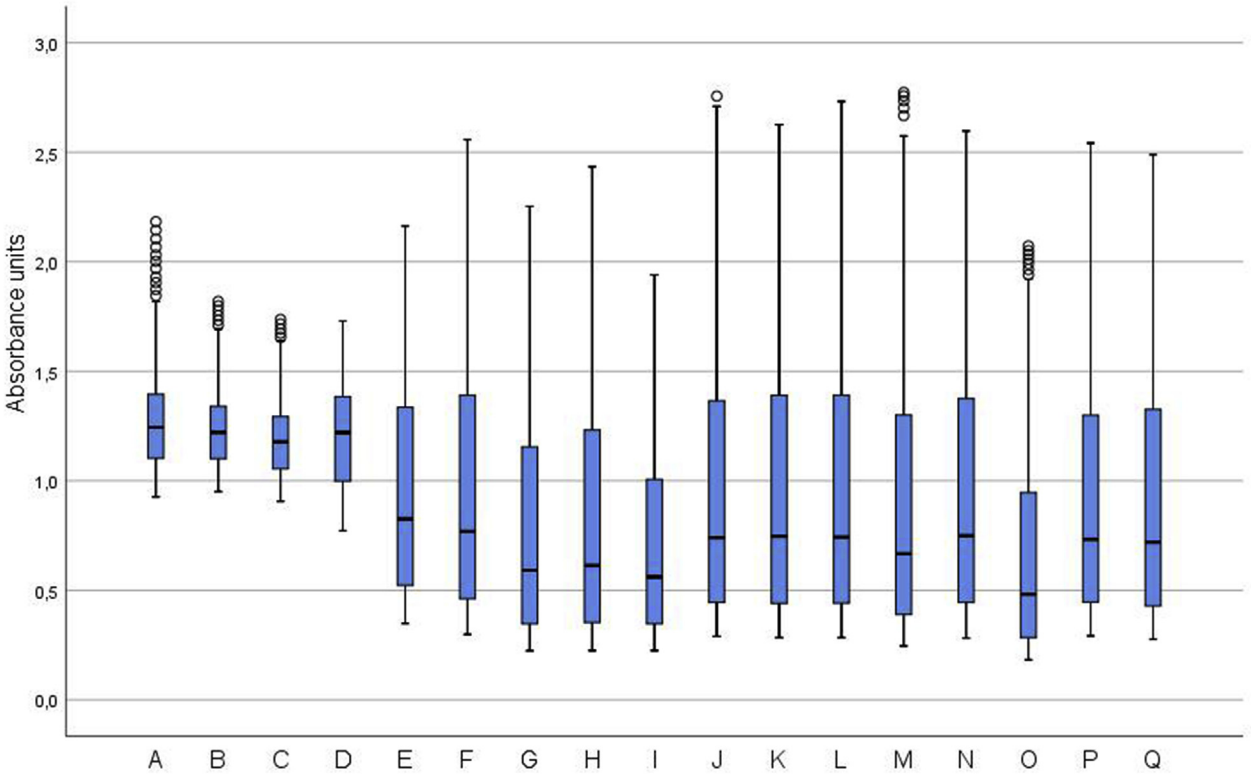

Figure 3. Microorganism absorbance unit ranges with silicon dioxide. *Kluyveromyces marxianus* (A), *Candida albicans* (B), *Candida glabrata* (C), *Candida krusei* (D), *Staphylococcus epidermidis* (E), *Enterococcus faecalis* (F), MSSA (G), MRSA (H), *Bacillus spizizenii* (I), *Proteus mirabilis* (J), *Enterobacter aerogenes* (K), *Salmonella enteritidis* (L), *Pseudomonas aeruginosa* (M), *Citrobacter freundii* (N), *Klebsiella pneumoniae* (O), *Moraxella catarrhalis* (P), *Escherichia coli* (Q).

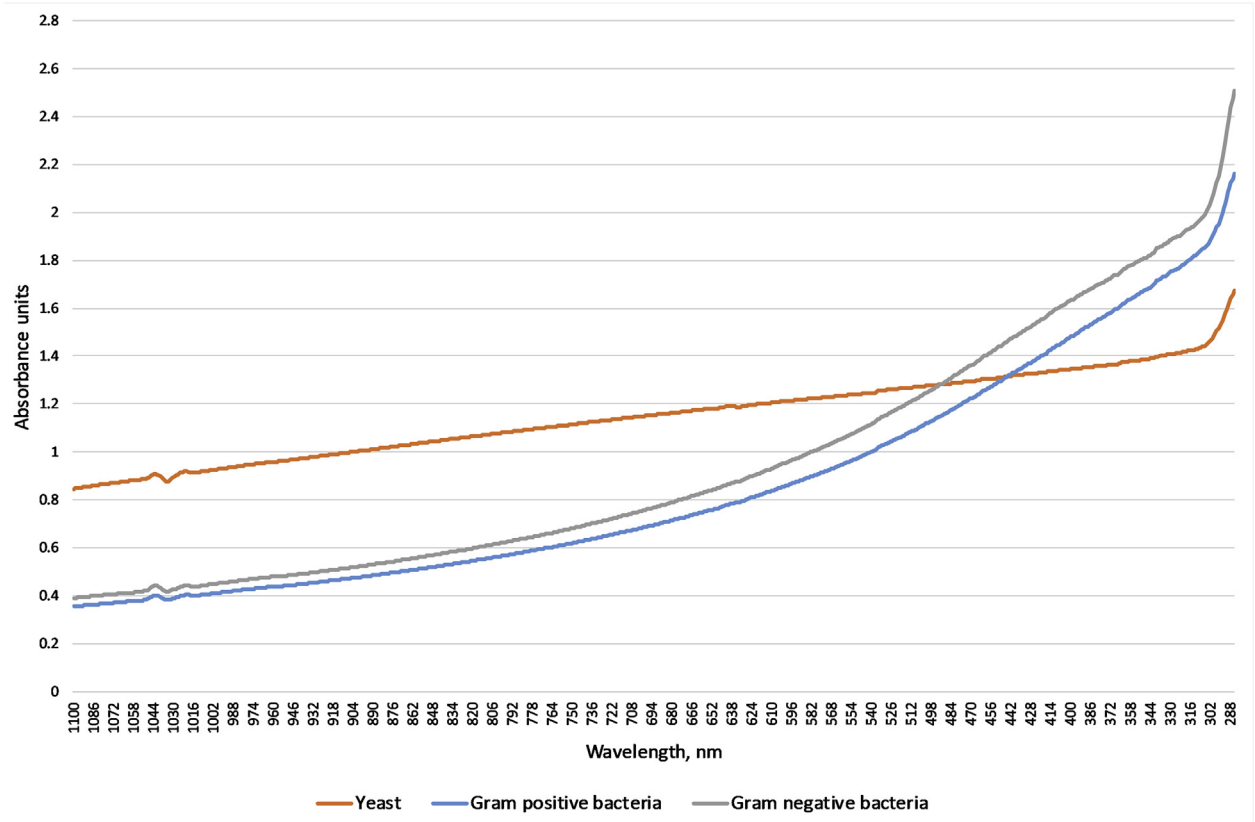

Figure 4. Average absorbance of all examined yeasts, Gram-positive and Gram-negative bacteria at 285–1100 nm with silicon dioxide.

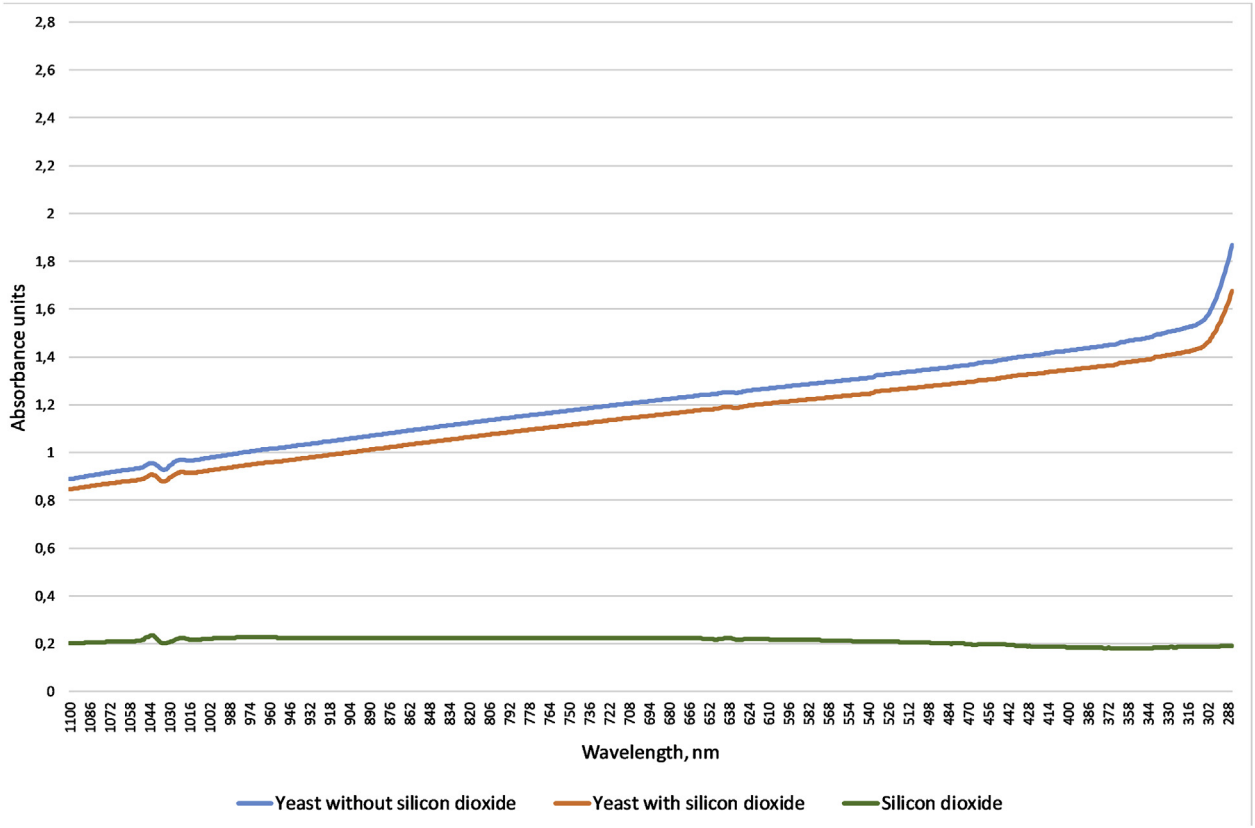

Figure 5. Average absorbance of all examined yeasts without and with silicon dioxide.

