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## THE ACOUSTIC EMISSIONS PRODUCED BY ESCHERICHIA COLI DURING THE GROWTH CYCLE

Traci Jane Cox  
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Traci Jane Cox, Student

Dr. Clair Hicks, Major Professor

Dr. David Harmon, Director of Graduate Studies

THE ACOUSTIC EMISSIONS PRODUCED BY ESCHERICHIA COLI DURING THE  
GROWTH CYCLE

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Agriculture, Food and Environment  
at the University of Kentucky

By

Traci Jane Cox

Louisville, Kentucky

Director: Dr. Clair Hicks, Professor of Animal and Food Sciences

Lexington, Kentucky

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## ABSTRACT OF THESIS

### THE ACOUSTIC EMISSIONS PRODUCED BY ESCHERICHIA COLI DURING THE GROWTH CYCLE

The objective of this study was to determine if acoustic emissions (AE) generated by three strains of *Escherichia Coli* (5024-parent strain, 8279-mutant strain and 8279-random/unrelated strain) could be used to differentiate each strain during their growth cycle. An acoustic sensor with an operating range of 35 kHz-100 kHz was inserted into the growth vessel and attached to a selected channel to capture AE data. The growth vessel was loaded with 60 ml of tryptic soy broth (TSB) (0.25% fructose) media with alginate (1.1%) or without alginate and inoculated with 1% ( $10^8$  CFU/ml) of an *E. coli* strain. The growth vessel was placed in a monitoring chamber and incubated at 32°C for 8-9 h. The AE's generated by each strain were collected throughout the growth cycle. All strains grown in media with and without alginate generated AE's within 5 min post inoculation. Strains grown in media without alginate generated stronger ( $P < 0.0001$ ) absolute energy (ABSE) and higher peak frequencies (PFRQ's), than in media with alginate. The AE's generated by strains 5024 and 8237 were stronger and easily distinguished from those generated by strain 8279. Strain 8237 generated 12% stronger ABSE from the 3<sup>rd</sup> to 8<sup>th</sup> h and 51% stronger PFRQ intensities than strain 5024 during 0-8 h. However, strain 5024 generated 15% stronger ABSE and 31% higher PFRQ's during the final hour of growth. Strain 5024 generated the highest PFRQ's from 5-50 kHz, while strain 8237 generated higher frequencies from 100-500 kHz. Fourteen distinguishable differences ( $P < 0.05$ ) in generated PFRQ's, between strains 5024 and 8237, were also observed in every 5 kHz increments from 100-500 kHz. Of these differences, strain 8237 generated higher frequencies within eight of the kHz ranges, while strain 5024 generated higher frequencies within six other kHz ranges. These data suggests that all bacteria may generate different AE's, thus producing a unique "fingerprint" of sound that will allow for its identification.

KEYWORDS: Acoustic Emissions, *Escherichia Coli*, Tryptic Soy Broth, Alginate, Absolute Energy, Peak Frequency

Traci Jane Cox

March 31, 2014

THE ACOUSTIC EMISSIONS PRODUCED BY ESCHERICHIA COLI DURING THE  
GROWTH CYCLE.

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Traci Jane Cox

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March 31, 2014

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(Date)

This thesis is dedicated in loving memory of my mother,  
Marilyn M. Cox

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# CHAPTER 1

## INTRODUCTION

Sound is generated by a vibrating object, propagating mechanical waves of energy through an elastic medium, causing the displacement and vibration of particles. The study of sound, acoustics includes four main categories: Earth science, Life science, Engineering and Arts, as described by “Lindsay’s Wheel of Acoustics” (Lindsay 1964). Each of these categories includes a broad spectrum of acoustics, including hearing, bioacoustics, seismic waves, noise, shock and vibration, music and communication. Another area of acoustics which has recently evolved is that from the acoustic emissions (AE’s) of bacteria.

Over the past fifty years, extensive research has provided insight into the intercellular communication of bacteria, known as chemical signaling or quorum sensing. Quorum sensing is a unique process, through which the production and release of chemical signals, known as autoinducers, allows bacteria to distinguish their population density, regulate gene expression and other bacterial behaviors. Though much attention has been given to chemical signaling, bacteria possibly communicate through another type of signaling, known as physical signaling.

Bacteria has been shown to not only respond to the physical signals of sound waves, electromagnetic radiation and electric currents, but they can generate them as well (Reguera 2011, Matsushashi et al. 1998 and Pelling et al. 2004). In 1998, Matsushashi et al. reported sonic emissions from *Bacillus subtilis* within the frequency range of 1-50 kHz.

Broad peak formations were observed at 8 to 10 kHz, 18 to 22 kHz and 27 to 43 kHz, and sharp peak formations at 16, 25 and 48 kHz. Pelling et al. (2004) examined the frequency of nanomechanical motions of *Saccharomyces cerevisiae* at 22°C and 30°C and found a 0.7 kHz increase in frequency with the higher temperature. The production of sound waves and the nanomechanical motions were suggested to be the result of metabolic processes and/or a communication pathway.

Though studies on the physical signaling of bacteria (particularly sound wave generation) are limited, the Department of Animal and Food Sciences at the University of Kentucky in collaboration with TriboFlow Separations (Lexington, KY) have undertaken a novel approach to research the acoustic emissions of bacteria. During the course of this research, three phases of studies have been performed, each utilizing a newly designed acoustic monitoring device (by TriboFlow Separations, Lexington, KY), along with piezoelectric sensors and AE monitoring software to collect the AE of bacteria. Preliminary studies from each phase have indicated AE during the lag, log and stationary phases of bacterial growth.

In the Phase 1 studies, Hicks et al. (2007) monitored the AE of two strains *Escherichia coli* (*E. coli*) and *Lactococcus lactis* ssp. *lactis* C2 using AE sensors with ranges between 20-50 kHz and 50-200 kHz. Peak emission frequencies from waveform “hits” were calculated using Fast Fourier Transform. The 20-50 kHz sensors detected AE activity within 5min after the medium was inoculated with *E. coli*, while the 50-200 kHz sensor detected activity in the log phase. Within the first hour, observations revealed notable differences in the averaged peak frequencies between the two strains. Detection of AE in *Lactococcus lactis* ssp. *lactis* C2 also occurred within 5min after inoculation

into the medium. Observations revealed notable differences in the average peak frequencies within the first hour between *E. coli* and *Lactococcus lactis* ssp. *lactis* C2. When *Lactococcus lactis* ssp. *lactis* C2 was inoculated with bacteriophage c2, shifting of peak frequencies was observed up to 150 min after inoculation. Though Wardini et al. (2010) did not detect any AE activity with *Lactococcus lactis* ssp. *lactis* C2 beyond the established threshold, AE peaks were detected at 33.2 and 40 min after it was infected with two different bacteriophages.

Though studies were performed during Phase 2, results have been reported. The only study performed during Phase 3 was by Ghosh et al. (2013), which also monitored the AE's of *Lactococcus lactis* ssp. *lactis* C2. Observations revealed those with initial cell counts of  $10^3$  cfu/ml had AE peak intensities starting at 172.5 to 278.5 min after inoculation, while those with  $10^6$  cfu/ml had intensities starting at 27.5 to 262.5 min after inoculation. When phages were added at 130 and 165 min post inoculation, AE peaks were observed within 8 min and continued up to 198 and 218 min, respectively.

While the studies on physical signaling are limited, the reported data appears to not only indicate AE's are generated by bacteria, as well as bacteriophages, but they are distinct from one another. These discoveries could possibly lead to acoustically "fingerprinting" or identifying bacteria based on their own unique AE signals. However, more research is indicated, as these studies have only investigated certain genus and strains of bacteria. The objectives of this study were to monitor the AE's generated from three strains of *Escherichia Coli* which included: a host strain, a mutant strain and a random/unrelated strain, during all growth phases in order:

1. To determine if the generated AE's could be differentiated between the three strains.
2. To determine if generated AE's could be differentiated between the host strain and mutant strain.

## CHAPTER 2

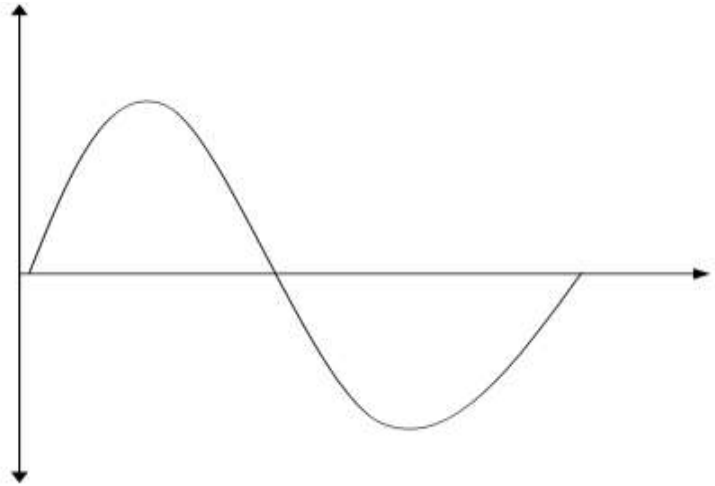
### LITERATURE REVIEW

#### 2.1. Fundamentals of Sound.

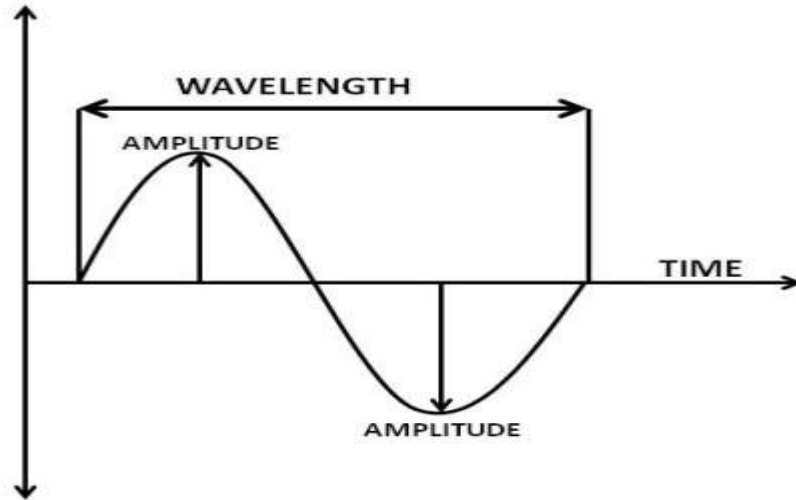
Sound is generated by a vibrating object and travels by waves of pressure, oscillating through a medium at various frequencies. A medium is required for sound to travel, as it is unable to travel within a vacuum. Mediums with elastic properties such as gas, liquid or solid allow for the propagation of sound. Sound waves are mechanical waves which transport energy through the medium, causing particles or matter to displace and vibrate back and forth.

**2.1.1. Properties of Sound Waves.** A sinusoid or sine wave is the basic waveform, as illustrated in **Figure 1**. A sound may contain only a single sine wave or several depending on the frequency. A sound wave is characterized by the following: wavelength, frequency and amplitude, as illustrated in **Figure 2**. Wavelength is the actual length of the wave or the distance between two exact points (peaks or troughs) on successive waves (Everest 2001). Frequency is the number of waves or cycles generated over a period of time (per second) and are measured in Hertz (Hz) (Everest 2001 and Raichel 2006). For example, if the frequency is 1Hz, one wave or cycle is occurring per one second. The pitch of the sound is determined by the frequency (Claybourne 2007 and Ostdiek et al. 2013). At low frequencies, vibrations are much slower and therefore produce a lower pitch, whereas high frequencies have faster vibrations and produce a





**Figure 1.** Sinusoid or Sine Wave. The basic wave form.



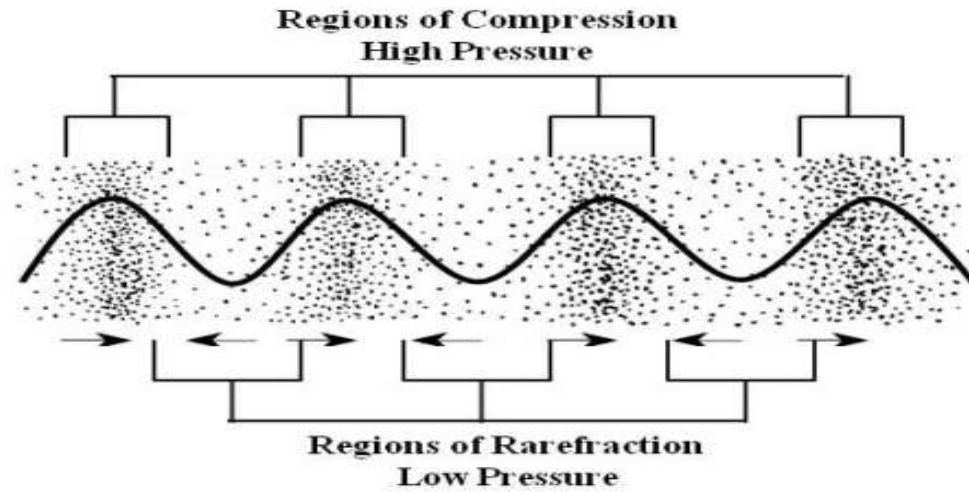
**Figure 2.** Sinusoid or sine wave properties. Wavelength is the length of the wave from the start point to the end point. Amplitude is the height of the peak or intensity.

higher pitch. The normal audio frequency range for humans is between 20 Hz to 20 kHz (Raichel 2006).