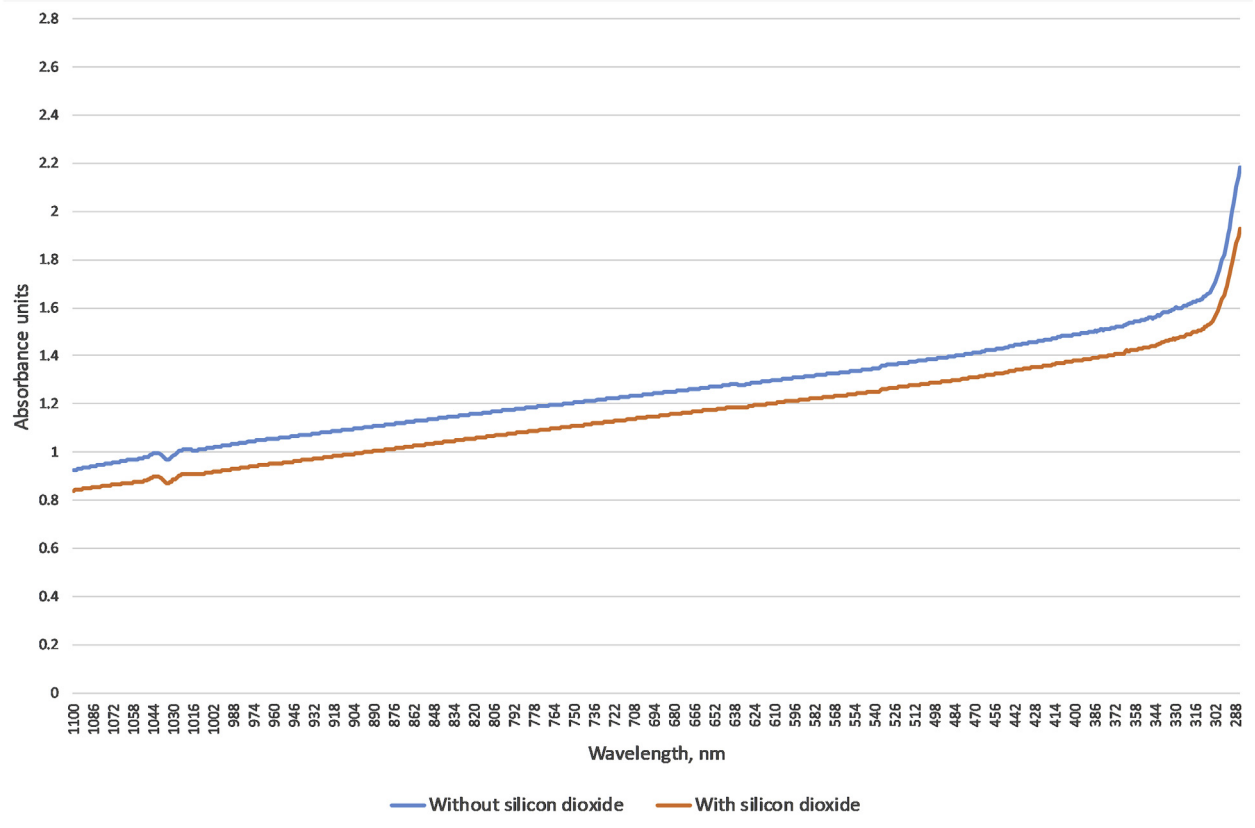

Figure 6. Absorbance of *Kluyveromyces marxianus* with and without silicon dioxide.

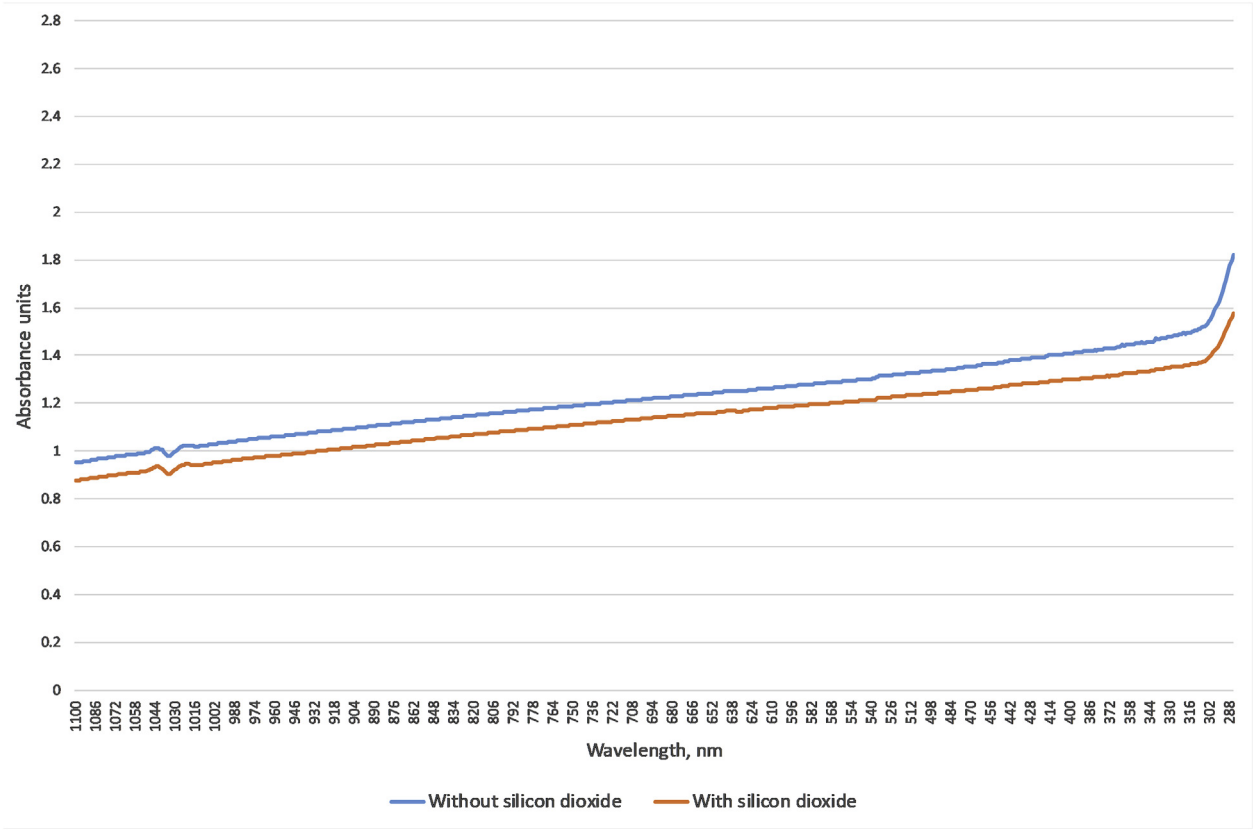

Figure 7. Absorbance of *Candida albicans* with and without silicon dioxide.

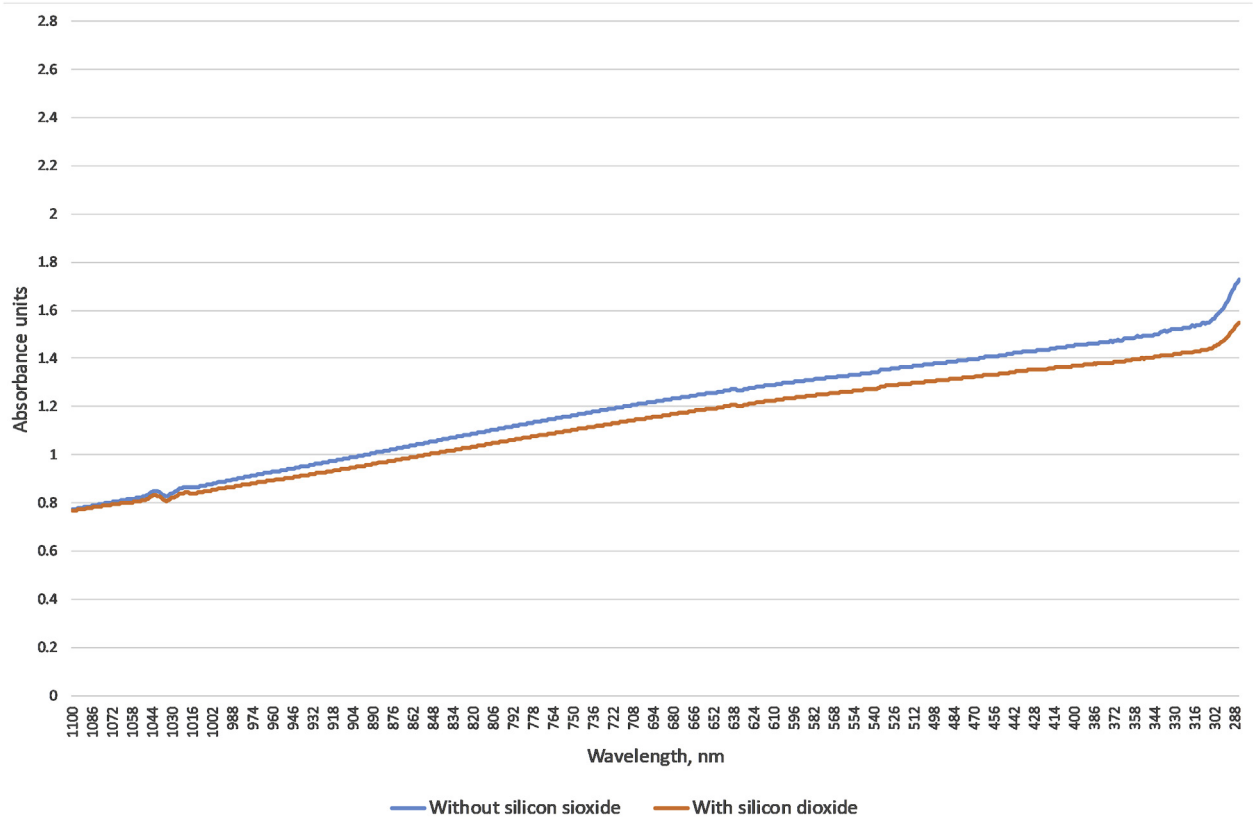

Figure 8. Absorbance of *Candida krusei* with and without silicon dioxide.

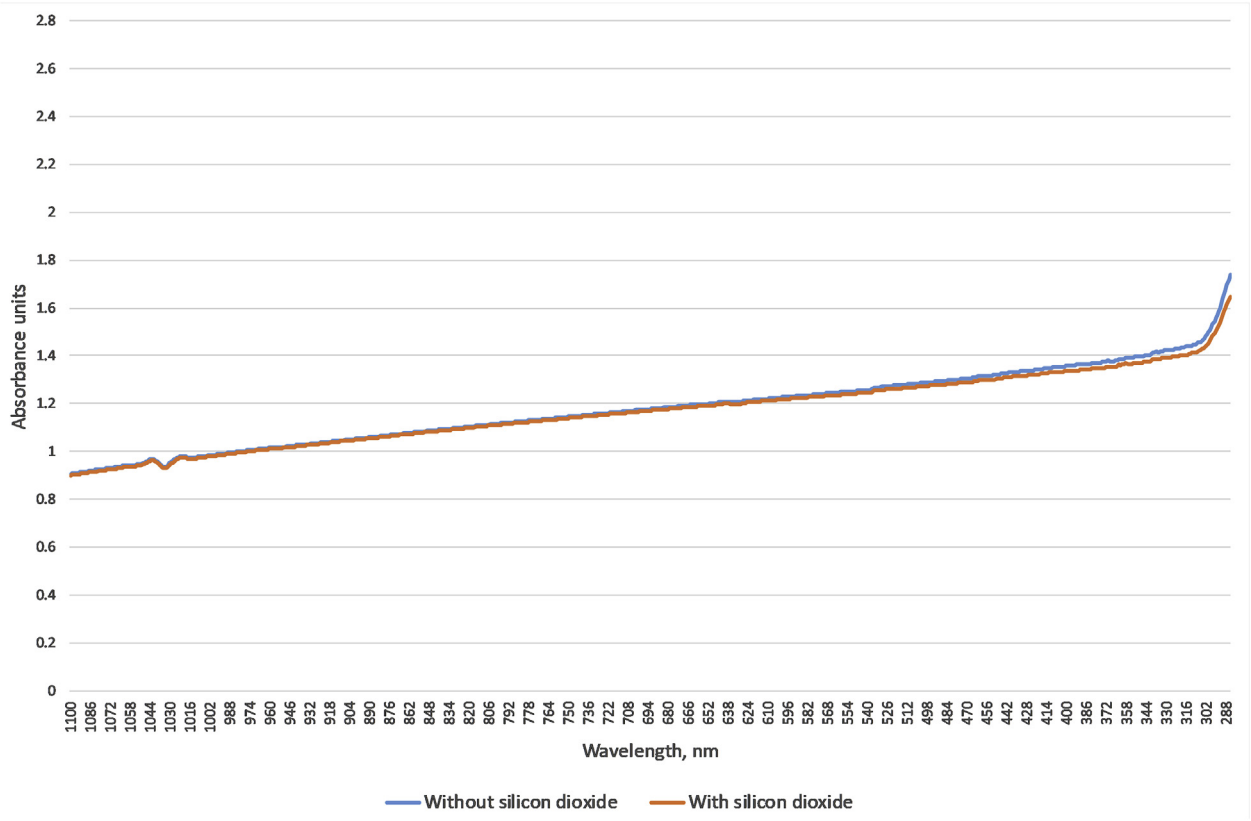

Figure 9. Absorbance of *Candida glabrata* with and without silicon dioxide.