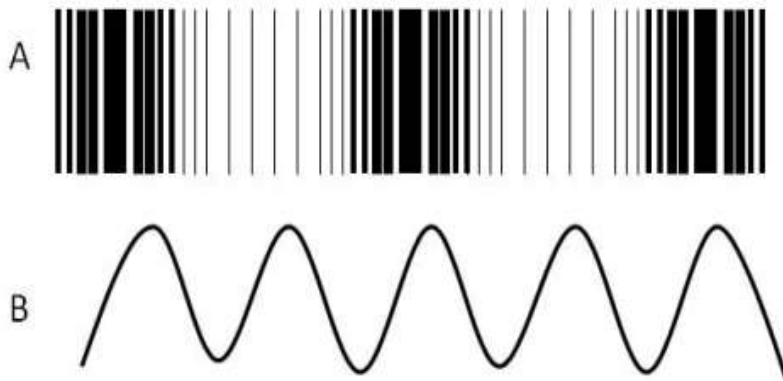
Amplitude is the intensity of the sound and is represented by the height of the wave (Claybourne 2007 and Ostdiek et al. 2013). A wave with great height will have higher amplitude, louder sound and thus greater intensity. The intensity of sound is measured in decibels (dB), with normal sound level ranges for humans being 0 dB (threshold of hearing) to greater than 120 dB (threshold of pain) (Ostdiek et al. 2013).

**2.1.2. Propagation of Sound Waves.** A sound wave is propagated by the displacement of particles in the medium. As the energy in the wave travels in a left to right formation, particles in the medium vibrate back and forth causing compression and rarefaction (Everest 2001), as shown in **Figure 3**. Compression occurs in a region of the wave comprised of high pressure where particles are more compact, whereas rarefaction occurs in a region comprised of low pressure where the particles are less compact (Everest 2001). Though compressed particles shift to the right and rarefaction particles to the left, all particles move back and forth of equal distance (Everest 2001).

Depending on the type of medium through which the energy travels, the motion of the displaced particles will vary, thus dictating the type of wave generated. There are two distinct types of sound waves: longitudinal and transverse. In longitudinal waves, the displaced particles in the medium are parallel to the propagation of the wave, while in transverse waves they are perpendicular (Everest 2001), shown in **Figure 4**. Sound is transmitted by longitudinal waves in gases and liquids (Isaacs 2005). In solids, however, it is transmitted by both longitudinal and transverse waves (Isaacs 2005).



**Figure 3.** Compression and rarefaction with wave propagation. As the wave travels forward, particles move back and forth in compression (high pressure) and rarefaction (low pressure) regions.



**Figure 4.** Types of mechanical waves. **(A)** Longitudinal wave forms propagate parallel to the displacement of medium and transmit sound in gases, liquids and solids. **(B)** Transverse wave forms propagate perpendicular to the displacement of medium and transmit sound only in solids.

**2.1.3. Speed of Sound Waves.** The actual speed of a sound wave is determined by the distance it travels within a certain time period. There are a few factors that can affect the speed of a wave. The speed at which sound waves travel, is dependent upon the density of the medium (Raichel 2006). Sound waves travel faster in mediums that are denser, than those that are less dense. Sound waves travel the fastest in solid mediums, followed by liquid and air, respectively (Claybourne 2007). Temperature also affects the wave speed in air, as the temperature rises, so too, does the wave speed (Claybourne 2007). **Table 1.** lists various mediums and their speed of sound.

**2.1.4. Sound Behaviors.** When a sound wave propagates, it travels outward in a straight line until it encounters some type of barrier such as a surface or an object or change in medium. A wave can take on different behaviors when this occurs: reflection, diffraction, refraction, and absorption. Reflection occurs when a wave strikes the surface of a barrier; the barrier absorbs some of the wave's energy, while the remainder is reflected (Raichel 2006). An echo is a well known example of sound reflection. When the direction of the wave's energy changes due to a barrier or obstacle, this behavior is known as diffraction (Everest 2001). An example of diffraction would be hearing a conversation that was taking place in another room. Refraction occurs when a wave is traveling from one medium to another medium of different density, causing a change in speed and the wave to bend or refract (Everest 2001 and Raichel 2006). If a sound wave traveling though air hits a concrete wall, the wave is refracted since the wall is much denser than air. Depending on the setting, all of these behaviors can occur at the same time during sound wave propagation.

**Table 1.** Speed of sound for various solids, liquids and gases.

<b>Substance</b>		<b>Speed of Sound (m/s)</b>
<b>Solids</b>		
Metals	Aluminum	5100
	Brass	4700
	Copper	3560
	Lead	1322
Various	Brick	3650
	Glass-pyrex	5640
	Rubber	1600
Wood	Ash	4670
	Maple	4110
	Oak	3850
<b>Liquids</b>	Ethanol	1724
	Glycerol	1904
	Mercury	1450
	Methanol	1103
	Water	1496
<b>Gases</b>	Air	331
	Air (20°C)	343
	Carbon Dioxide	259
	Hydrogen	1284
	Oxygen	316

Information obtained from the CRC HandBook of Chemistry and Physics, 71<sup>st</sup> Edition, 1990-1991, David R. Lide.

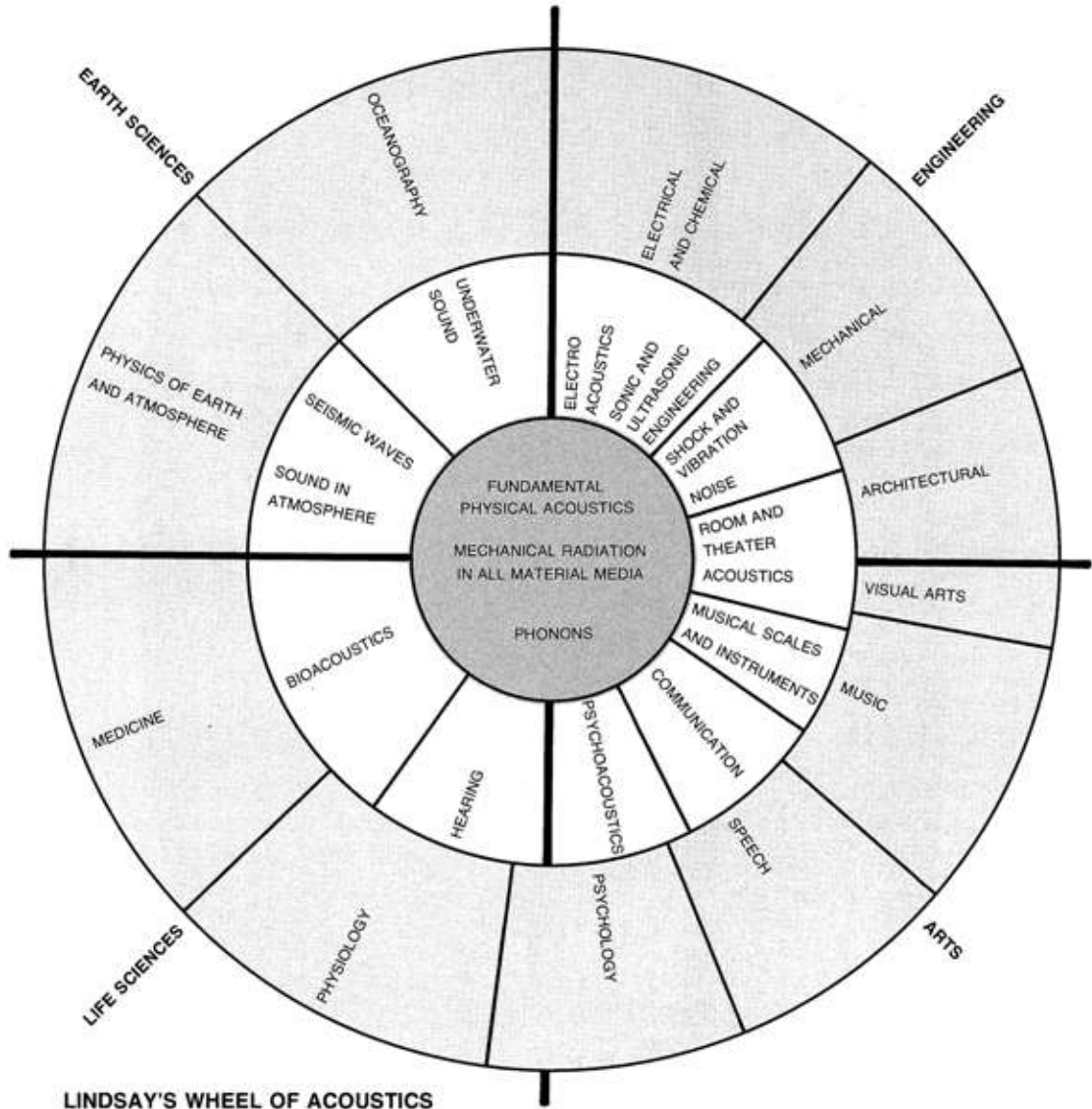
## **2.2. Acoustics.**

Acoustics is an interdisciplinary science that involves the study of production, control, transmission, reception and effects of sound (Britannica 2013). Sound is everywhere, which makes the field of acoustics quite diverse. In 1964, R. Bruce Lindsay published the “Lindsay’s Wheel of Acoustics,” (**Figure 5.**) which portrays four broad areas of acoustics: Earth Sciences, Life Sciences, Engineering and Arts. Each specific area is host to numerous acoustic disciplinarians such as, hearing, bioacoustics, seismic waves, noise, shock and vibration, music and communication. Within the last two decades, an evolving disciplinary shown to generate sound through acoustic emissions (AE’s), is that of bacteria.

**2.2.1. Acoustic Emission.** AE is defined by Muravin as a “phenomenon of sound and ultrasound wave generation by materials that undergo deformation and fracture processes.” Understanding the production of AE’s is an important aspect of science and technology. AE’s are stress waves generated by a surge of energy released within an area of a material, due to internal structural changes (Miinshiou et al. 1998 and Ozevin et al. 2003). Materials that are subject to fractures or cracking, such as metal, concrete, composites and plastics are primary sources of AE (Muravin 2009).

**2.2.2. Acoustic Emission Testing.** Acoustic emission testing (AET) is a technique used in evaluating and detecting structural defects and damage (Miinshiou et al. 1998 and Ozevin et al. 2003). The process involves the application of an acoustic emission system. The typical AE system consists of sensors, preamplifiers and a data collection device. The data collection device performs signal filtering, analog-digital conversion, hit (or wave) detection, data analysis, graphs and charts. Contact AE sensors





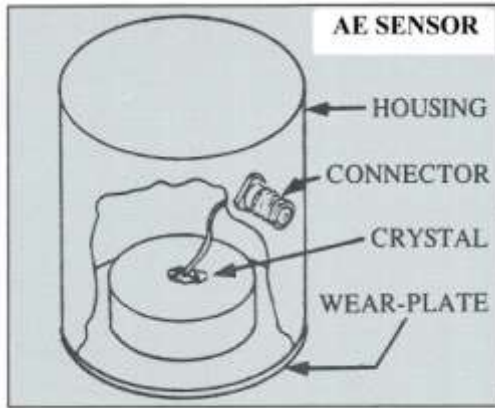
LINDSAY'S WHEEL OF ACOUSTICS

**Figure 5.** Lindsay's Wheel of Acoustics. (R. Bruce Lindsey in J. Acoust. Soc. Am. V. 36, p. 2242 (1964)).

are attached and secured to locations on the material. AE signals detected by the sensor are preamplified and then filtered to minimize noise interference and strengthen the signal. The filtered signal is passed through analog – digital conversion and becomes digitized. The waveform is detected, where it converts the signal into AE hits.

**2.2.3. Acoustic Sensor.** The purpose of AE sensors is to detect mechanical stress waves produced within materials caused by structural change. A crucial component in AE sensors is the transducer. The transducer is responsible for converting mechanical wave energy into an electrical signal (Emerterio et al. 2008). The most common type of contact transducer used in AET is piezoelectricity (Ozevin et al. 2003). Piezoelectricity is defined as the electric charge that is produced when a material such as crystal (quartz), is subjected to mechanical stress (Fraden 2010). When mechanical waves strike the surface of the AE sensor, an electrical signal is generated from the piezoelectric crystal. The signal is then preamplified and continues through the AE monitoring system for further processing.

Contact AE sensors are typically comprised of stainless steel, while the face material can be of either ceramic or stainless steel. The piezoelectric crystal is housed within the stainless steel chamber, as shown in **Figure 6**. The frequencies of AE's are generally range between 150-300 kHz (Hellier et al. 2003). Sensors used in AET have a typical operating frequency range between 20 kHz-1 MHz (Hellier et al. 2003 and Muravin 2009). The R6a sensors (Physical Acoustics Corporation (PAC), Princeton Junction, NJ.) are general purpose acoustic sensors with an operating frequency of 35-100 kHz. Per PAC, these sensors are typically used on metal and fiber reinforced



**Figure 6.** AE sensor indicating crystal location. (Adapted from Physical Acoustics Corporation. Princeton Junction, NJ. 2005.)

polymer (FRP) structures such as pipelines and storage tanks, due to their high sensitivity and low resonance. The R6 $\alpha$  sensor and its operating frequency are shown in **Figures 7 and 8**, respectively.

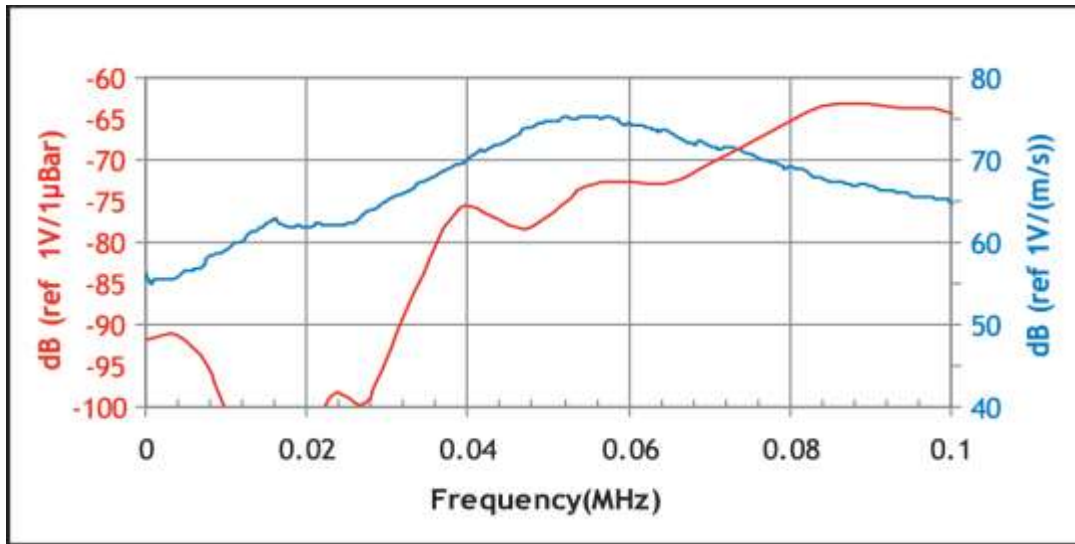
### **2.3. Bacteria Communication.**

**2.3.1. Chemical Signaling Factors.** The intercellular communication of bacteria is known as quorum sensing. Quorum sensing is a unique process that enables bacteria, to determine its population density and regulate gene expression based on chemical signaling molecule concentrations (Gray 1997, Schauder and Bassler 2001, Federle and Bassler 2003 and Bhattacharyya and Choudhury 2008). Bacteria produce and release these chemical signaling molecules, known as autoinducers, into their environment. The concentration of autoinducers is dependent upon the population density (Federle and Bassler 2003). When population densities are low, so too are the concentration of autoinducers and therefore will typically diffuse and go undetected (Gray 1997 and Bhattacharyya and Choudhury 2008). However, in higher density populations or growing population, the autoinducer concentrations are increased to threshold levels, thus activating gene expression (Gray 1997 and Bhattacharyya and Choudhury 2008). Quorum sensing is also involved in the regulation of other bacteria behaviors, including symbiosis, virulence, antibiotic production and biofilm formation (Schauder and Bassler 2001 and Sifri 2008).

**2.3.2. Physical Signaling Factors.** Over the past few decades, the focus of bacteria communication has concentrated on the chemical signaling process or quorum sensing. Though research is somewhat limited, early evidence indicates bacteria possibly



**Figure 7.** The R6 $\alpha$  ceramic acoustic sensor from PAC, Princeton Junction, NJ.



**Figure 8.** Frequency Response of the R6 $\alpha$  acoustic sensor. (Adapted from Physical Acoustics Corporation. Princeton Junction, NJ. 2005.)

communicate through another type of signaling, known as physical signaling. Through physical signaling, bacteria have been shown to respond to sound waves, electromagnetic radiation and electric currents (Reguera 2011). Not only do bacteria respond to physical signals, they can generate them as well. Studies (Matsushashi et al. 1998 and Pelling et al. 2004) have shown bacteria and other microorganisms are capable of producing sound waves, which may be indicative of intracellular motions caused by essential cellular activity (Reguera 2011).

The production of sound waves by bacteria was initially reported in 1998 by Matsushashi et al. In this study, a sensitive pressure condenser microphone connected to a preamplifier was used to detect sound waves in *Bacillus subtilis*. Sonic emissions were scanned at frequencies between 1-50 kHz. Broad peak formation was reported at 8 to 10 kHz, 18 to 22 kHz and 27 to 43 kHz, while sharp peaks were reported at 16, 25 and 48 kHz. Matsushashi et al. suggest the production of sound waves were possibly from repeated expansion and contraction of intracellular structures undergoing metabolic processes.

Pelling et al. (2004) investigated the nanomechanical motions of yeast cells using an atomic force microscope (AFM). *Saccharomyces cerevisiae* were studied at the end of log phase and examined for nanomechanical motion at 22°C and 30°C. Fourier transform calculations revealed the frequency of nanomechanical motions significantly increased from 0.9 kHz to 1.6 kHz due to the increase in temperature. The motions were thought to be propagating from metabolic activity. To determine if this was true, the cells were treated with sodium azide, which inhibits metabolic activity and all motions ceased. The

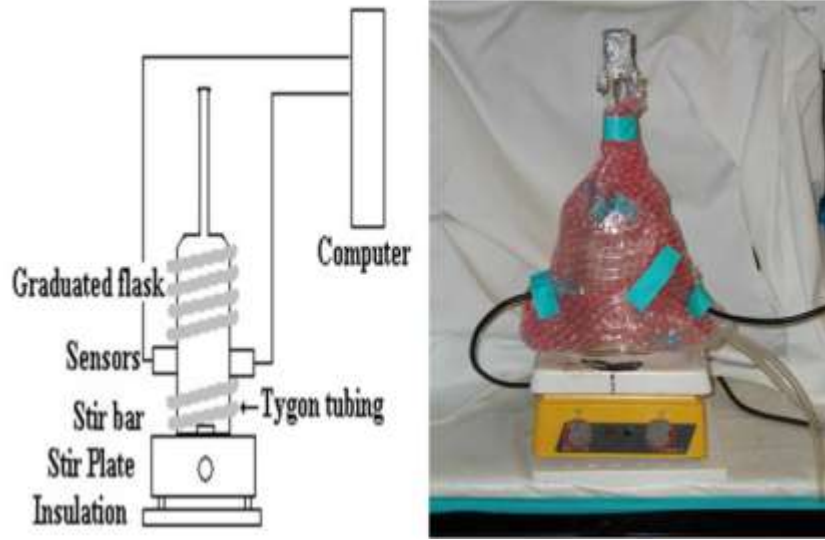
results of this study suggest nanomechanical motions may result from a communication pathway or metabolic processing.

#### **2.4. Acoustic Emission Studies at the University of Kentucky.**

Over the last decade, the Department of Animal and Food Sciences at the University of Kentucky and TriboFlow Separations (Lexington, KY) have initiated cooperative research approaches to determine whether bacteria and bacteriophages emit AE's during the growth phase. Several preliminary studies have been performed using various strains of *Escherichia coli* and *Lactococcus lactis* ssp. *lactis* strains, as well as bacteriophages. These studies have been categorized as Phase 1, 2 and 3 since different acoustic monitoring devices were used in each study. Subsequently after technological challenges were discovered with phase 1 and 2 monitoring devices, newly designed models were constructed. Despite these challenges, data from these preliminary studies have reported AE's during the lag, log and stationary growth phases.

**2.4.1. Phase 1 Acoustic Monitoring Device and Procedure.** Initially, the monitoring device, shown in **Figure 9**, was comprised of a square glass flask onto which was attached two solid state piezoelectric AE sensors (model R6 $\alpha$ ) purchased from Physical Acoustics Corporation (PAC, Princeton Junction, NJ). The sensors, with operating frequencies between 20-50 kHz, were positioned and attached with rubber bands on opposite sides of the flask. Tygon tubing was wrapped around the flask in a spiral fashion starting from the neck and ending at the base. The tubing was connected to a water bath that maintained a temperature of 32°C. The entire flask, including the AE sensors and tubing, was wrapped in 1cm bubble wrap for insulation to maintain





**Figure 9.** Phase 1 AE monitoring device.

temperature. The AE sensors were attached to an AE computerized monitoring system, which was calibrated prior to inoculation of the media to eliminate any background noise. A sterilized magnetic stir bar along with sterile media was placed in the flask, inoculated and stirred for two min to evenly disperse the bacteria. Every 30-60 min the data monitoring system was paused, the stir bar was turned on and samples were collected for optical density (OD) measurements. OD's for 1ml aliquots were read by a spectrophotometer at 600nm. Although the AE device was able to detect acoustic signals, the strength of the signal was impeded by multiple interfaces and therefore, the repeatability was questioned.

**2.4.2. Phase 1 AE Studies.** Three separate studies were performed during phase 1, which monitored the AE's from various strains of *Escherichia coli* (*E. coli*), *Lactococcus lactis* ssp. *lactis* and bacteriophages. The aforementioned AE monitoring device and procedure were carried out in each of these studies.

In the first 2007 study, Hicks et al. monitored two strains (15q and 15cc) of *E. coli* grown in tryptic soy broth (TSB) medium at 32°C for > 5h, using two types of AE sensors with sensitivities of 20 to 50 kHz and 50 to 200 kHz. When bacteria generated AE's, the waveform was classified as a "hit." The peak emission frequency for each "hit" was calculated using fast Fourier transform. AE activity was detected by the 20 to 50 kHz sensor within five min after inoculation. However, detection of AE activity with the 50 to 200 kHz sensor occurred during the log phase. Shifting of peak frequencies were noted throughout all phases of growth among both strains of *E. coli*. Notable differences in average peak frequencies were observed within the first hour between the strains of *E. coli*, as 15q produced seven peaks and 15cc only produced five peaks. Post

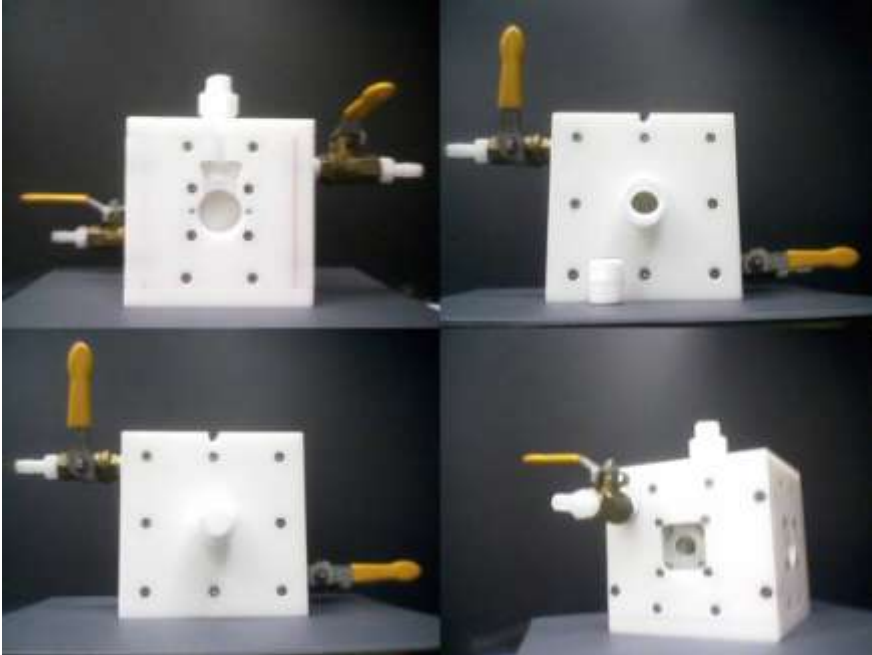
incubation revealed 15cc produced three peak domains (one being defined, along with two smaller ones), while 15q produced only one broad peak. This study showed that different strains of bacteria are able to produce different AE signals.

Another 2007 study by Hicks et al. monitored the AE's of *Lactococcus lactis* ssp. *lactis* C2 and bacteriophages, using M17 medium and M17 agar at 32°C for 11h. The AE sensors were the same as the ones used in the previous study and fast Fourier transform was also used to analyze average peak frequencies. As seen with *E. coli*, the *Lactococcus lactis* ssp. *lactis* C2 also showed AE activity within the first 5 min after inoculation in the M17 medium. AE activity in the M17 agar medium increased from the time of inoculation up to nine hours and then decreased in the hours from nine to eleven. AE activity was also reported on *Lactococcus lactis* ssp. *lactis* C2 grown in M17 broth and inoculated with bacteriophage c2. Shifts in average peak frequencies were noted from inoculation up to 150 min, consistent with the transition from lag to log phase. The average peak frequency data from *Lactococcus lactis* ssp. *lactis* C2 was compared to that of *E. coli* 15q and found differing peak frequencies among them within the first hour of growth. These findings indicate that bacteriophages are able generate AE's and different species of bacteria produce different AE signals.

In 2010, Wardini et al. performed a similar study monitoring *Lactococcus lactis* ssp. *lactis* C2 and bacteriophages (sk1 and ml3) in M17 medium at 26°C for 8h. This study used AE sensors with a range of 5 to 50 kHz. The medium was inoculated with *Lactococcus lactis* ssp. *lactis* C2 and approximately 90 min later was infected with phages sk1 and ml3. *Lactococcus lactis* ssp. *lactis* C2 was also grown in medium without phage infection. Sound intensity was measured in attojoules and the energy rates

for detected sound waves were analyzed. A standard threshold of 3sigma was set and any peaks above this were considered significant. Results from the study revealed *Lactococcus lactis* ssp. *lactis* C2 without phage infection did not produce any AE beyond the 3 sigma threshold. However, *Lactococcus lactis* ssp. *lactis* C2 infected with the sk1 and ml3 phages did produce AE's beyond 3 sigma. Initial peaks were observed at 33.2 and 40 min after infection for sk1 and ml3 phages, respectively. The ml3 phage also showed a burst of two large peaks at 50 min and again at 240 min after infection. However, the sk1 phage showed no other peak production from the initial peak at 33.2 min. The results of this study revealed different bacteriophages produce different AE signals.