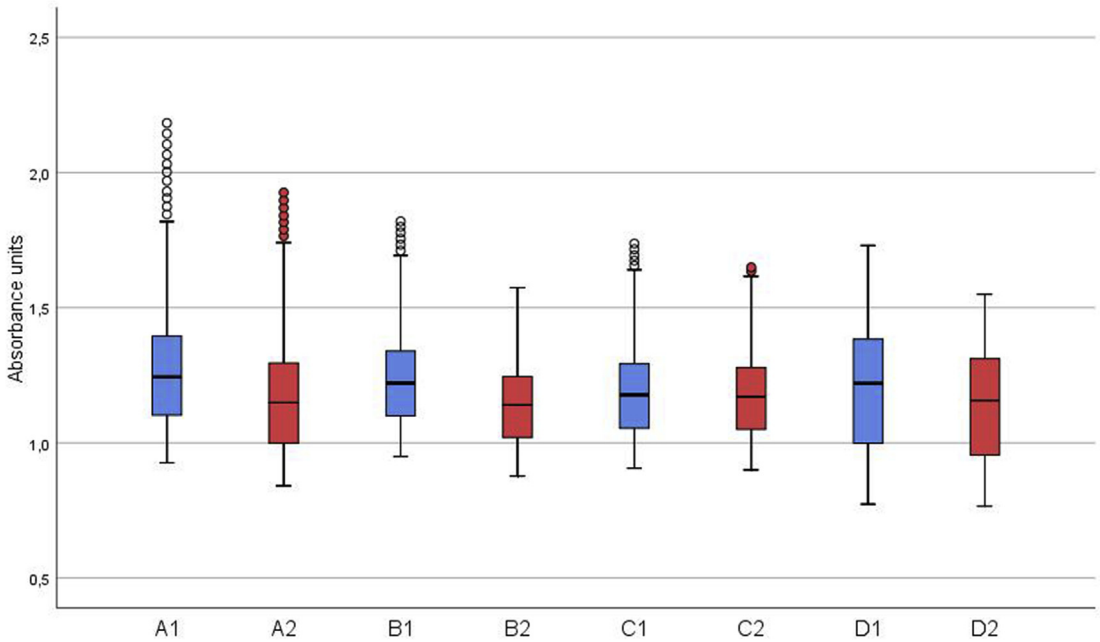

Figure 10. Absorbance unit ranges for yeasts. *Kluyveromyces marxianus* (A1), *Kluyveromyces marxianus* with SiO_2 (A2), *Candida albicans* (B1), *Candida albicans* with SiO_2 (B2), *Candida glabrata* (C1), *Candida glabrata* with SiO_2 (C2), *Candida krusei* (D1), *Candida krusei* with SiO_2 (D2).

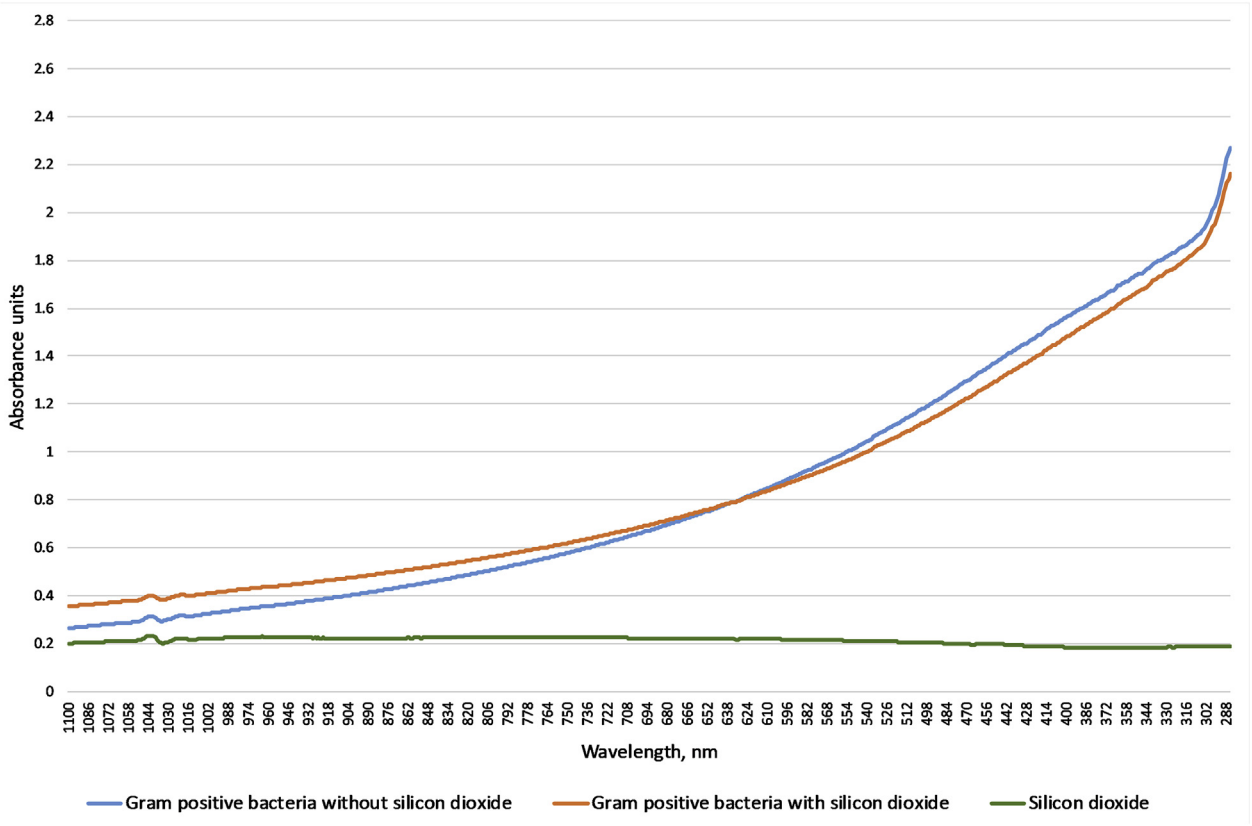

Figure 11. Average absorbance of all examined Gram-positive bacteria with and without silicon dioxide.

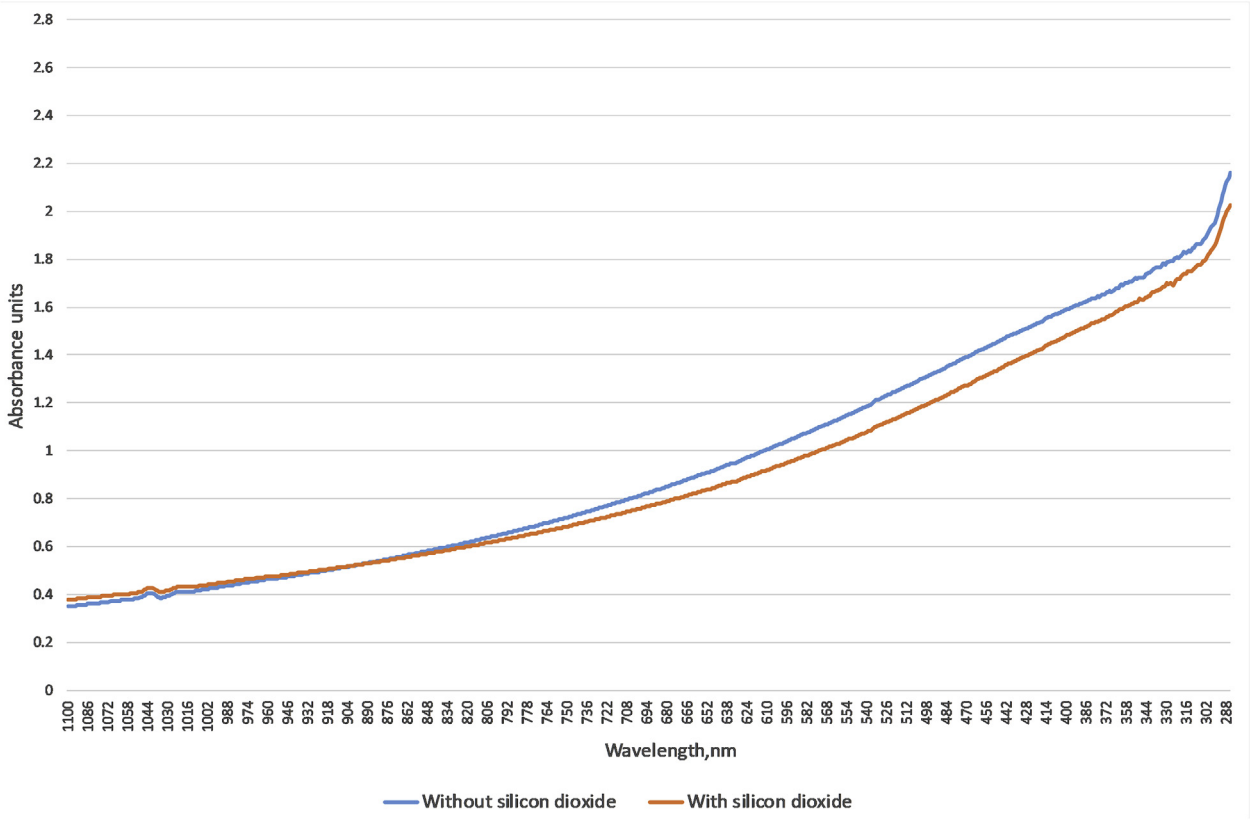

Figure 12. Absorbance of *Staphylococcus epidermidis* with and without silicon dioxide.

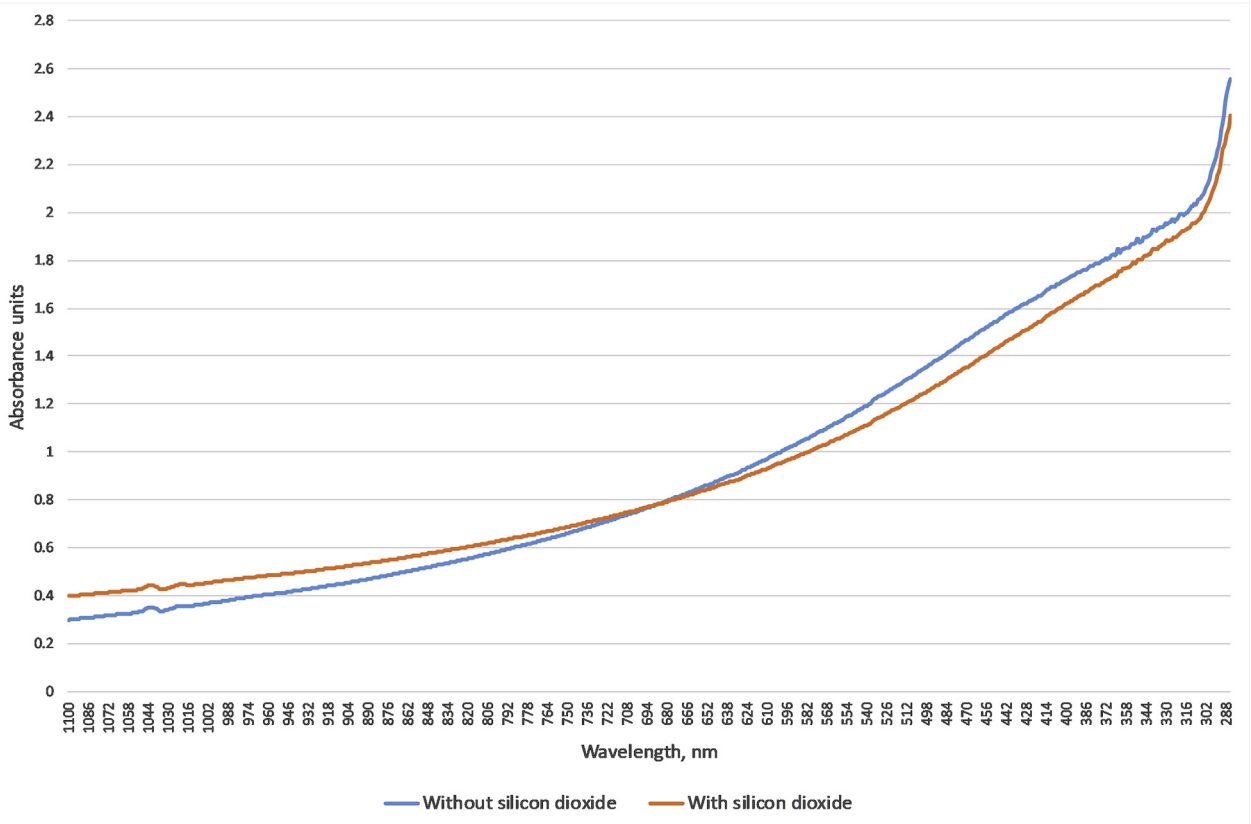

Figure 13. Absorbance of *Enterococcus faecalis* with and without silicon dioxide.