**2.4.3. Phase 2 Acoustic Monitoring Device and Procedure.** When sound impedance was identified with the phase 1 monitoring device, a new device was custom designed by TriboFlow Separations (Lexington, KY). This device was a unique 5 inch cube, constructed with a two wall design and comprised of ¼ inch jacketed delrin as shown in **Figure 10**. Within the device was a chamber that was accessed by a plastic screw mounted on the top side. Engineered within the walls, on opposite sides of the chamber, were mountings for the R6α sensors. Ball valves were also installed on each of these two sides and were connected to a water bath that pumped and recirculated water at 32°C between the two walls. Sterile media was placed inside the chamber, inoculated with bacteria and the top mounted screw was secured in place. The AE sensors were attached to the AE computerized monitoring system, which were calibrated prior to inoculation of the media to eliminate any background noise. Every 30-60 min the data monitoring system was paused, the stir bar was turned on and samples were

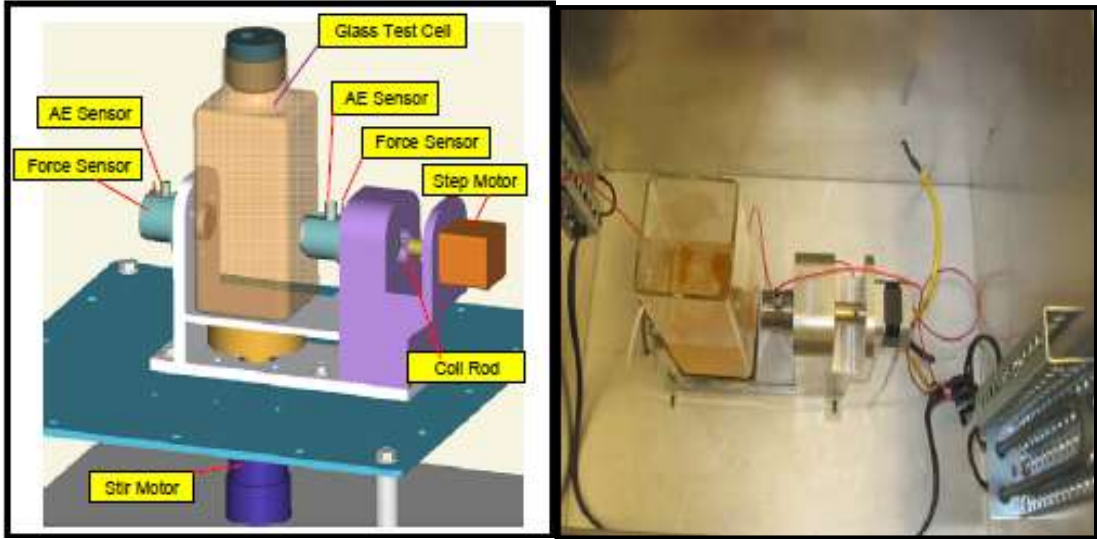


**Figure 10.** Phase 2 AE monitoring device.

collected for OD measurements. OD's for 1ml aliquots were read by a spectrophotometer at 600nm. Limitations with this device involved the acoustic noise produced from the consistent flow of water and the recirculating pump. Results from any of the studies utilizing this device have not been reported.

**2.4.4. Phase 3 Acoustic Monitoring Device and Procedure.** For phase 3, TriboFlow Separations (Lexington, KY) designed and developed a rectangular compartment comprised of stainless steel. The compartment houses two separate chambers that are identical and equipped with a hinged top door for easy access. Within each compartment are stainless steel devices that support a square glass flask, to which an R6 $\alpha$  sensor (PAC, Princeton Junction, NJ) having sensitivity between 20-100 kHz, was mounted to opposite sides of the outer walls of the flask. The AE sensors were attached to the AE computerized monitoring system. Encapsulated within each device is a stirring motor. Each compartment has two heaters attached to the wall with thermocouples to maintain a temperature of 32°C. The outer frame of the compartment is sound insulated with a one inch thick black rubber/foam material. A picture of the compartment is shown in **Figure 11**. Sterile media was placed inside the flask and inoculated with bacteria. Every 30min collection of data was paused, media was stirred and a 1ml aliquot was collected. OD's for the aliquots were read by a spectrophotometer at 600nm.

**2.4.5. Phase 3 AE Studies.** In a 2013 study by Ghosh et al., utilizing the phase 3 monitoring device and procedure, AE's were detected from *Lactococcus lactis* ssp. *lactis* C2 with differing initial cell counts. From those with 10<sup>3</sup> cfu/ml, AE peaks intensities beyond 3sigma were observed at 172.5, 262.5 and 278.5 min after inoculation. Those with 10<sup>6</sup> cfu/ml, had AE peaks as early as 27.5 to 262.5 min after inoculation. AE's



**Figure 11.** Phase3 AE monitoring device.

were also observed before and after the addition of bacteriophage c2 to *Lactococcus lactis* ssp. *lactis* C2. Phage additions occurred at 130 and 165 min post inoculation of bacteria. When the phage was added at 165min, the first AE peaks with intensities  $> 3$  sigma were detected within 8-43 min and continued up to 198 min after the addition. For phage added at 130 min, intensities were observed 8 min after addition and continued up to 218 min. These findings support the aforementioned studies using *Lactococcus lactis* ssp. *lactis* C2 and bacteriophage and the production of AE's.

Though studies on physical signaling, particularly sound wave production, are limited, the findings have indicated bacteria and bacteriophages do produce AE's and are distinct from one another. Preliminary findings from the ongoing research at the University of Kentucky have concluded:

1. Different bacterial strains produce different AE signals.
2. Different bacterial genera produce different AE signals.
3. Different bacteriophages produce different AE signals.

These discoveries may potentially be a valuable and unique approach to identifying or “fingerprinting” bacteria.



## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1. Bacterial Strains.

*Escherichia coli* (*E. coli*) strains of W1485, MG1655 and MC1060 (coli genetic stock culture numbers (CGSC#) 5024, 8237 and 8279, respectively) were obtained from the *E. coli* Genetic Stock Center at Yale University (New Haven, CT). Strains will be identified in this manuscript by their CGSC#. Each strain of *E. coli* was transported and received on a ¼ inch filtered disc. These strains are all derivatives of *E. coli* K-12. The characteristics of each strain are listed in **Table 2**. Strain 5024 is the parent strain of 8237. Though each strain's genotype differs, strain 5024 is the only one that possesses the F+ plasmid (sex pilus) and rpoS396 (the sigma factor=  $\sigma^s$ ). The  $\sigma^s$  is the master regulator in the general stress response of *E. coli* (Hengge-Aronis, 2002 and Kazmierczak, et al. 2005).

#### 3.2. Media and Biological Supplies.

All media and biological supplies were purchased from Fisher Scientific (Pittsburgh, PA). **Table 3**. lists the item, along with its brand name, reference and lot numbers.

**Table 2.** Characteristics of *E. coli* strains.

Strain	CGSC# <sup>a</sup>	Source	Genotype <sup>b</sup>
W1485	5024	J. Lederberg	F+, $\lambda^-$ , <i>rpoS396(Am)</i> , <i>rph-1</i>
MG1655	8237	J. E. Wertz	F-, $\lambda^-$ , <i>rph-1</i>
MC1060	8279	J. E. Wertz	F-, $\Delta$ ( <i>co dB-lacI</i> )3, <i>galK16</i> , <i>galE15(GalS)</i> , $\lambda^-$ , <i>e14-</i> , <i>mcrA0</i> , <i>relA1</i> , <i>rpsL150(strR)</i> , <i>spoT1</i> , <i>mcrB1</i> , <i>hsdR2</i>

<sup>a</sup> *E. coli* Genetic Stock Center, Yale University, New Haven, CT.

<sup>b</sup> Berlyn 1998 Microbiol.Mol.Biol.Rev. 62:814.

**Table 3.** Media and biological supplies.

Item	Brand	Reference Number	Lot Number
Plate Count Agar (PCA)	BD <sup>a</sup>	247940	2195425
Tryptic Soy Broth (with dextrose)	BD	286220	3042060
Tryptic Soy Broth (without dextrose)	BD	211768	6198191
Tryptic Soy Agar (TSA)	BD	211043	6304083
Skim Milk	BD	232100	7320448
L-grade Fructose	Fisher Science Education	S25332A	3ABAF12010412
Alginate	Fluka	71238	138708722308055
Cryovial	Fisherbrand	0566964	929645268
Microcentrifuge tube	Fisherbrand	02-681-286	06300216
Petriefilm™-Aerobic Count Plate	3M™		2012-8TM

<sup>a</sup> Brand name for Becton, Dickinson and Company.

### **3.3. Sub-Culture Media and Preparation.**

Plate count agar (PCA) and tryptic soy broth (TSB) (0.25% dextrose) were prepared according to the manufacturer's instructions. Both mediums were autoclaved at 121°C for 15 min. The PCA was placed in plastic petri dishes and allowed to solidify at room temperature. All mediums were refrigerated at 4°C until time of use. Using sterile tweezers, the filtered discs containing each strain of *E.coli* were placed separately in the center of a PCA plate. TSB was pipetted onto each disc until it was completely saturated. A sterile plastic streaking utensil was used to gently streak the excess TSB out and away from the disc. The plates were inverted and incubated at 37°C for 24h. Growth was visually observed on all plates.

### **3.4. Culture Media and Preparation.**

TSB (0.25% dextrose), tryptic soy agar (TSA) and skim milk powder were prepared according to the manufacturer's instructions. Using a sterile pipette, three tubes were prepared with 7 ml of TSB. A total of ninety culture slants (thirty slants for each strain of *E. coli*) were prepared using 7ml of TSA agar. Plastic caps were placed on each TSB tube and screw caps were secured on each TSA tube prior to autoclaving at 121°C for 15 min. The tubes of TSB were stored at 4°C until use. The rack of tubes containing TSA agar were propped at a 30° angle using a metal slant, allowed to solidify at room temperature and stored at 4°C until use. A culture was prepared for each individual strain of *E. coli*. For each culture, a sterile loop was used to obtain several isolated colonies from the PCA plate, then inserting and gently mixing into 7 ml TSB. All cultures were incubated at 37°C for 24 h. Stock cultures were prepared using the TSA slants. The

inoculation method for the TSA slants involved the insertion of a sterile wire needle into the TSB culture, stabbing the needle into the agar slant and then streaking the surface of the agar slant. Thirty TSA slants were inoculated with each individual strain of *E. coli* and incubated at 37°C for 24 h. Post incubation, 1ml of sterile skim milk was injected onto the TSA slant. Using a sterile wire needle, the skim milk was gently swept over the top of the agar slant, pipetted into a cryovial and stored at -80°C until use.

### **3.5. Working Culture Preparation.**

TSB (0.25% dextrose) was prepared according to the manufacturer's instructions. The broth was autoclaved at 121°C for 15 min. Working cultures were prepared by inoculating 25 ml of TSB with 1 ml of frozen *E. coli* stock cultures. The mixture was incubated in a 32°C water bath for 16 h. Twenty-five, 1 ml aliquots were produced for each *E. coli* strain and were stored in microcentrifuge tubes at -80°C until use.

### **3.6. Media Preparation for AE Monitoring.**

**3.6.1. TSB with Dextrose and Alginate.** TSB (0.25% dextrose) was prepared according to the manufacturer's instructions. Approximately 11 gms (1.1%) of alginate was mixed into the broth until the solids were completely dissolved. The TSB was autoclaved at 121°C for 15 min.

**3.6.2. TSB with Fructose and Alginate.** TSB (without dextrose) was prepared according to the manufacturer's instructions. Approximately 11 gms (1.1%) of alginate and 2.5 gms (0.25%) of fructose were mixed into the broth until the solids were completely dissolved. The TSB was autoclaved at 121°C for 15min.

**3.6.3. TSB with Fructose.** TSB (without dextrose) was prepared according to the manufacturer's instructions. Approximately 2.5 gms (0.25%) fructose was mixed into the broth until the solids were completely dissolved. The TSB was autoclaved at 121°C for 15 min.

### **3.7. Current Acoustic Monitoring Device.**

The most recent AE monitoring device used in this study was designed and developed by TriboFlow Separations (Lexington, KY). It is a rectangular compartment comprised of stainless steel, housing two separate chambers that are identical and temperature controlled. A picture of the compartment is shown in **Figure 12**. Each chamber is equipped with a hinged top door for easy access and acoustic sensor connectors to the PCI-2 Based AE System (PAC, Princeton Junction, NJ). The outer frame of the compartment is sound insulated with a one inch thick black rubber/foam material. The environment of the compartment is also sound and vibration isolated. The compartment and the computer are housed in a storage room, located on the ground floor of the Charles E. Barnhart building, on the University of Kentucky campus. The compartment and computer sit directly on top of a well constructed rectangle shaped table top (**Figure 13**), comprised of wood, sand and granite. Four columns of cinder blocks (stacked three high) stand on a concrete floor and support each corner of the tabletop. The tabletop is constructed of plywood and has a 6 inch wall of wood, rising up



**Figure 12.** Acoustic monitoring compartment (designed and developed by TriboFlow Separations, Lexington, KY).



**Figure 13.** Computer set up and acoustic monitoring compartment.



and boxing in the table's entire surface. The boxed surface is completely filled with sand and is covered with a 3 inch piece of solid granite.

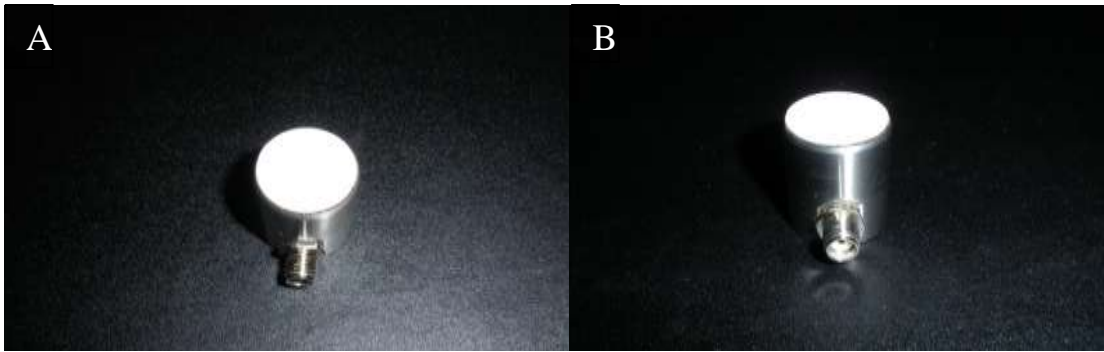
**3.7.1. Acoustic Monitoring Tubes.** Devices for the containment of media and bacteria were designed out of PVC tubing and are shown in **Figure 14**. Four tubes were constructed, two from standard white and two from clear PVC tubing. Initially, the standard white PVC tubes were used for this study, but were replaced with clear PVC in order to monitor for the production of gas bubbles. Each tube was measured and cut from a larger segment of tubing, then placed on a lathe where one end was machined for o-ring placement. The dimensions of the standard white PVC tubing were as follows: 203.3mm in length, OD of tubing ranged from 26.77 to 26.82mm, ID from 20.57 to 20.63mm and a wall thickness from 3.12 to 3.25mm. The o-ring dimensions were as follows: OD of 23.88mm, ID of 18.68mm and a wall thickness of 2.64mm. The dimensions of the clear PVC tubing were as follows: 203.3mm in length, OD of tubing ranged from 26.22 to 26.72mm, ID from 20.32 to 20.41mm and a wall thickness from 3.10 to 3.18mm. The o-ring dimensions were precisely the same as stated for the standard white tubing.