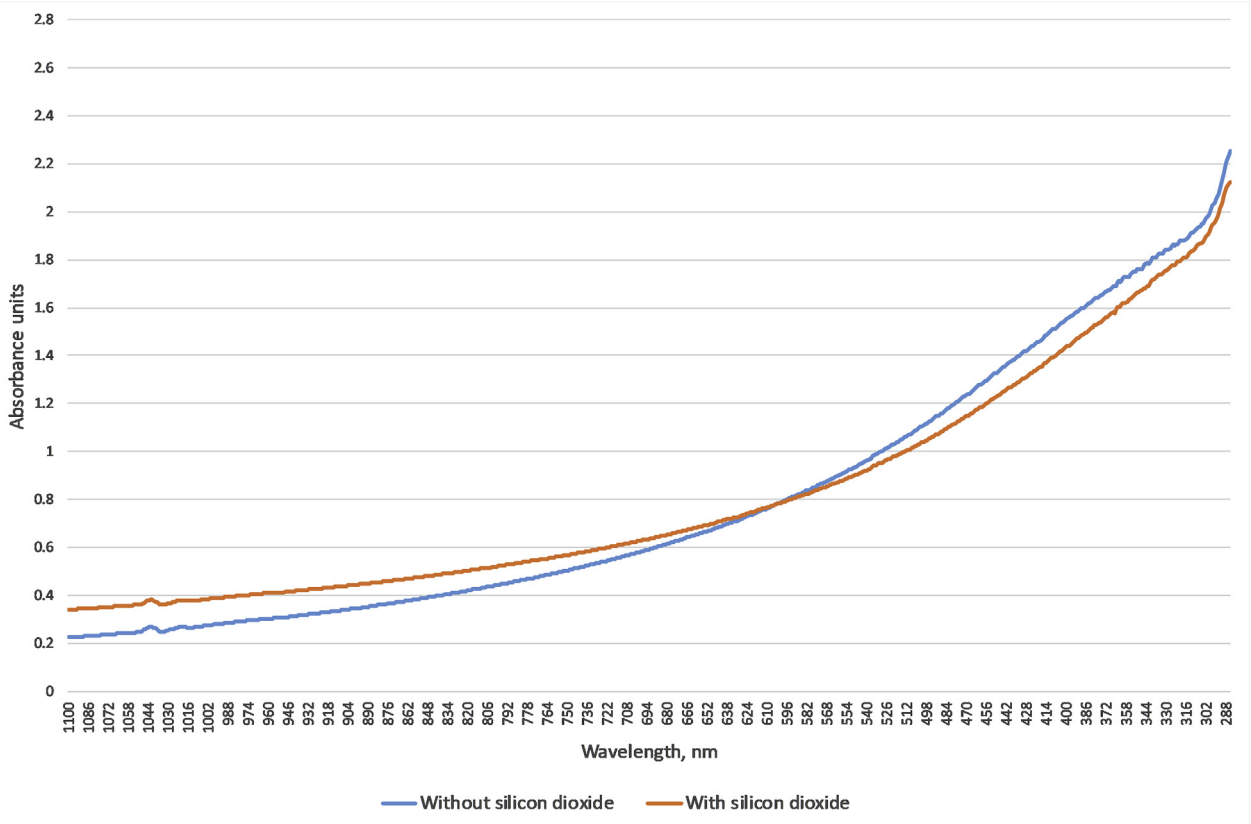

Figure 14. Absorbance of MSSA with and without silicon dioxide.

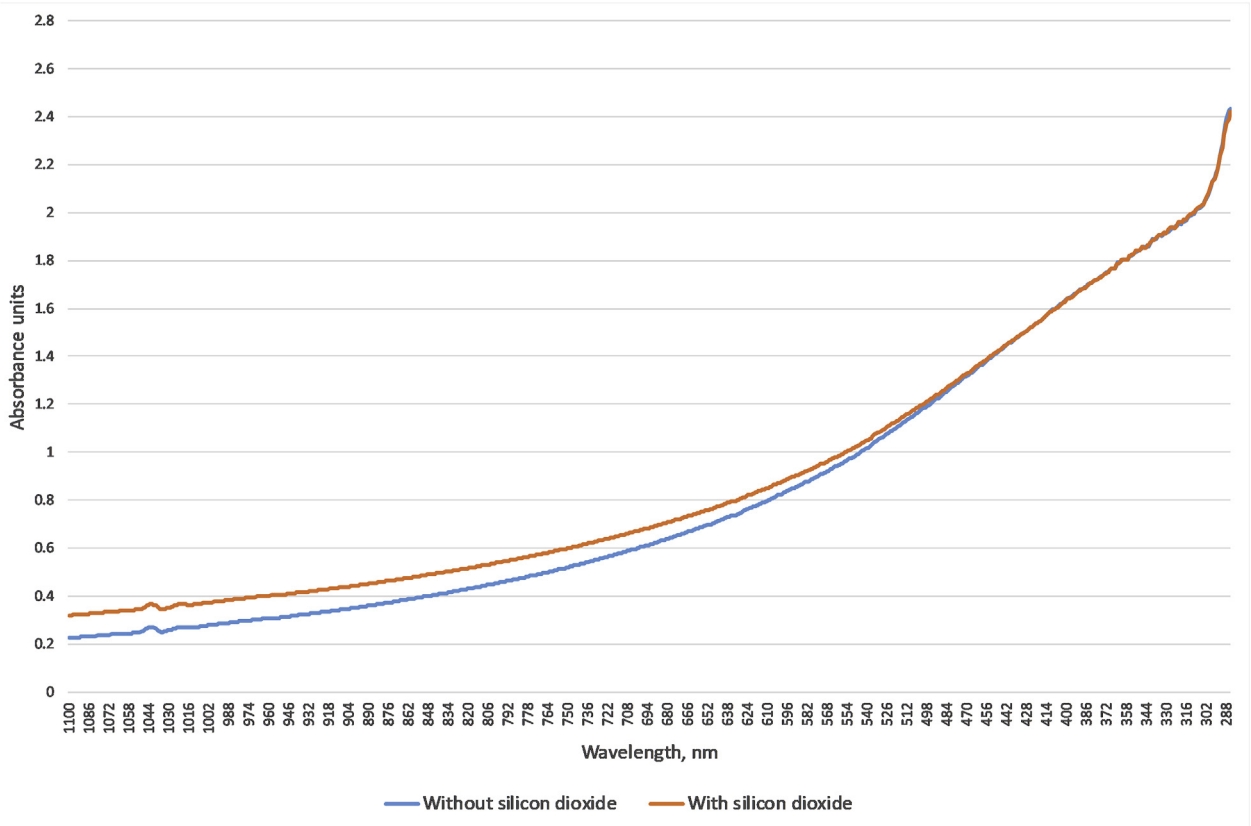

Figure 15. Absorbance of MRSA with and without silicon dioxide.

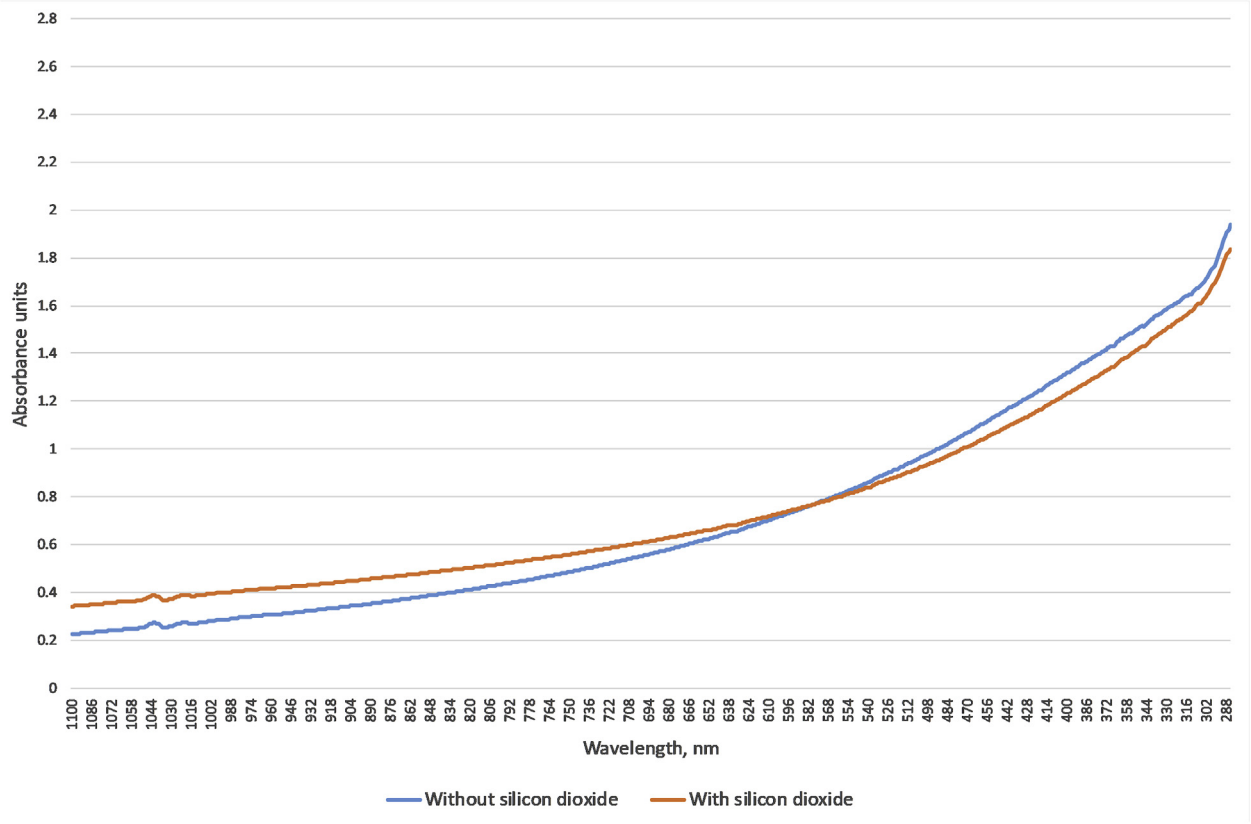

Figure 16. Absorbance of *Bacillus spizizenii* with and without silicon dioxide.

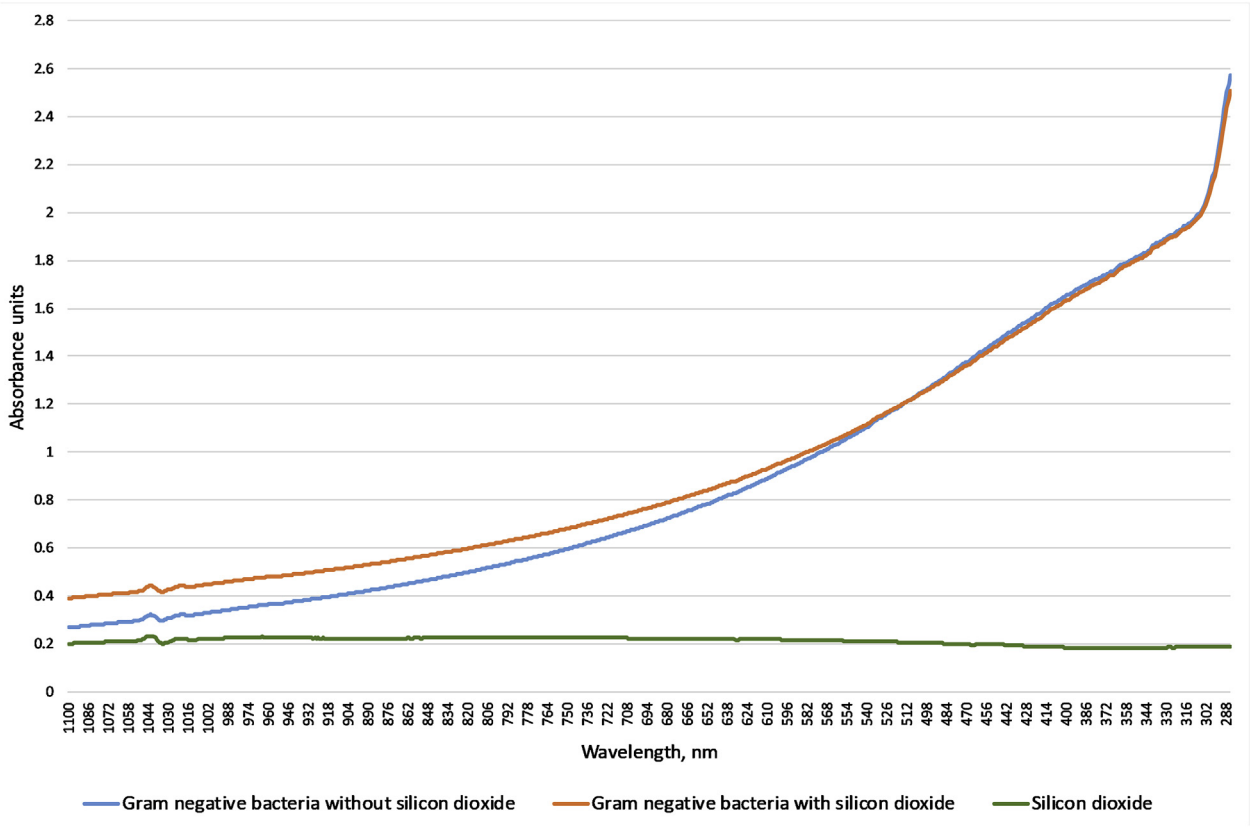

Figure 17. Average absorbance of all examined Gram-negative bacteria with and without silicon dioxide.

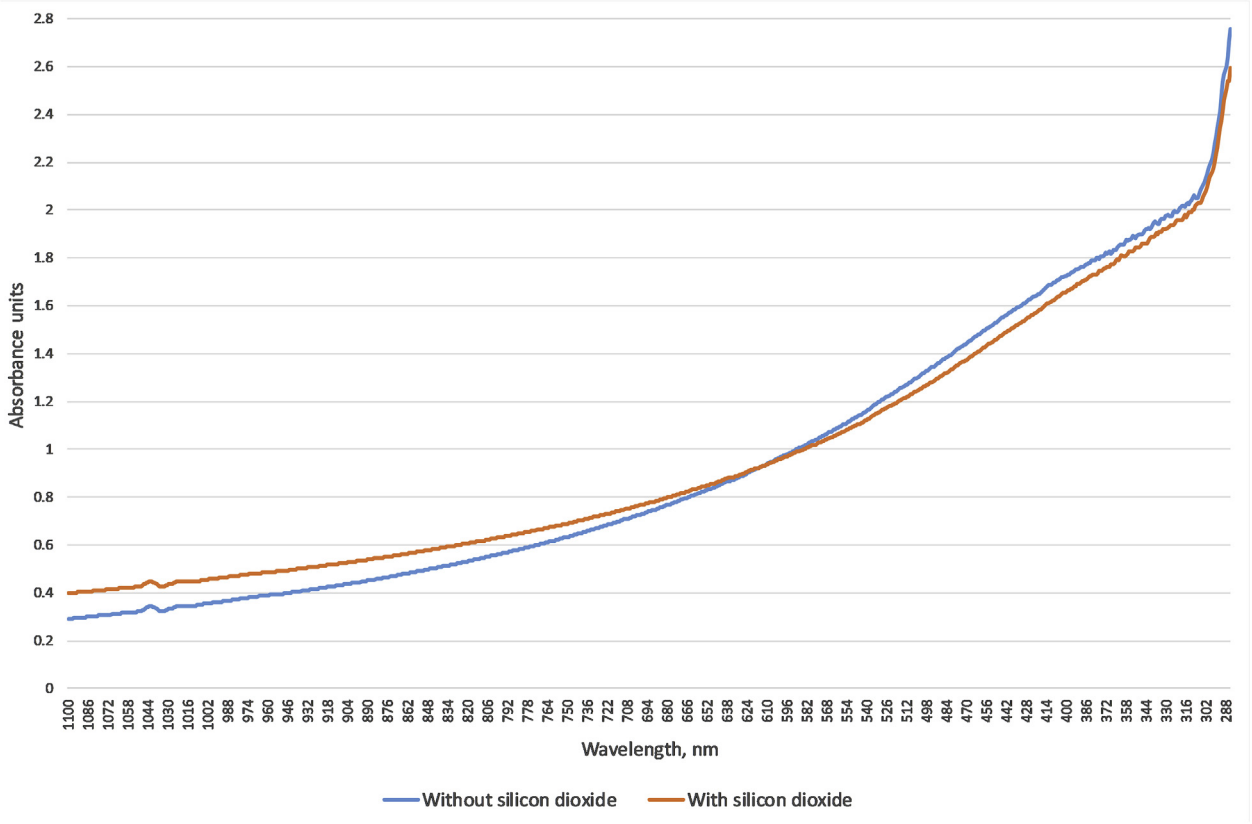

Figure 18. Absorbance of *Proteus mirabilis* with and without silicon dioxide.

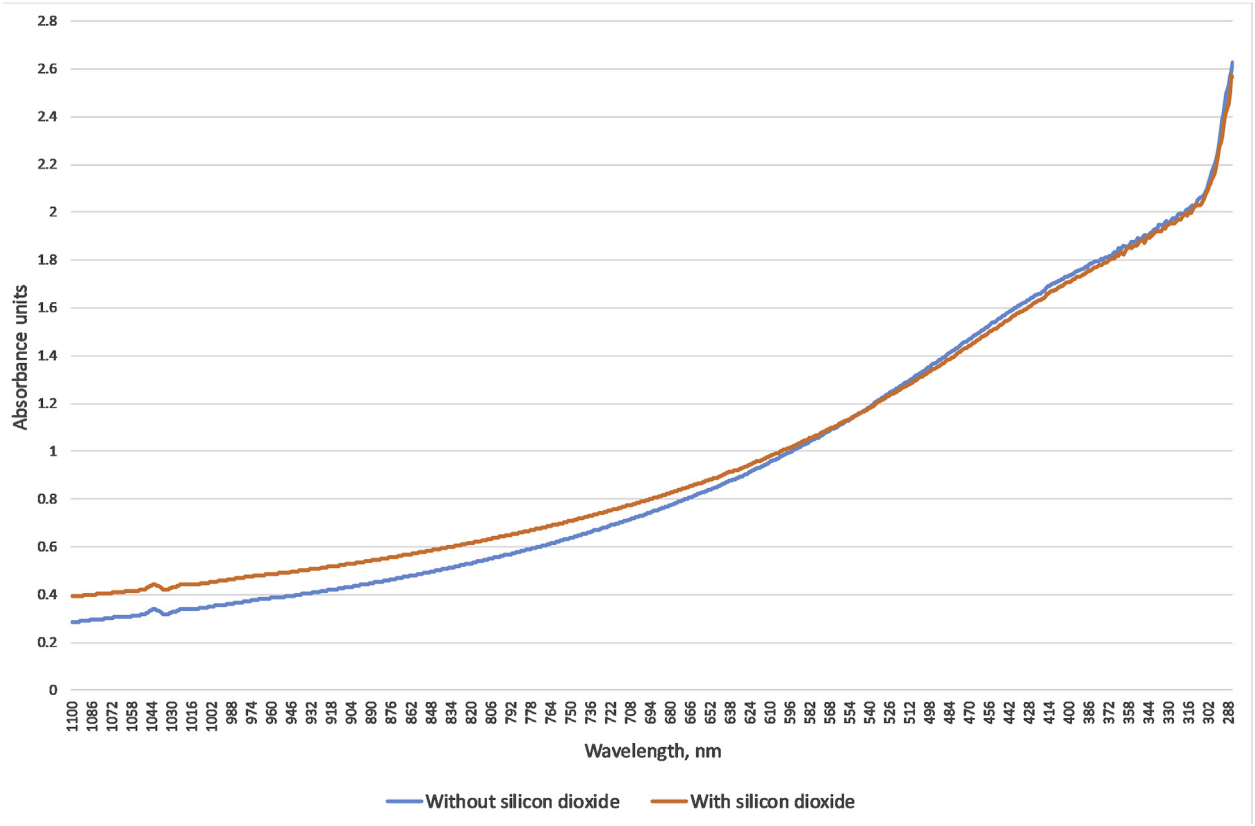

Figure 19. Absorbance of *Enterobacter aerogenes* with and without silicon dioxide.

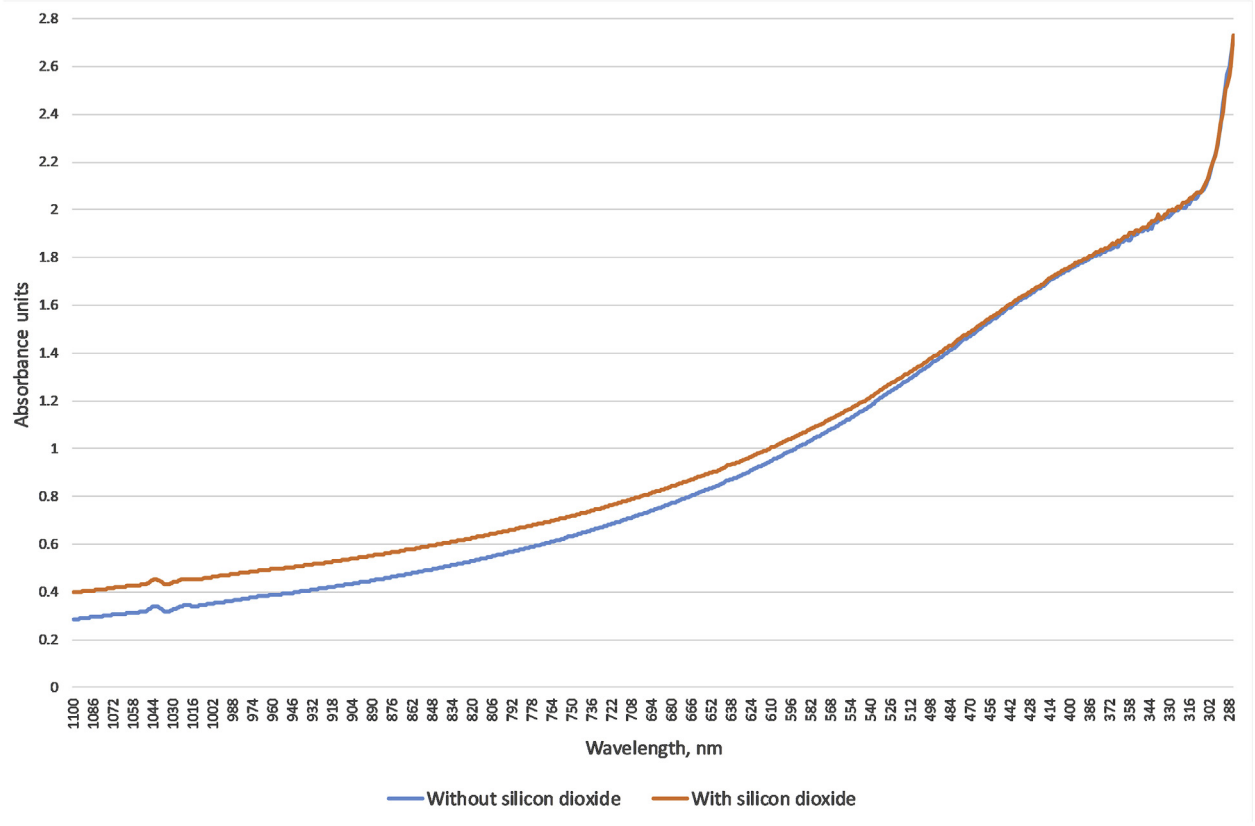

Figure 20. Absorbance of *Salmonella enteritidis* with and without silicon dioxide.