**3.7.2. Acoustic Sensors.** Two acoustic ceramic sensors, R6 $\alpha$  models SNAD52 and SNAD54 (PAC, Princeton Junction, NJ) were selected for this study and are shown in **Figure 15**. Each acoustic sensor is handcrafted, that is each piezoelectric crystal requires a characteristic evaluation prior to assembling within the sensor. The operating frequency range of the R6 $\alpha$  sensor was between 35-100 kHz.

**3.7.3. Set Up of Apparatus.** Prior to tube insertion, a thin layer of silicon grease was applied around the outer edge of the ceramic surface of each R6 $\alpha$  sensor, to prevent



**Figure 14.** Acoustic Monitoring Tubes and Sensors. (A) White monitoring tube with view of o-ring placement and model R6 $\alpha$  sensor, (B) White monitoring tube with R6 $\alpha$  sensor fully inserted, (C) Clear monitoring tube with view of o-ring placement and R6 $\alpha$  sensor and (D) Clear monitoring tube with R6 $\alpha$  sensor fully inserted.



**Figure 15.** Acoustic Sensors. (A) The top view and (B) side view of the R6 $\alpha$  sensor (PAC, Princeton Junction, NJ).

seepage of media into the device. Each sensor was inserted at the end of the tube with the o-ring, thus allowing a secure and leak proof placement, and connected to a selected channel equipped to the AE monitoring system. The tubes were positioned vertically (with the open end at the top and the connected sensor at the bottom), secured on a biuret stand and placed in each of the monitoring chambers, as shown in **Figure 16**. The thermostat for each heating element in the monitoring chambers were set at 32°C, which were monitored by internal thermometers, located in each chamber, throughout each experiment.

**3.7.4. AE Data Collection.** Data was collected and analyzed using AEwin™ software (PAC Princeton Junction, NJ). Each R6α sensor was connected to a 40 dB preamplifier (model 1220A, PAC, Princeton Junction, NJ) and a designated channel in the AE monitoring system.

Prior to each experiment, a trial run was performed to establish the most appropriate dB level for each type of sensor. The R6α sensor threshold levels ranged between 21- 26 dB. A layout was formatted, which included the established threshold level and preamplification of 40 dB. The analog filter which allows the capturing of signals in a designated range was set at 1 kHz for the lower and 3 MHz for the upper. The waveform streaming included a sampling rate = 1MSPS (one million samples per second), with a record length of millisecond = 100.3520.

Once the criteria had been established for an AE monitoring session, the program was initiated. AE was detected when a waveform struck the acoustic sensor, signaling a “hit.” For each “hit”, specific components of the waveform above the AE threshold were extracted, including the time of the hit, along with its AE amplitude, rise time, AE



**Figure 16.** Set up of apparatus inside one of the AE monitoring chambers.

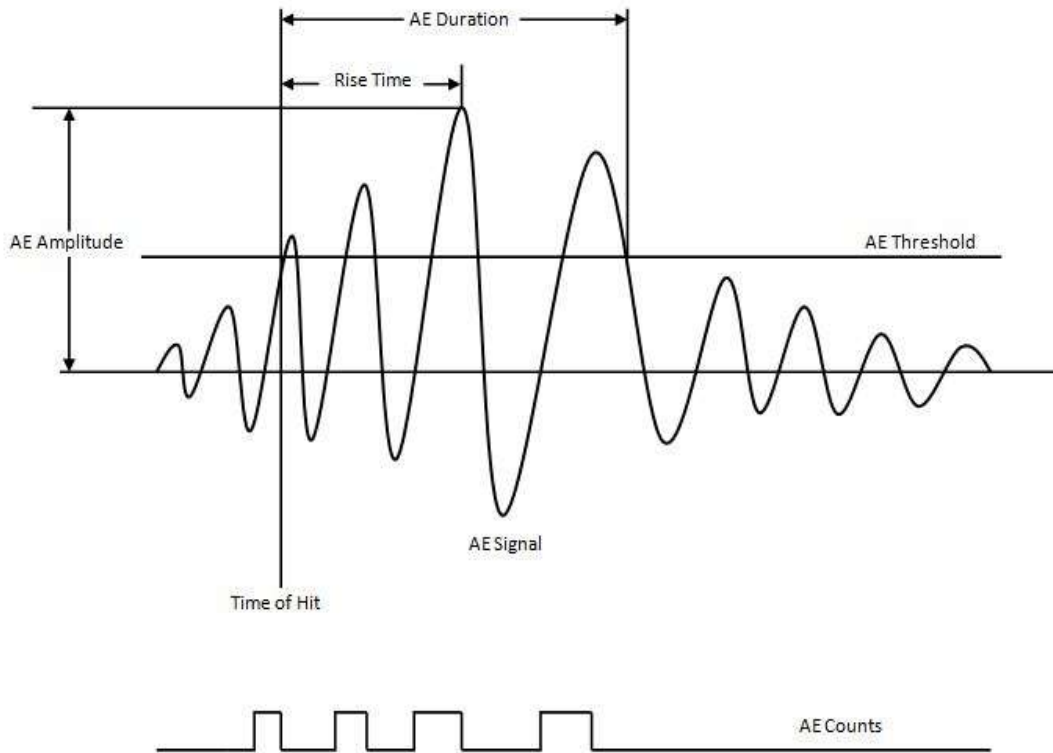
duration, absolute energy (ABSE) and peak frequency (PFRQ), shown in **Figure 17**.

ABSE is measured in attoJoules (aJ) and is the “true” energy measure of the AE hit (PAC Manual, 2003). PFRQ is measured in kilohertz (kHz) and is the point at which the largest peak formation occurs (PAC Manual, 2003). The AEwin™ software performs Fast Fourier Transform calculations on raw data, converting it to frequency based data, shown in **Figure 18**. Upon completion of each experiment, the data was separated for each channel using the Winport function within the AEwin™ software system. Individual channel data was exported to Microsoft® Office Excel 2007, where the data was manipulated to only include the following: time (hours), number of hits, ABSE and PFRQ.

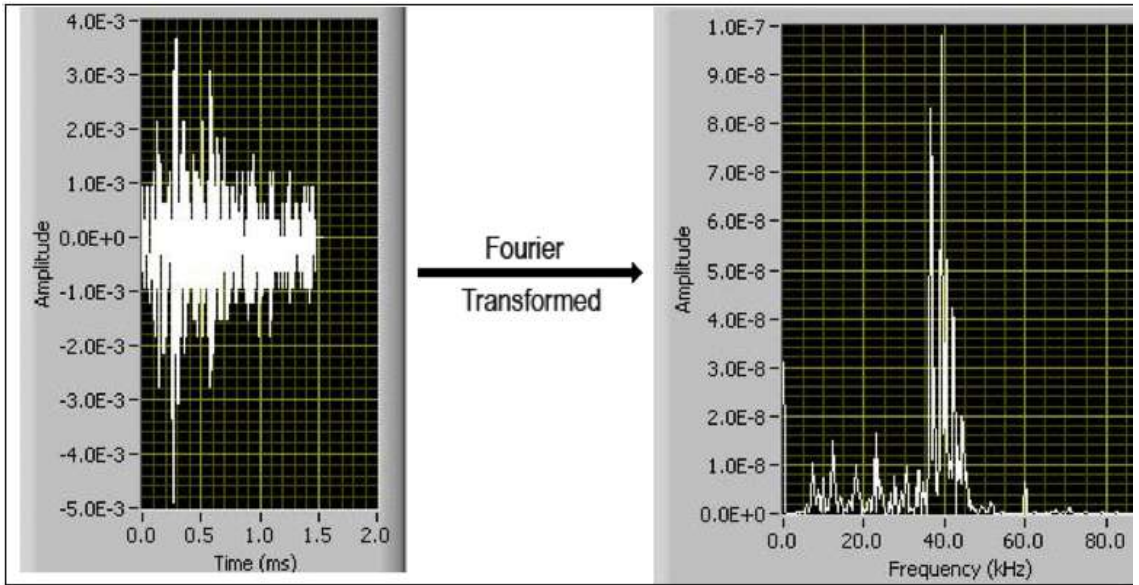
### **3.8. AE Monitoring.**

**3.8.1. AE Monitoring with Controls.** Distilled water and each of the three types of TSB media (dextrose with alginate, fructose with and without alginate) were used as controls to establish baseline “noise” frequencies. AE monitoring was performed on each control in each type of PVC tubing (standard white and clear), using the R6α sensor. Each tube was filled with 60 ml of a control and monitored for a minimum of 2 h at 32°C.

**3.8.2. TSB with Dextrose and Alginate.** The initial set up for AE monitoring included the use of the standard white PVC tubing and the R6α sensors. Each tube was filled with 60 ml of sterile TSB (0.25% dextrose and 1.1% alginate) medium and inoculated with 0.6 ml of the same strain of *E. coli*. The mixture was gently stirred with a sterile utensil to disperse the bacteria throughout the media. AE monitoring and data



**Figure 17.** Diagram of AE Hit Extraction. (Adapted and modified from PCI-2 Based AE System User's Manual, 2003, PAC, Princeton Junction, NJ.)



**Figure 18.** Conversion of raw data from time to frequency using Fast Fourier Transform.



collection was initiated on channels 4 and 6, both utilizing 24 dB levels and continued for up to 9 h. One experiment was performed on all three strains of *E. coli*. Optical densities (OD's) were taken every hour, on each tube, during each of these experiments.

**3.8.2.1. Durham Test.** A Durham test, which detects the production of gas, was performed on each strain of *E. coli*. The test was performed by placing an inverted Durham tube within each of three test tubes. Each test tube was loaded with 25 ml of sterile TSB (0.25% dextrose and 1.1% alginate) medium and autoclaved at 121°C for 15 min. Each individual test tube was then inoculated with 1 ml of an *E. coli* culture and incubated at 37°C for 24 h.

**3.8.3. TSB with Fructose and Alginate.** Experiments were performed using the clear PVC tubing and the R6 $\alpha$  sensors. Each tube was filled with 60 ml of sterile TSB (with 0.25% fructose and 1.1% alginate) and inoculated with 0.6 ml of the same strain of *E-coli*. The mixture was gently stirred with a sterile pipette to disperse the bacteria throughout the media. AE monitoring and data collection was initiated on channel 3, 4 and 6 utilizing dB levels of 26, 21 and 26, respectively, and continued for up to a minimum of 8 h. A total of five experiments were performed on strain 5024, seven were performed on strain 8237 and six were performed on strain 8279. Hourly OD's were obtained on each strain of *E. coli* during two of the experiments.

**3.8.4. TSB with Fructose and No Alginate.** Experiments were performed using the clear PVC tubing and the R6 $\alpha$  sensors. Each tube was filled with 60 ml of sterile TSB (with 0.25% fructose and no alginate) and inoculated with 0.6 ml of the same strain of *E. coli*. The mixture was gently stirred with a sterile pipette to disperse the bacteria throughout the media. AE monitoring and data collection was initiated on channels 4 and

6 both utilizing dB levels of 25, which continued for up to a minimum of 8 h. Two experiments on each strain of *E. coli* were performed, with hourly OD's obtained during both experiments.

### **3.9. Monitoring Cell Growth.**

**3.9.1 Optical Density.** OD's were obtained hourly on each bacterium up to at least 8 h. Prior to the start of each experiment, a 1 ml aliquot of sterile media was used as the blank. When obtaining a sample, the AE system was paused and the media was stirred with a sterile utensil for approximately 15 sec. A 1 ml aliquot was extracted using a sterile pipette and transferred to a crystal cuvette (StarnaCells, Inc., Atascadero, CA). The cuvette was placed in the spectrophotometer (Unico 1000 series, United Products & Instruments, Inc., Dayton, NJ) and OD's were determined at 600 nm. Once the sampling was complete, the AE monitoring system was resumed.

**3.9.2. Average OD at Time of ABSE Increase.** OD's were estimated at the time in which the ABSE increased and were compared for any differences between the strains of *E. coli*. Calculations were performed on two runs with each media type: TSB with fructose (with alginate) and TSB with fructose (without alginate), along with each strain of *E. coli* and on both channels.

**3.9.3. Gram Stain.** Individual experiments were performed by inoculating 60 ml of sterile TSB (0.25% dextrose and 1.1% alginate) with 0.6 ml of strain 5024 or 8237 and incubating within the 32°C chamber for 8 h. At the conclusion of each experiment, a 1 ml aliquot of solution was collected, from each chamber, using a sterile pipette and placed into separate microcentrifuge tubes. A sterile loop was used to obtain the bacteria

from the microcentrifuge tube, which was then gently mixed into a drop of sterile water that was placed in the middle of a glass slide. Each slide was held by a wooden clothespin and passed through the flame of a Bunsen burner until the solution was heat fixed. Gram stain reagents purchased from Fisher Scientific (Pittsburgh, PA) were used to perform a conventional gram stain method on each slide. Using a Micromaster™ microscope (Fisher Scientific, Pittsburgh, PA), each slide was observed as having pink rod shaped cells, indicative of *E. coli*.

**3.9.4. Enumeration of *E. coli* Cells.** Individual experiments were performed by inoculating 60 ml of sterile TSB (0.25% dextrose and 1.1% alginate) with 0.6 ml of strain 5024 or 8237 and incubating within the 32°C chamber for 8 h. At the conclusion of each experiment, a 1 ml aliquot of solution was collected, from each tube, using a sterile pipette and placed into separate microcentrifuge tubes. Serial dilutions of  $10^{-6}$ ,  $10^{-3}$  and  $10^{-2}$  were prepared for each strain. In duplicate, a 1ml aliquot from each dilution was obtained and placed directly in the center of a Petrifilm™ Aerobic Plate Count. A flat plastic spreader was placed atop Petrifilm™, thus allowing the solution to slowly disperse across the grid. The Petrifilm™ was incubated at 37°C for 24 h. The bacterial cell colonies were counted on each Petrifilm™ using a manual colony counter (AmericanOptical Darkfield, Quebec).