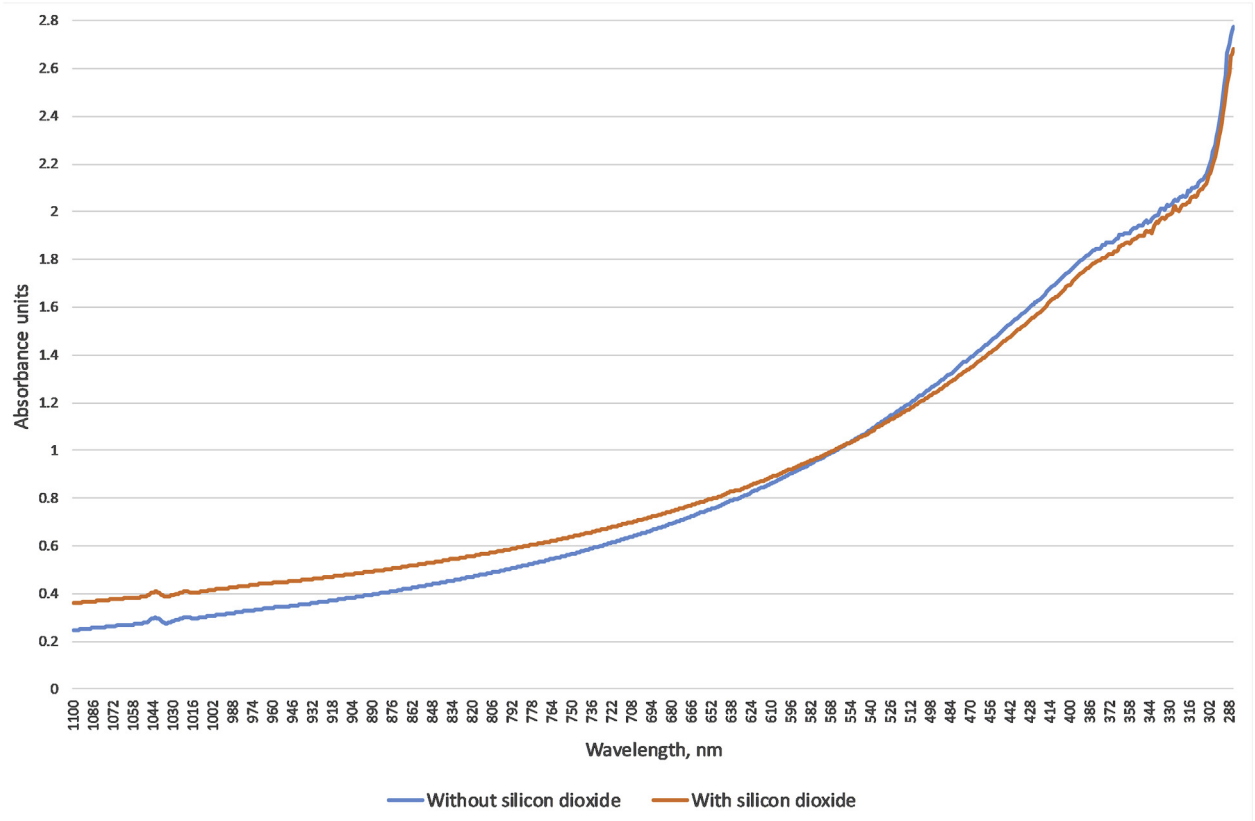

Figure 21. Absorbance of *Pseudomonas aeruginosa* with and without silicon dioxide.

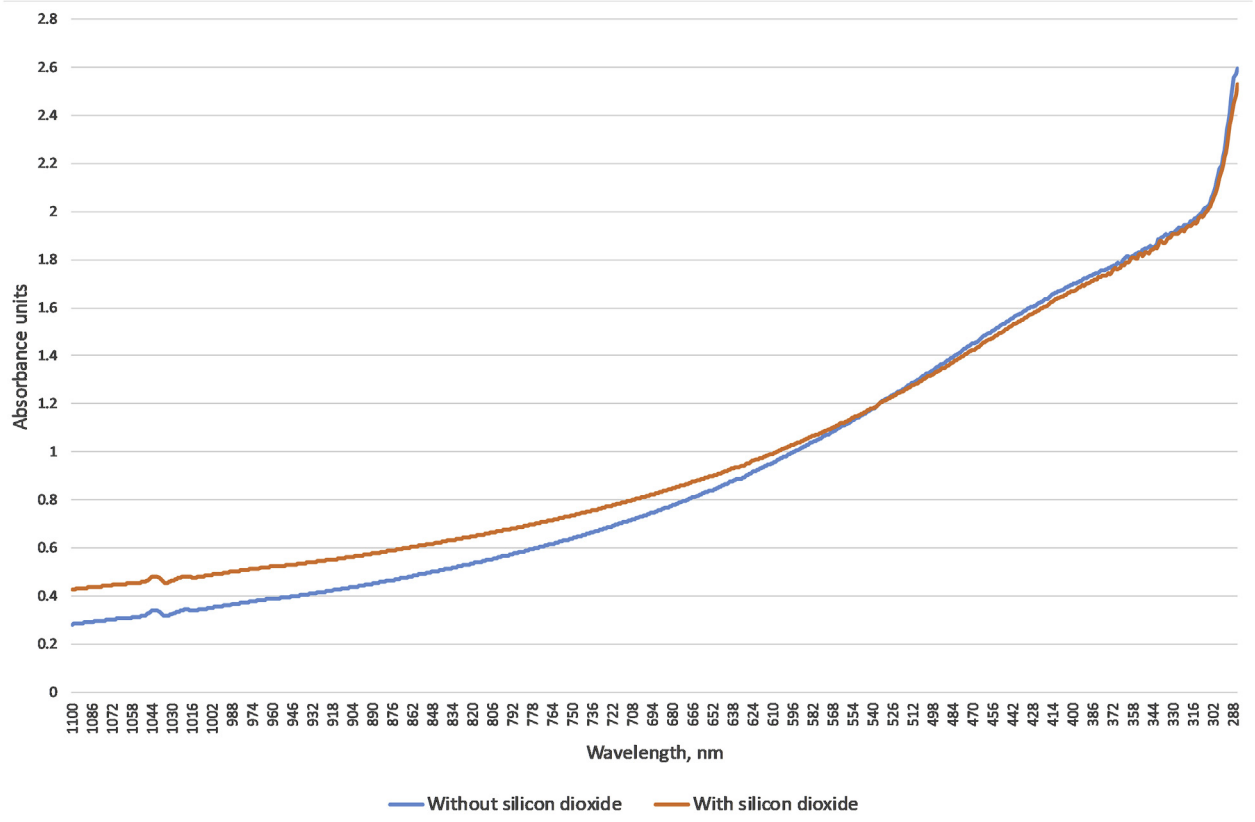

Figure 22. Absorbance of *Citrobacter freundii* with and without silicon dioxide.

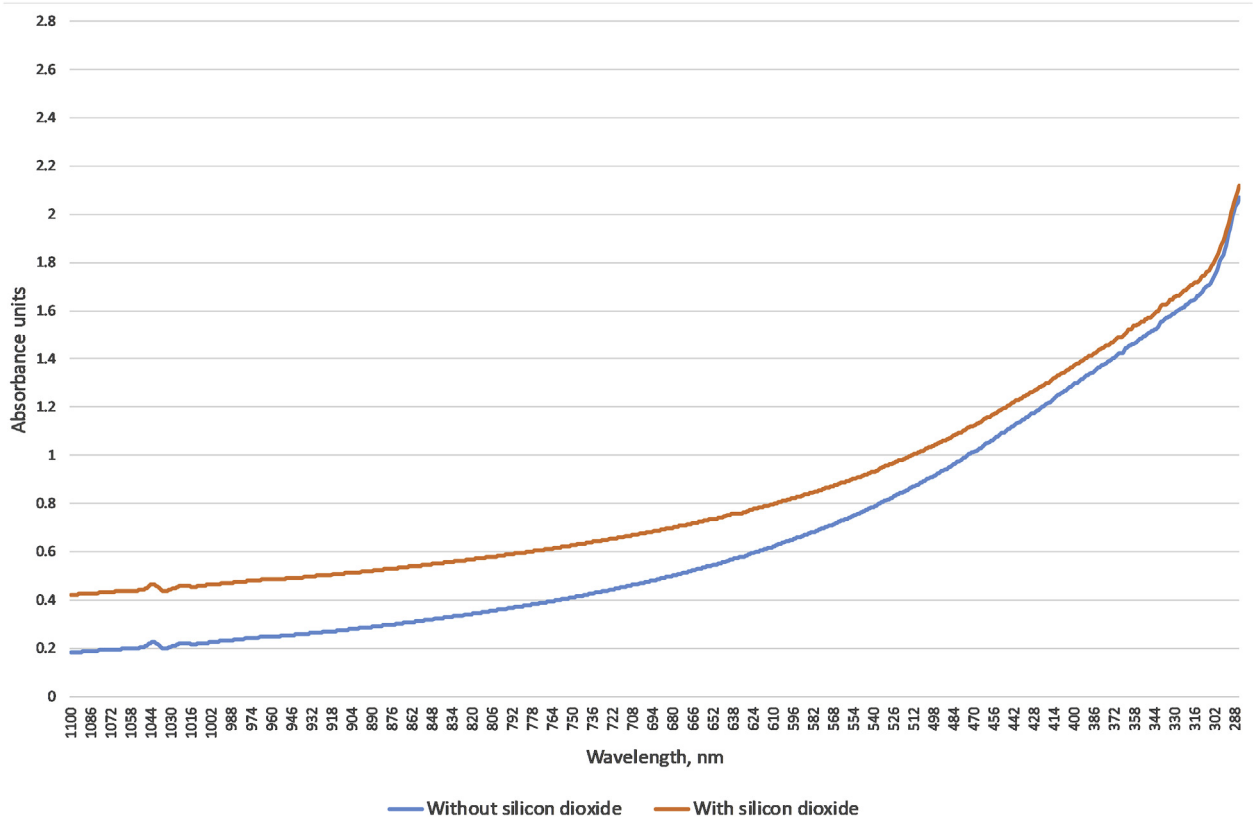

Figure 23. Absorbance of *Klebsiella pneumoniae* with and without silicon dioxide.

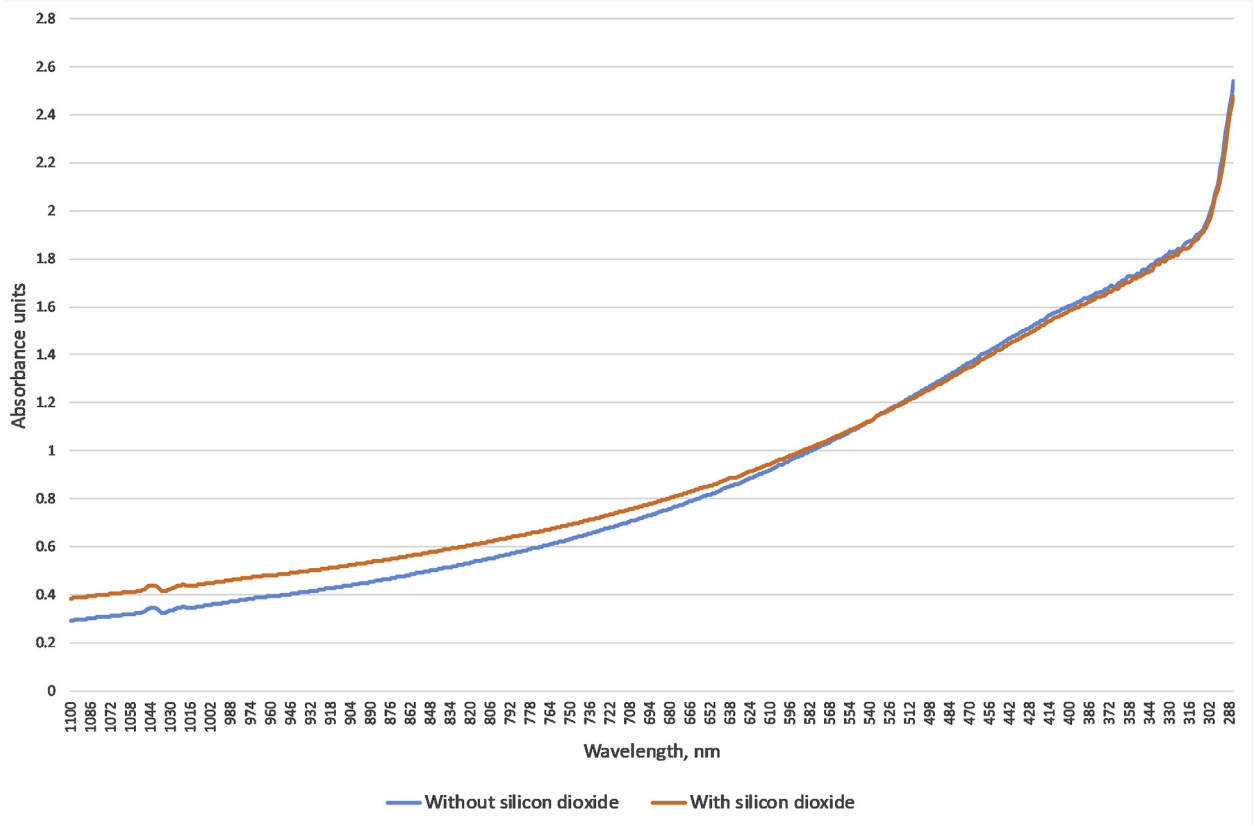

Figure 24. Absorbance of *Moraxella catarrhalis* with and without silicon dioxide.