**3.9.5. Growth of *E. coli* Cells.** Individual experiments were performed for by inoculating 60 ml of sterile TSB (0.25% fructose and 1.1% alginate) with 0.6 ml of strain 5024 or 8237 or 8279. The solution of each strain was incubated in the chamber at 32°C. Serial dilutions of  $10^4$  and  $10^2$  were prepared for plate counts at 0, 60, 120 and 180 min. Assuming cell growth reached  $10^8$  by the 4<sup>th</sup> h, the solution was diluted to  $10^5$  and serial

dilutions of  $10^3$  and  $10^2$  were prepared for the plate counts at 240 and 300 min. A 1 ml aliquot from each dilution was obtained and placed directly in the center of a Petrifilm™ Aerobic Plate Count. A flat plastic spreader was placed atop Petrifilm™, thus allowing the solution to slowly disperse across the grid. The Petrifilm™ was incubated at 37°C for 24 h. Colony formation was measured in  $\text{Log}_{10}$  CFU/ml using a Flash & Go™ Plate Reader (IUL, Farmingdale, NY, USA). OD's were also obtained initially and hourly, for 5 h.

### **3.10. Visual Analysis of AE Data.**

OD's obtained from the experiments when each *E. coli* strain was grown in TSB with fructose (with and without alginate) were plotted against time using graphs designed in Microsoft® Office Excel 2007 and analyzed for differences between each strain and media type.

Means were calculated on the generated ABSE and PFRQ from each strain (grown in media with TSB with fructose: with and without alginate), on each channel, during all the experiments. For each strain, the two ABSE and PFRQ means that were the most similar were selected for visual analysis. The ABSE and PFRQ generated from those strains were plotted against time using graphs designed in Microsoft® Office Excel 2007 and analyzed for differences between the strains and media type.

### **3.11. Statistical Analysis of AE Data.**

Statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Significant differences between the *E. coli* strains, type of media, channel, sensor,

ABSE and PFRQ were determined by a Two Way Factorial ANOVA design which included a General Linear Model and Least Square Means. Differences were considered significant if the LS-Mean was ( $P < 0.05$ ).

Using the same analysis design, significant differences were also determined between *E. coli* species 5024 and 8237, when grown in TSB with fructose (without alginate) and the generated ABSE and PFRQ at different time periods, as well as, hourly and the generated PFRQ's at different kHz ranges. Differences were considered significant if the LS-Mean was ( $P < 0.05$ ).

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1. Changes in Media.

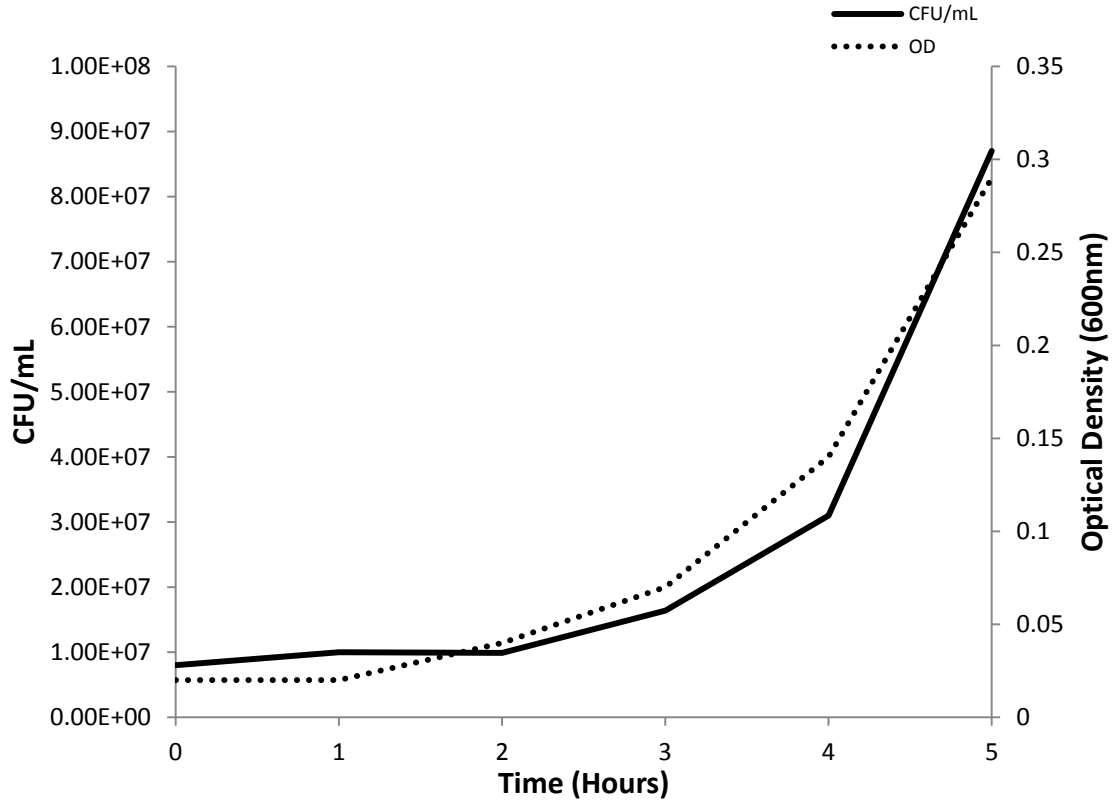
**4.1.1. Media with Dextrose.** At the conclusion of each experiment, using the medium, tryptic soy broth (TSB) with dextrose and alginate, bubbles were observed at the surface of the medium, indicating possible gas formation. A Durham test was performed on each strain of *E. coli* and all were negative (no visible CO<sub>2</sub> bubble in the inverted Durham tube). Since the results of the Durham tests were negative, another experiment was performed and once again, at the conclusion of the experiment, bubble formation was observed at the medium's surface. This was a cause for concern, since the formation of gas could produce unwanted sounds during acoustic emission (AE) monitoring (Stencel 2009). Gas formation was thought to be a by-product of dextrose fermentation and therefore a different carbohydrate source was selected for media preparation. Fructose was substituted for dextrose in the preparation of TSB media for the experiments that followed and the growth vessel was changed to clear PVC for ease of monitoring gas bubble formation. Although AE data was collected on media containing dextrose, no analysis was performed due to the possibility of gas formation, which could confound the interpretation of the data.

**4.1.2. Media with Fructose.** With the substitution of fructose for dextrose in the media, all tubes were visually inspected at the conclusion of each experiment for the formation of gas bubbles. The observations concluded there was no visible formation of

gas bubbles with the use of fructose. All of the experiments in this study were performed using media: TSB (0.25% fructose and 1.1% alginate) and TSB (0.25% fructose and no alginate).

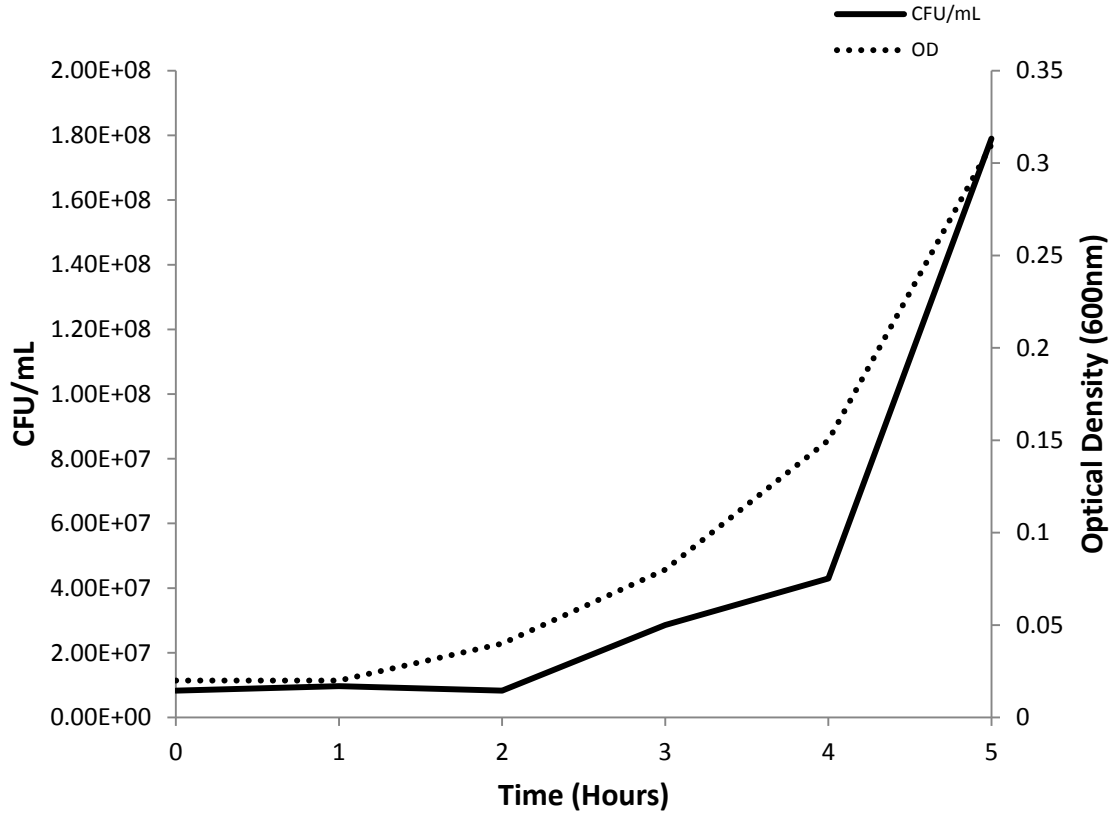
## 4.2. Cell Growth.

**4.2.1. Bacterial Strains.** The five hour growth curves indicate each strain of *E. coli* had initial cell counts of  $10^6$  CFU/ml (colony forming units (CFU)). **Figure 19-21** shows the five hour growth curves for each strain. Strain 8237 appeared to grow the fastest, producing a population of  $1.79 \times 10^8$  CFU/ml by the 5<sup>th</sup> h, followed by 5024 and 8279. The rate of cell growth was an important aspect to the AE signal detection. In this research, a 1% inoculum was added to the TSB medium. Since the culture added to the growth vessel had an initial population of  $10^8$  CFU/ml and once added to the growth medium would have been approximately  $10^6$  CFU/ml. Within the first 5 min after inoculation (**Figure 22**), the generated absolute energy (ABSE) from all three strains, whether grown in media with or without alginate, was 16% greater ( $P < 0.02$ ) than the background noise. Strain 5024 was the only strain that generated stronger ( $P < 0.02$ ) ABSE in media without alginate, than with alginate, shown in **Figure 23**. This was also true with peak frequency (PFRQ) observations, as all three strains generated frequencies 13% higher ( $P < 0.0001$ ) than the background noise, shown in **Figure 24**. Strains 8237 and 8279 grown in medium without alginate (**Figure 25**), generated higher frequencies ( $P < 0.0001$ ) than those grown in medium with alginate. Personal communication from Hicks (2010), discovered during previous AE testing that adding the hydrocolloid, alginate, to a medium, maintained the suspension of cells and prevented cells from

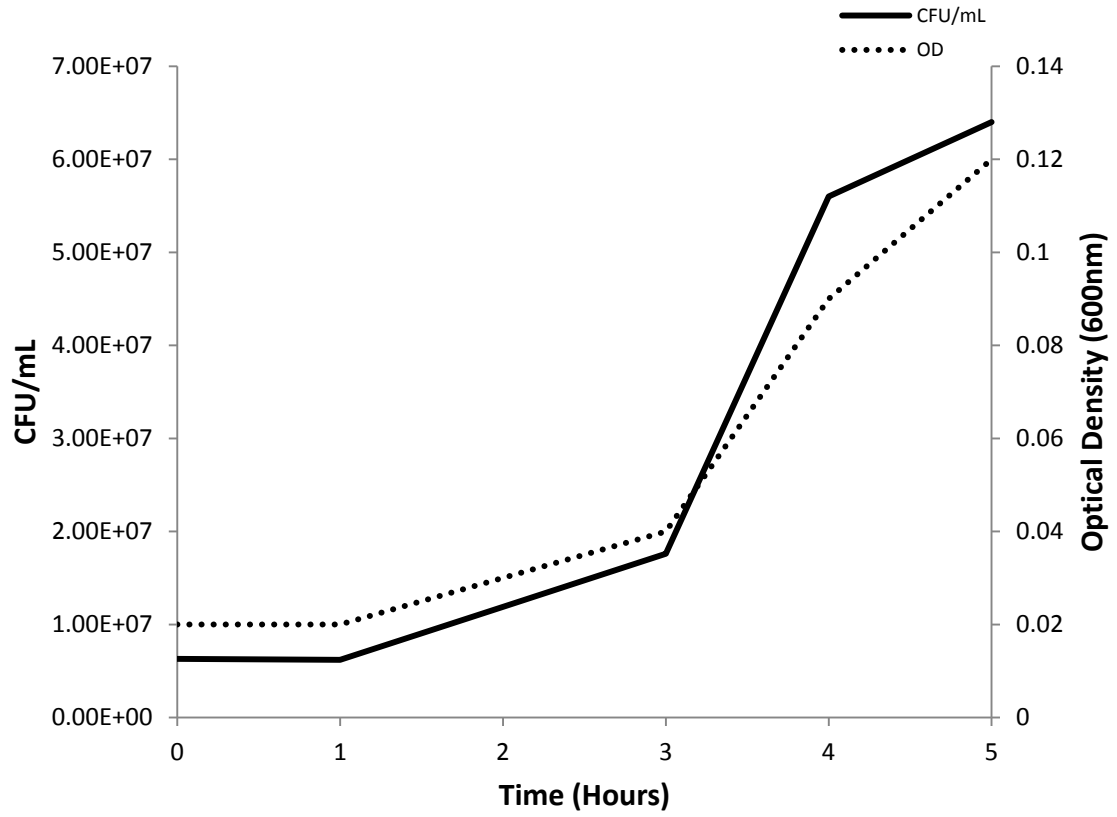


**Figure 19.** Five hour growth curve with hourly OD's for *E. coli* strain 5024.

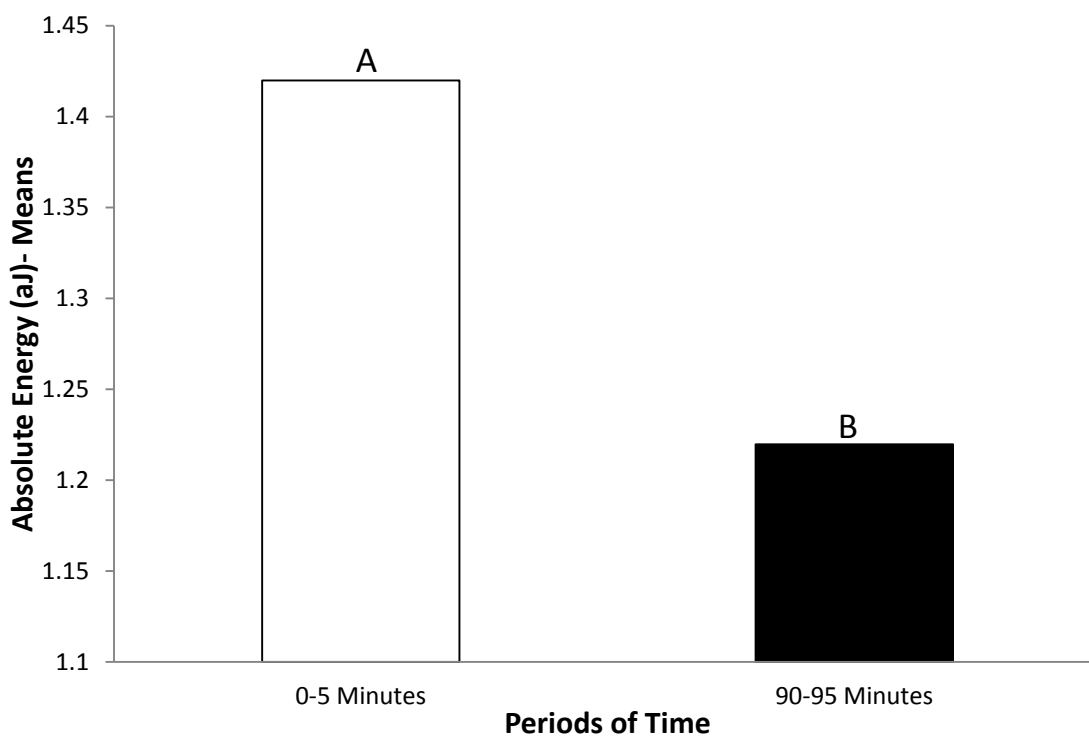




**Figure 20.** Five hour growth curve with hourly OD's for *E. coli* strain 8237.



**Figure 21.** Five hour growth curve with hourly OD's for *E. coli* strain 8279.

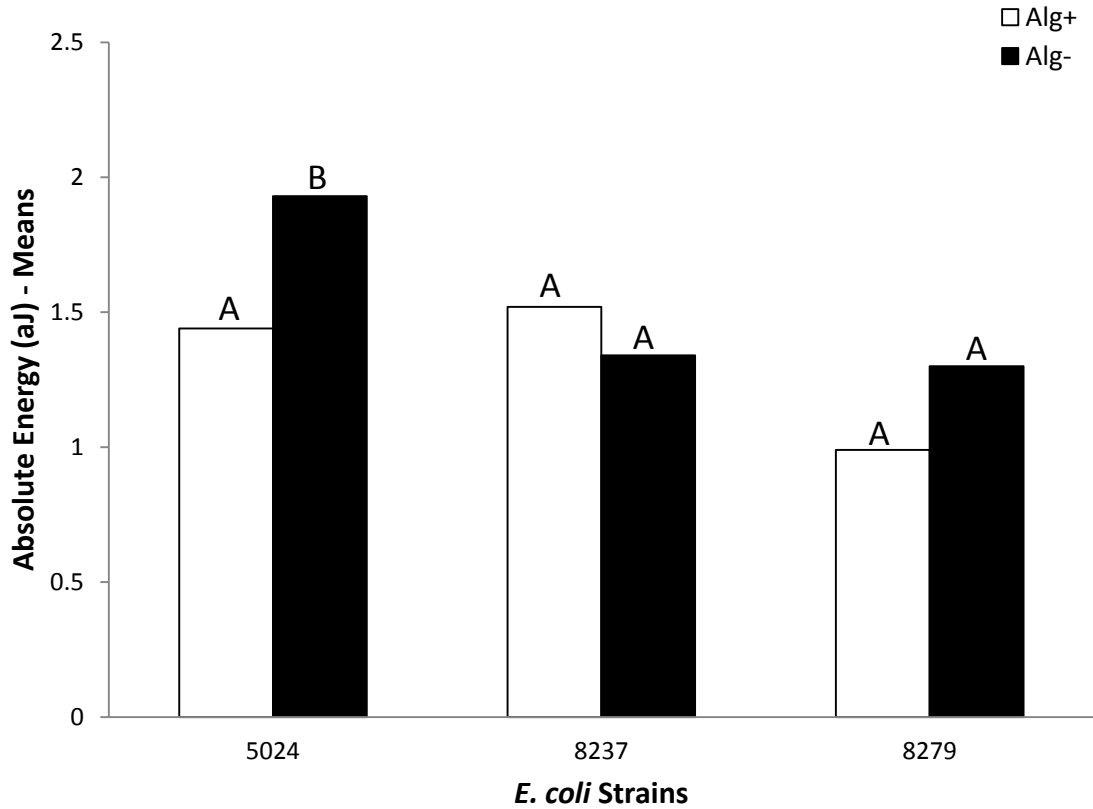


**Figure 22.** Comparison of the average ABSE between 0-5 min and 90-95 min (background noise) generated from all *E. coli* strains grown in media with and without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ).between each range of time.

\*Standard error = 0.06 for 0-5 min according to the least square mean.

\*Standard error = 0.06 for 90-95 min according to the least square mean.

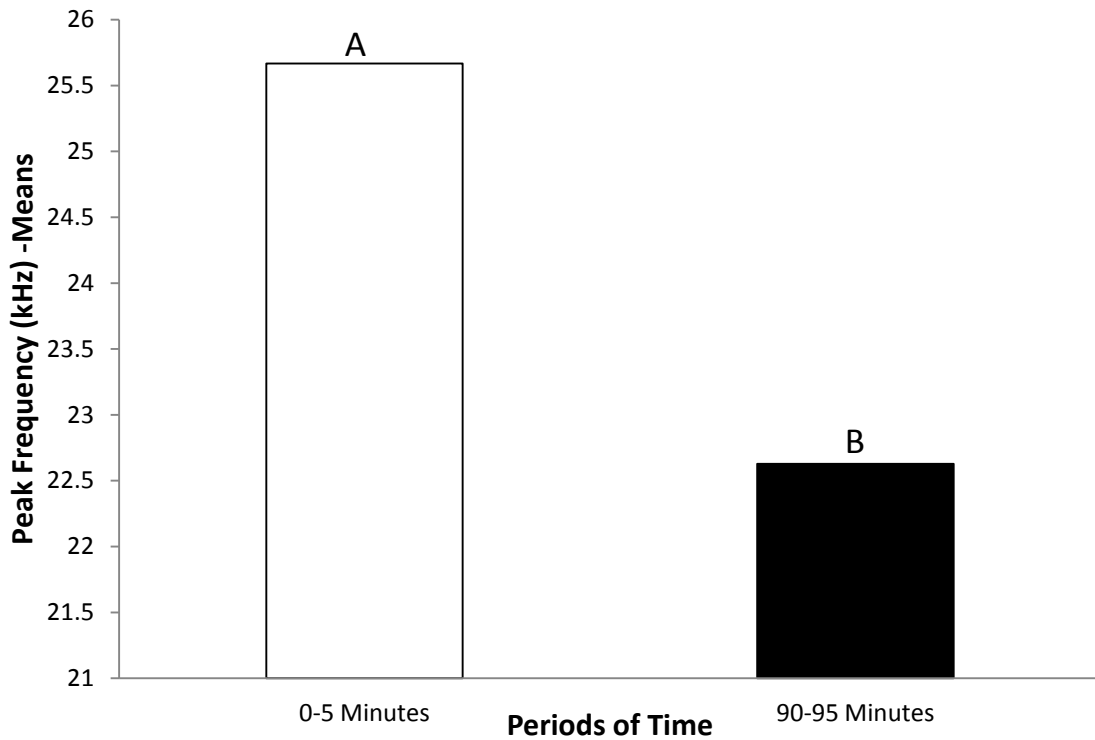


**Figure 23.** Comparison of the average ABSE between media type within each *E. coli* strain from 0-5 min.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ).within each *E. coli* strain

\*Standard error = 0.15 for strain 5024 with alginate according to the least square mean.

\*Standard error = 0.13 for strain 5024 without alginate according to the least square mean.

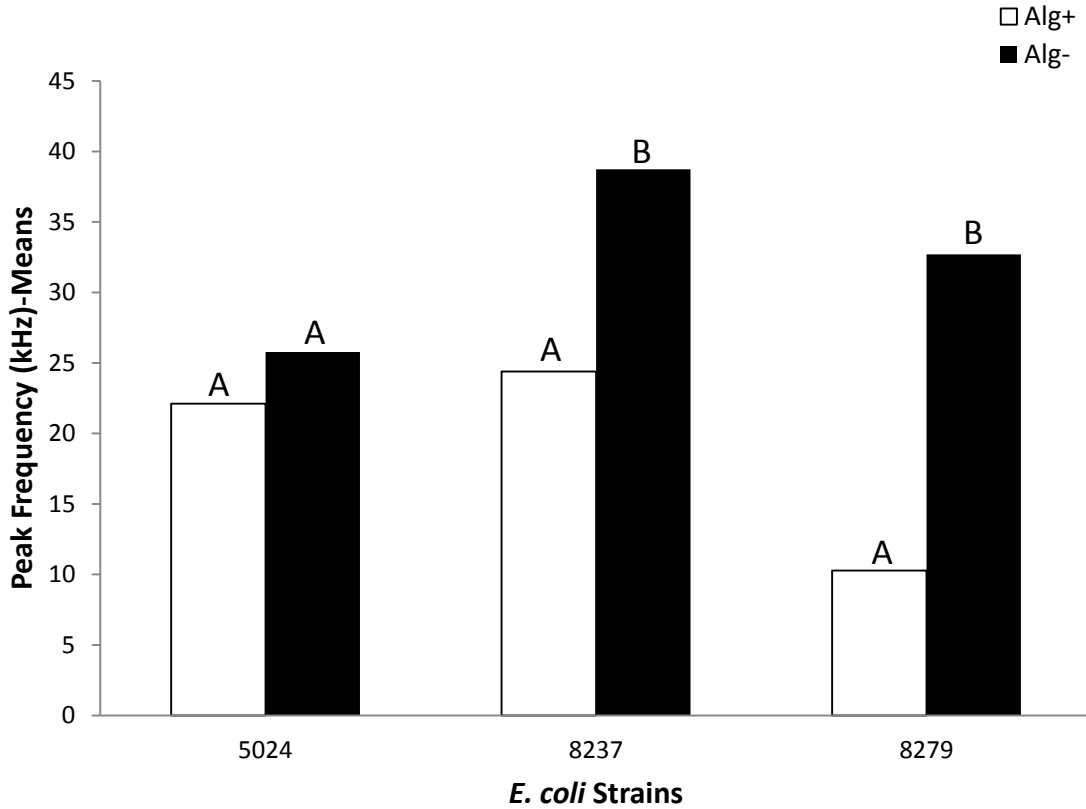


**Figure 24.** Comparison of the average PFRQ's between 0-5 min and 90-95min (background noise) generated from all *E. coli* strains grown in media with and without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ).between each range of time.

\*Standard error = 0.64 for 0-5 min according to the least square mean.

\*Standard error = 0.65 for 90-95 min according to the least square mean.



**Figure 25.** Comparison of the average PFRQ's between media type within each *E. coli* strain from 0-5 min.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ).within each *E. coli* strain

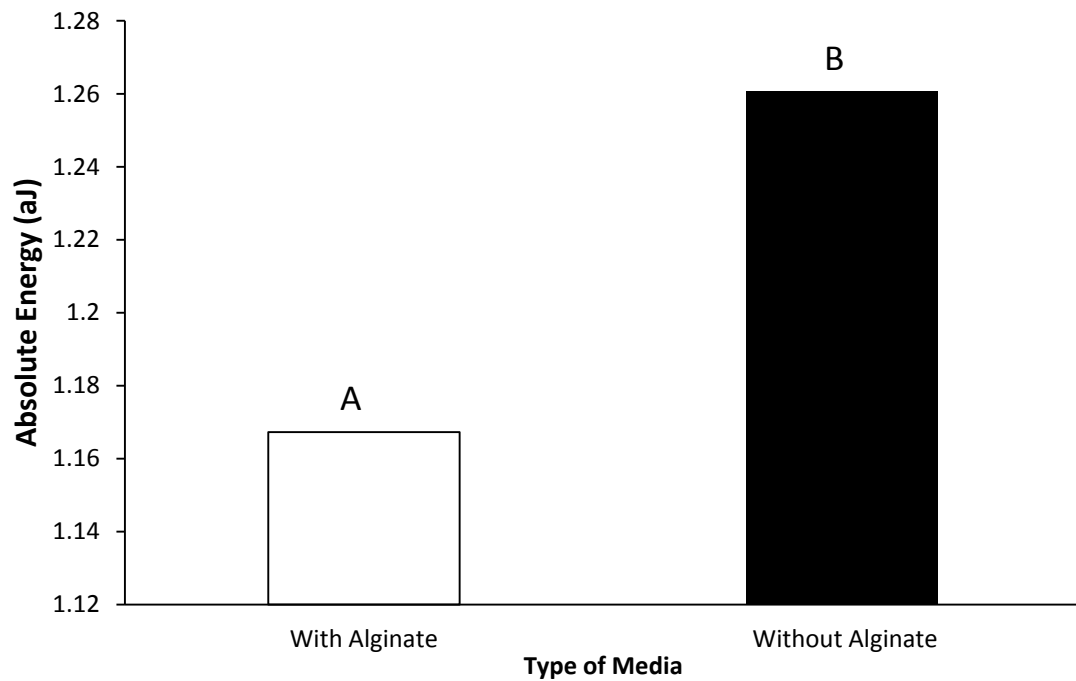
\*Standard Error = 1.78 for strain 8237 with alginate according to the least square mean.  
 \*Standard Error = 1.39 for strain 8237 without alginate according to the least square mean.