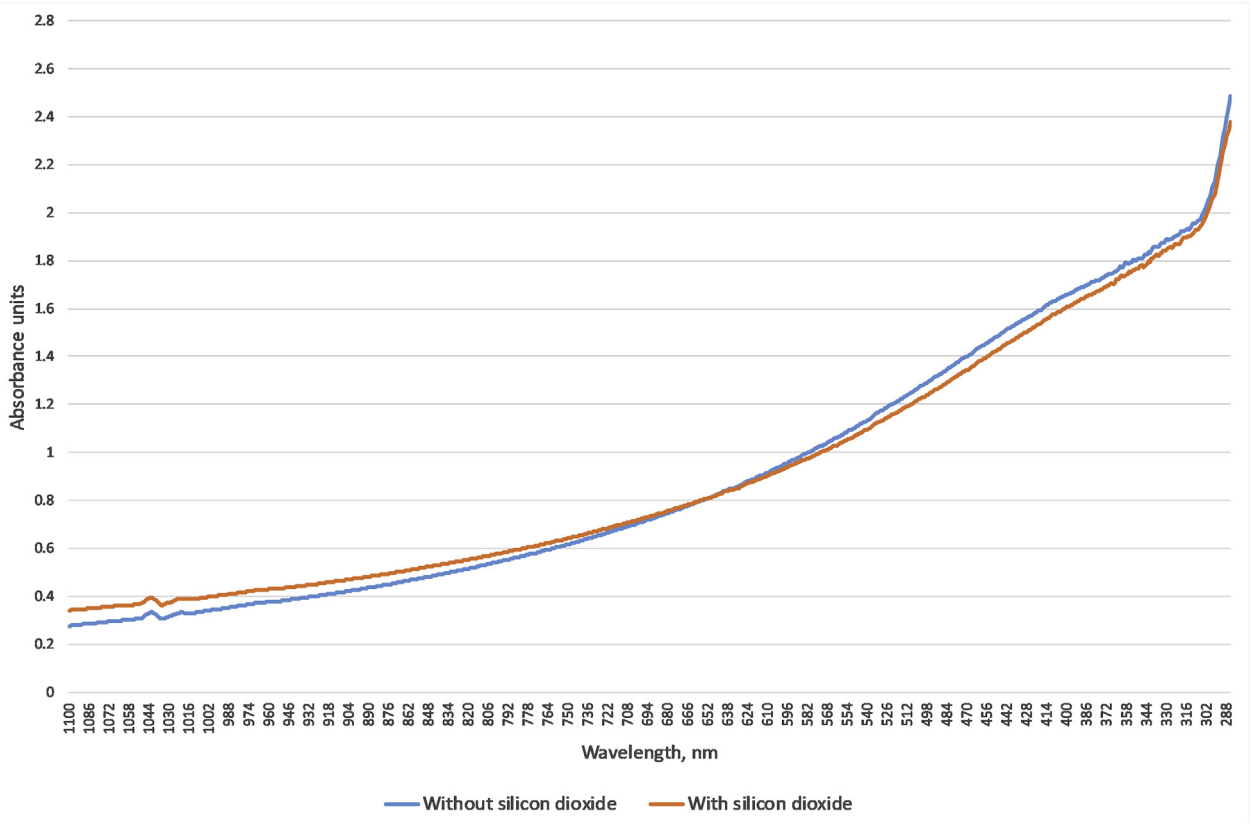

Figure 25. Absorbance of *Escherichia coli* with and without silicon dioxide.

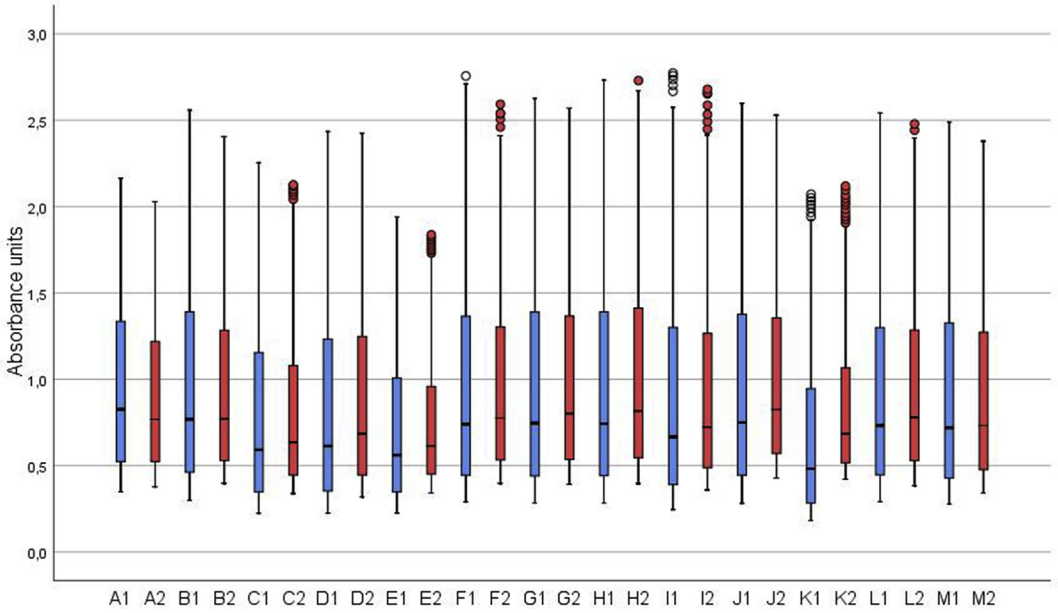

Figure 26. Absorbance unit ranges for bacteria. *Staphylococcus epidermidis* (A1), *Staphylococcus epidermidis* with SiO_2 (A2), *Enterococcus faecalis* (B1), *Enterococcus faecalis* with SiO_2 (B2), MSSA (C1), MSSA with SiO_2 (C2), MRSA (D1), MRSA with SiO_2 (D2), *Bacillus spizizenii* (E1), *Bacillus spizizenii* with SiO_2 (E2), *Proteus mirabilis* (F1), *Proteus mirabilis* with SiO_2 (F2), *Enterobacter aerogenes* (G1), *Enterobacter aerogenes* with SiO_2 (G2), *Salmonella enteritidis* (H1), *Salmonella enteritidis* with SiO_2 (H2), *Pseudomonas aeruginosa* (I1), *Pseudomonas aeruginosa* with SiO_2 (I2), *Citrobacter freundii* (J1), *Citrobacter freundii* with SiO_2 (J2), *Klebsiella pneumoniae* (K1), *Klebsiella pneumoniae* with SiO_2 (K2), *Moraxella catarrhalis* (L1), *Moraxella catarrhalis* with SiO_2 (L2), *Escherichia coli* (M1), *Escherichia coli* with SiO_2 (M2).

Table 8. Effects of silicon dioxide on the absorbance of all examined microorganisms at a wavelength interval of 285–1100 nm.

Microorganism	Wavelengths at which the absorbance increased after the addition of silicon dioxide
Kluyveromyces marxianus	Low during the entire measurement
Candida albicans	Low during the entire measurement
Candida glabrata	Low during the entire measurement
Candida krusei	Low during the entire measurement
Staphylococcus epidermidis	900–1100
Enterococcus faecalis	690–1100
MSSA	605–1100
MRSA	430–1100
Bacillus spizizenii	580–1100
Proteus mirabilis	610–1100
Enterobacter aerogenes	550–1100
Salmonella enteritidis	295–1100
Pseudomonas aeruginosa	560–1100
Citrobacter freundii	535–1100
Klebsiella pneumoniae	285–1100
Moraxella catarrhalis	540–1100
Escherichia coli	650–1100

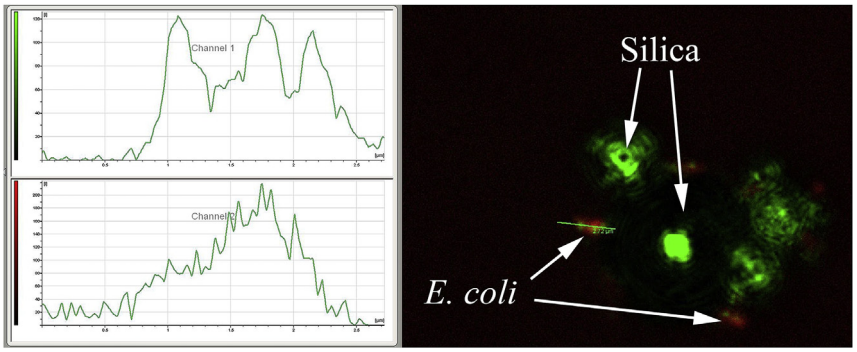

Figure 27. *Escherichia coli* adhesion with silicon dioxide using fluorescent microscopy.

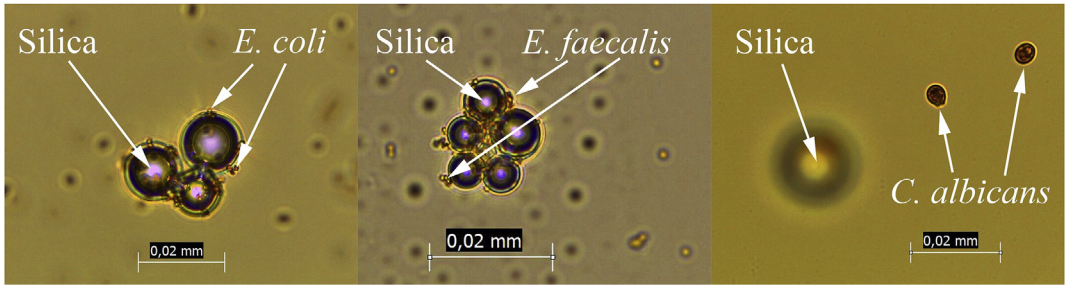

Figure 28. *Escherichia coli, Enterococcus faecalis,* and *Candida albicans* adhesion with silicon dioxide using light microscopy with iodine.

4. Discussion

4.1. Comparing yeast with bacteria

In this study, we demonstrated that spectrophotometry can be used to distinguish bacteria from yeasts at a wavelength interval of 285–1100 mm by measuring their absorbance.

Unpaired t-test's showed that adding silicon dioxide did not alter the probability of distinguishing bacteria from yeast at a wavelength interval of 285–1100 nm. A shorter wavelength interval of 285–700 nm also did not increase the likelihood of distinguishing bacteria from yeast, although by visually examining the graph it was possible to distinguish between the two. The most favourable conditions at which yeast-bacteria comparisons without silicon dioxide could be made occurred at 285–1100 nm. This suggests that a larger wavelength interval is best suited for comparing yeasts with bacteria.

4.2. Comparing bacteria to one another

Decreasing the wavelength interval from 285-1100 nm to 285–700 nm improved the likelihood of distinguishing bacteria from one another. At 285–1100 nm without silicon dioxide, only 52.6% of the bacterial-bacteria comparisons demonstrated a significant difference, but at 285–700 nm without silicon dioxide the results improved to 71.8%. A similar pattern occurred when silicon dioxide was added, as the results improved from 69.2% to 74.6%. This indicates that a smaller wavelength interval is best suited for comparing bacteria with each other. When

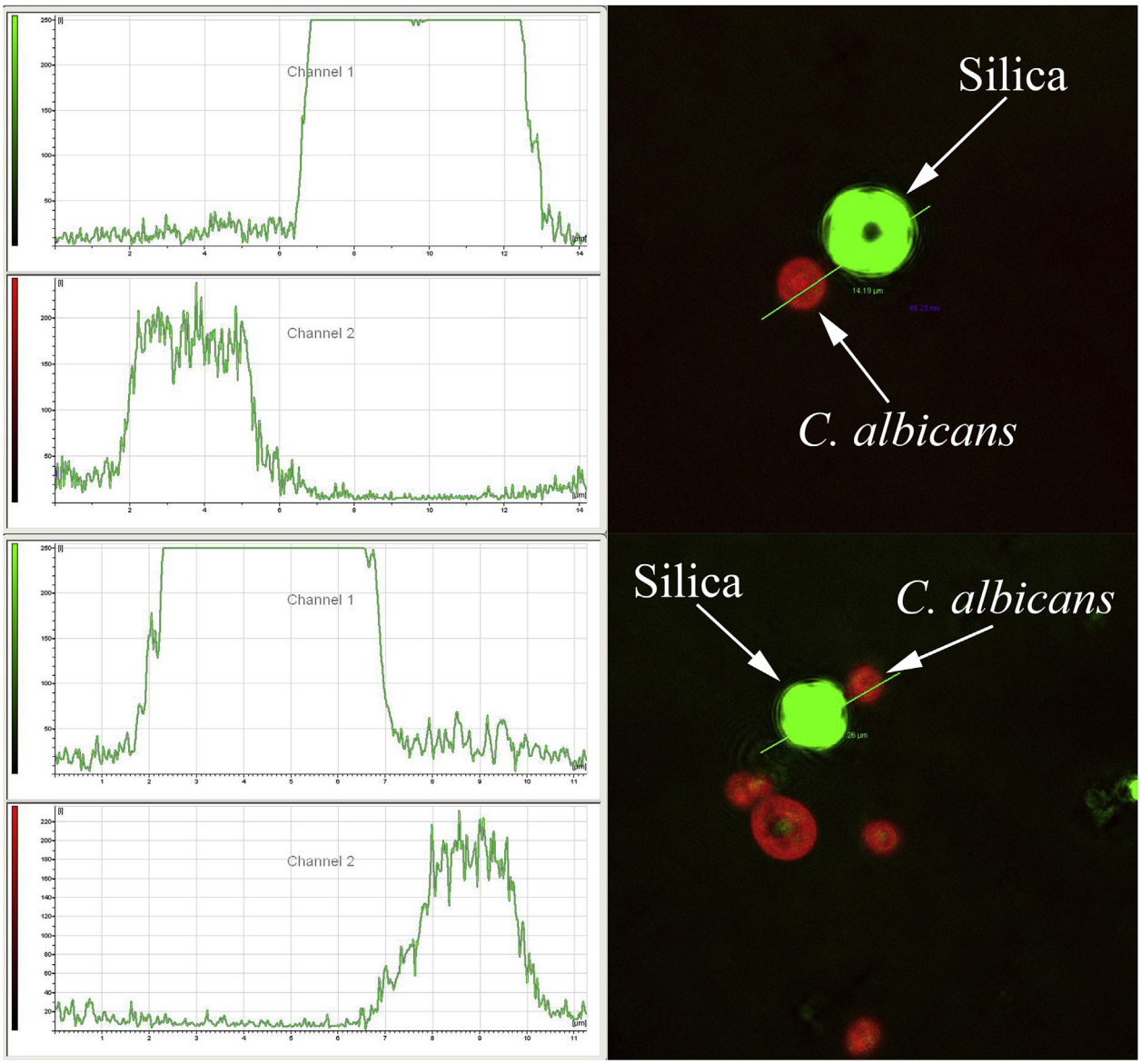

Figure 29. *Candida albicans* adhesion with silicon dioxide using fluorescent microscopy.

comparing Gram-positive to Gram-negative bacteria, the best results occurred at the 285–700 nm interval (without the need to take into account the addition of silicon dioxide). The probability of distinguishing between bacterial types with and without silicon dioxide was 80%.

4.3. Comparing yeasts to one another

The most favourable wavelengths at which yeast should be compared is between 285-700 nm with silicon dioxide, even though silicon dioxide did not provide a substantial light absorption change for yeast. At 285–700 nm without silicon dioxide it was possible to distinguish one yeast from another in 100% of the comparisons, but after the addition of silicon dioxide this possibility decreased to 83.3%; a 16.7% decrease. Likewise, at the measurement interval 285–1100 nm it was possible to distinguish one yeast from another in 83.3% of the comparisons, but when silicon dioxide was added the probability decreased to 50%; a 40% decrease. These results suggest that silicon dioxide should not be applied when comparing yeasts, and a wavelength of 285–700 nm is an appropriate interval for comparisons among yeasts as opposed to 285–1100 nm.

5. Conclusions

5.1. Potential practical applications

This study can provide a foundation for a new method of distinguishing microorganisms in a short period of time. To achieve this, there should be a database that contains each microorganism's absorbance within a specific substance and specific optical density.

This study may also provide the foundations for a new validation method for determining whether microorganisms bind to a specific substance, especially as fluorescence microscopy and electron microscopy, which are currently used to make these validations, can be time consuming.

5.2. Microorganism adhesion with silicon dioxide

Adding silicon dioxide increased the average absorbance of all examined bacteria at long wavelengths. The average absorbance of silicon dioxide is 0.211 absorbance units. The Beer-Lambert law states that a

mixture contains the combined absorbance of both samples, but this would only be the case if the concentration of each measured component is the same in the mixture as in pure samples [20]. If a suspension with high absorbance, such as bacteria or yeast, is mixed with a suspension with low absorbance and a sample is then taken, it is expected that the new absorbance would be less than that of the bacteria or yeast, as the mixed suspension would contain a smaller amount of bacteria or yeast per ml than the pure samples. However, for bacteria, this was not the case. The increase of absorbance at near infrared wavelengths, and for some bacteria at the visible light and ultraviolet wavelengths, indicates that bacteria made complexes with the silicon dioxide that exhibited an altered absorbance from the pure samples. A control measurement of *Escherichia coli* with fluorescence microscopy using a Leica TCS SL confocal microscope confirmed co-localization of *Escherichia coli* and silicon dioxide (Figure 27), while light microscopy with iodine confirmed *Escherichia coli* and *Enterococcus faecalis* adhesion to the silicon dioxide (Figure 28). For yeasts, however, the results were different. The absorbance did drop during the entire measurement for all of the yeasts, although with a barely noticeable drop for *Candida glabrata*. This suggests that the yeasts in this experiment either did not adhere to the silicon dioxide or did not form complexes that exhibited an altered absorbance. A control measurement of *Candida albicans* with fluorescence microscopy confirmed these suspicions as no noticeable co-localization was observed (Figure 29). *Candida albicans* adhesion with silicon dioxide was also not observed using light microscopy with iodine (Figure 28). This result is in accordance with another study claiming that silica nanoparticles reduces the attachment of *Candida albicans* to surfaces [21]. Our results suggest that bacteria have an adherence for silicon dioxide, and that it may be a poor choice of material for use in implantable prostheses. Our results also suggest that spectrophotometry might be used to confirm microorganism adhesion to inorganic surfaces as well as distinguish bacteria from yeast. For future studies we suggest using different inorganic materials to evaluate which types of microorganisms might adhere to them and whether such adhesions can be proven using spectrophotometry. Moreover, analysing one sample of each microorganism using fluorescent microscopy to confirm their adhesion to microparticles is suggested.

Declarations

Author contribution statement

Roberts Lozins: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tūrs Selga: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Dzintars Ozoliš: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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References

[1] L. Hall-Stoodley, J. Costerton, P. Stoodley, Bacterial biofilms: from the Natural environment to infectious diseases, Nat. Rev. Microbiol. 2 (2) (2004) 95–108.
[2] C. Chenu, G. Sotzky, Interactions between Microorganisms and Soil Particles, Wiley & Sons, 2002, pp. 9–13.
[3] P. Loskill, H. Hähl, T. Faidt, S. Grandthyll, F. Müller, K. Jacobs, Is adhesion superficial? Silicon wafers as a model system to study van der Waals interactions, Adv. Colloid Interface Sci. 179–182 (2012) 107–113.
[4] R.D. Simoni, R. Hill, M. Vaughan, H.A. Tabor, Classic instrument: the beckman DU spectrophotometer and its inventor, arnold O. Beckman, J. Biol. Chem. 278 (49) (2003).
[5] A. Abbas, A. Lichtman, S. Pillai, Cellular and Molecular Immunology, eighth ed., Elsevier Inc., 2015, pp. 503–504.
[6] [Internet], Utilizing Spectrophotometry in a Worldwide Approach to Medical Aid - HunterLab Horizons Blog, HunterLab Horizons Blog, 2018 [cited 14 July 2018]. Available from: https://www.hunterlab.com/blog/color-pharmaceuticals/using-spectrophotometry-in-medicine-a-worldwide-approach-to-medical-aid/.
[7] D. Ozoliņš, A. Žileviča, Higher speed of yeast adherence in comparison with speed of bacteria adherence to silica microparticles, Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci. 72 (5) (2018) 298–301.
[8] G. Lorite, R. Janissen, J. Clerici, C. Rodrigues, J. Tomaz, B. Mizaikoff, et al., Surface physicochemical properties at the micro and nano length scales: role on bacterial adhesion and xylella fastidiosa biofilm development, PloS One 8 (9) (2013), e75247.
[9] K. Anusavice, R. Phillips, C. Shen, H. Rawls, Phillips' Science of Dental Materials, twelfth ed., Elsevier Inc., 2013, pp. 307–339.
[10] A. Folpe, C. Inwards, Bone and Soft Tissue Pathology, Saunders/Elsevier, Philadelphia, Pa, 2010, pp. 255–275.
[11] G. Muzio, M. Miola, S. Perero, M. Oraldi, M. Maggiora, S. Ferraris, et al., Polypropylene prostheses coated with silver nanoclusters/silica coating obtained by sputtering: biocompatibility and antibacterial properties, Surf. Coating. Technol. 319 (2017) 326–334.
[12] W. Zimmerli, A. Trampuz, P.E. Ochsner, Prosthetic-joint infections, N. Engl. J. Med. 351 (2004) 1645–1654.
[13] S. Cross, J. Underwood, Underwood's Pathology, seventh ed., Elsevier Inc., 2019, pp. 27–52.
[14] Van der Waals Forces, Chemistry LibreTexts [Internet], 2019 [cited 4 September 2019]. Available from: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Physical_Properties_of_Matter/Atomic_and_Molecular_Properties/Intermolecular_Forces/Van_der_Waals_Forces.
[15] S. Halder, K. Yadav, R. Sarkar, S. Mukherjee, P. Saha, S. Haldar, et al., Alteration of Zeta potential and membrane permeability in bacteria: a study with cationic agents, SpringerPlus 4 (1) (2015).
[16] A. Gordienko, I. Kurdish, Surface electrical properties of bacillus subtilis cells and the effect of interaction with silicon dioxide particles, Biophysics 52 (2) (2007) 217–219.
[17] G. Chen, H. Zhu, Bacterial adhesion to silica sand as related to Gibbs energy variations, Colloids Surf. B Biointerfaces 44 (1) (2005) 41–48.
[18] C. Alupoaei, L. García-Rubio, An interpretation model for the UV-VIS spectra of microorganisms, Chem. Eng. Commun. 192 (2) (2005) 198–218.
[19] Y. Hu, N. Zhao, T. Gan, J. Duan, H. Yu, D. Meng, et al., Analytic method on characteristic parameters of bacteria in water by multiwavelength transmission spectroscopy, J. Spectrosc. 2017 (2017) 1–7.
[20] [Internet], Spectrophotometry, Web.mst.edu., 2019 [cited 3 January 2019]. Available from: https://web.mst.edu/~gbert/Color_Lg/spec/Aspec.html.
[21] B. Cousins, H. Allison, P. Doherty, C. Edwards, M. Garvey, D. Martin, et al., Effects of a nanoparticulate silica substrate on cell attachment of Candida albicans, J. Appl. Microbiol. 102 (3) (2007) 757–765.