\*Standard error = 1.42 for strain 8279 with alginate according to the least square mean.  
 \*Standard error = 1.45 for strain 8279 without alginate according to the least square mean.

settling atop the AE sensor. This data supports their discovery, as evidenced by the medium without alginate allowed faster settling of cells atop the sensor, thus producing a stronger signal.

Observations from 5 to 100 min revealed the generated ABSE were strongest ( $P < 0.03$  and  $P < 0.0001$ ) from all strains grown in medium without alginate (**Figure 26**) and within 5-15 min post inoculation (**Figure 27**), respectively. The generated ABSE from 5-100 min for strains 5024 and 8237 were significantly stronger ( $P < 0.0001$ ) than strain 8279, shown in **Figure 28**. The generated PFRQ was also significantly higher ( $P < 0.0001$ ) in all strains grown in medium without alginate (**Figure 29**) and within 5-15 min post inoculation (**Figure 30**). Once again, strains 5024 and 8237 generated higher frequencies ( $P < 0.0001$ ) than strain 8279 from 5-100 min, shown in **Figure 31**. The generated PFRQ's from strain 8237 differed significantly ( $P < 0.0001$ ) from strain 5024 and continued to generate the highest frequencies throughout the first 100 min after inoculation. It is important to understand that prior to the inoculation into new medium; the bacteria cultures were in the stationary phase and within the first 5 min and continuously up to 100 min post inoculation, their cellular activity generated observable ABSE and PFRQ's. Indeed, all three strains of *E. coli* generated ABSE and PFRQ's that differed from each other. Thus, it was important to understand whether these differences in AE were due to differing cell numbers or cellular activities.

Two of the *E. coli* strains selected for these experiments were characteristically quite similar. Strain 5024 is the parent strain of the mutant strain 8237. If both strains could be grown at similar rates, then the ABSE and PFRQ differences would be due to differences in their cellular activity. Strain 8279 was genetically quite different than



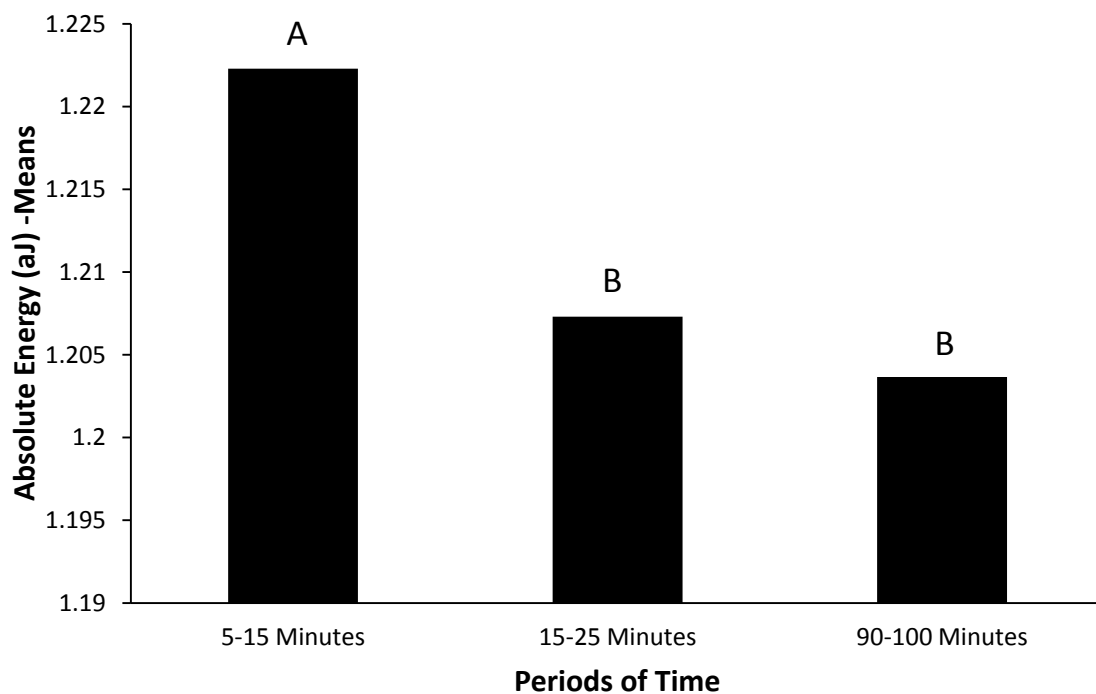
**Figure 26.** Comparison of the average ABSE between media type from 5-100 min.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ),between media type.

\*Standard Error = 0.0042 for media with alginate according to the least square mean.

\*Standard Error = 0.0038 for media without alginate according to the least square mean.





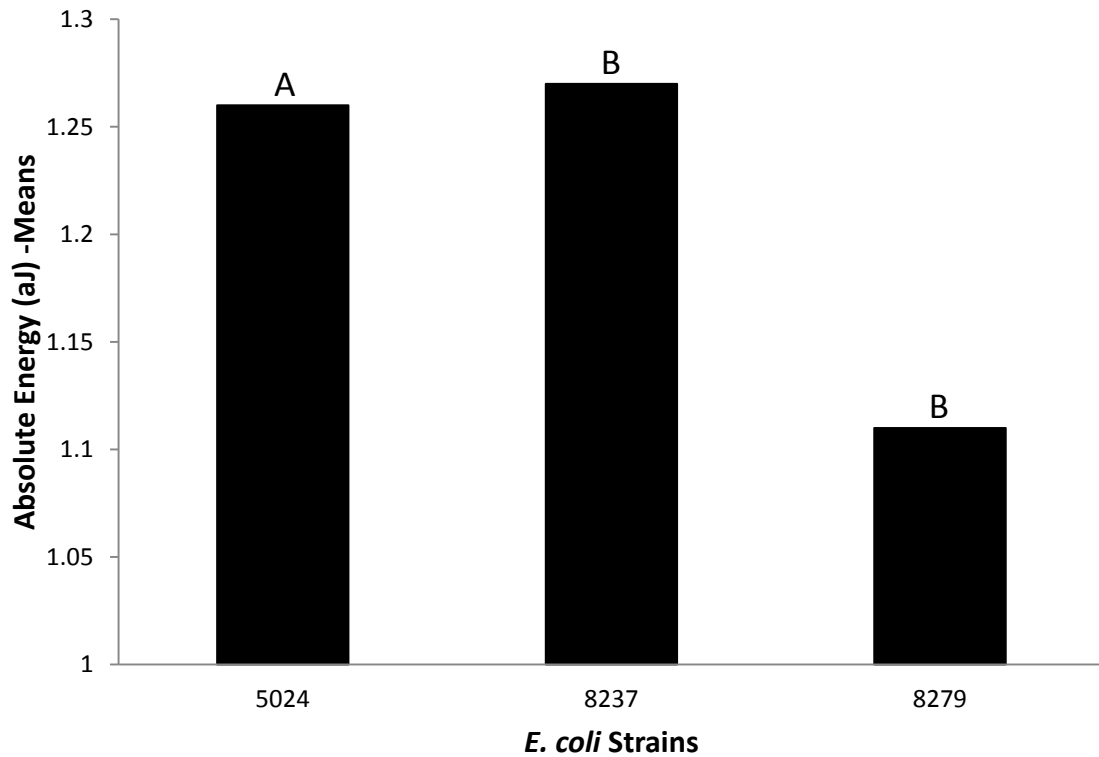
**Figure 27.** Comparison of the average ABSE generated by all *E. coli* strains, grown in media with and without alginate, between select periods of time from 5-100 min.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ).between each time period.

\*Standard Error = 0.00487 for 5-15 min according to the least square mean.

\*Standard Error = 0.00484 for 15-25 min according to the least square mean.

\*Standard Error = 0.00497 for 90-100 min according to the least square mean.



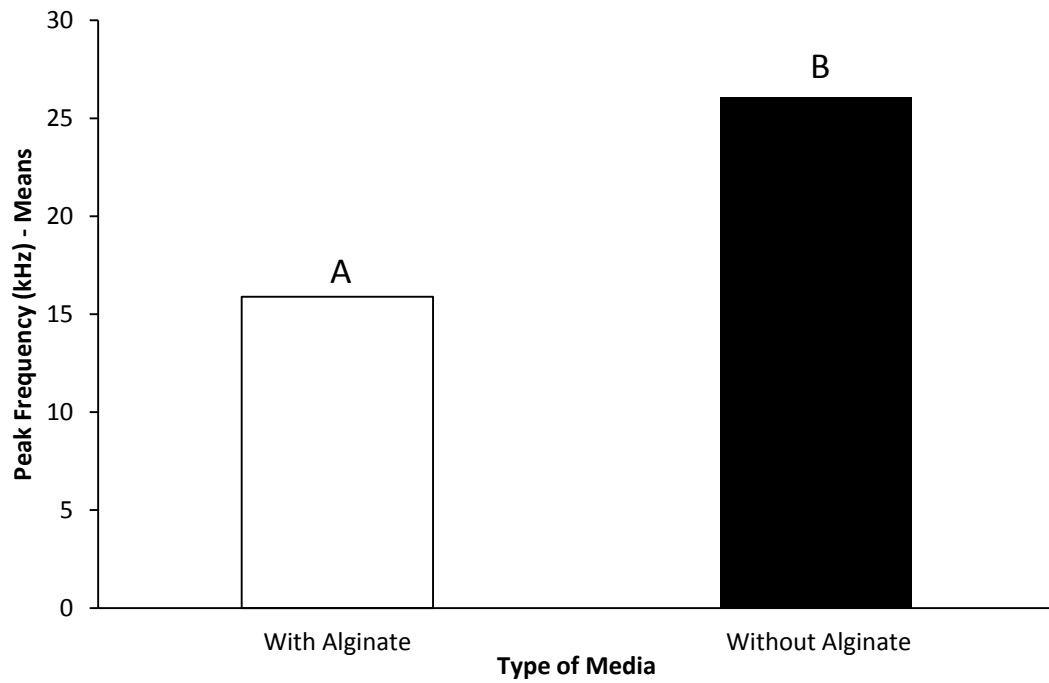
**Figure 28.** Comparison of the average ABSE between each *E. coli* strain, grown in media with and without alginate, from 5-100 min.

ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.0052 for strain 5024 according to the least square mean.

\*Standard Error = 0.0049 for strain 8237 according to the least square mean.

\*Standard Error = 0.0045 for strain 8279 according to the least square mean.

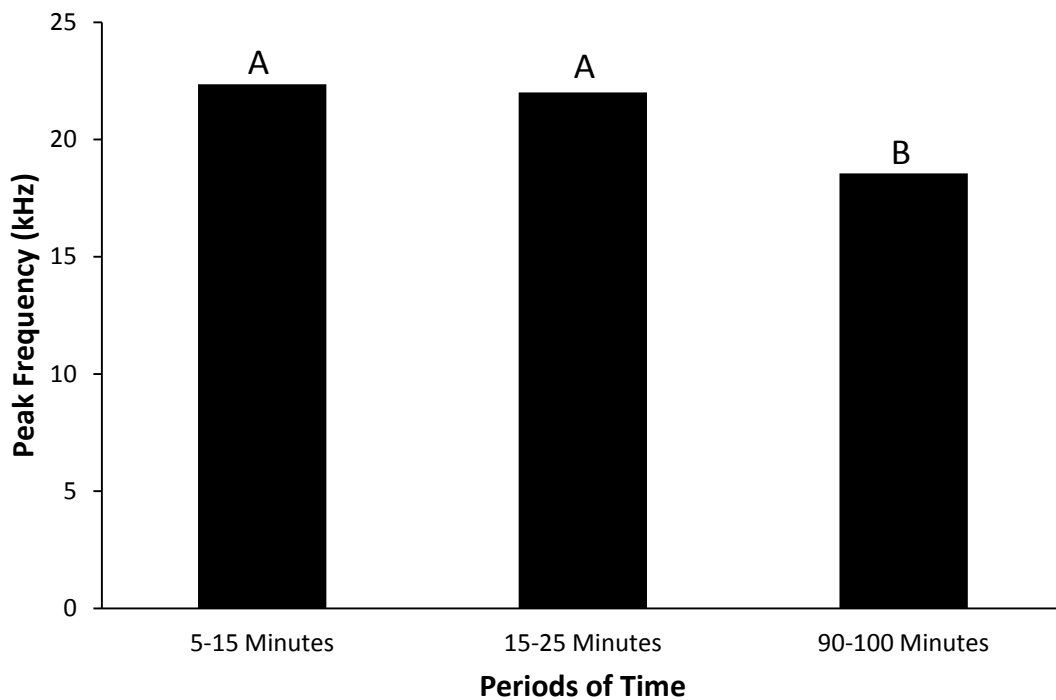


**Figure 29.** Comparison of the average PFRQ's between media type from 5-100 min.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ).between media type.

\*Standard Error = 0.3667 for media with alginate according to the least square mean.

\*Standard Error = 0.3254 for media without alginate according to the least square mean.



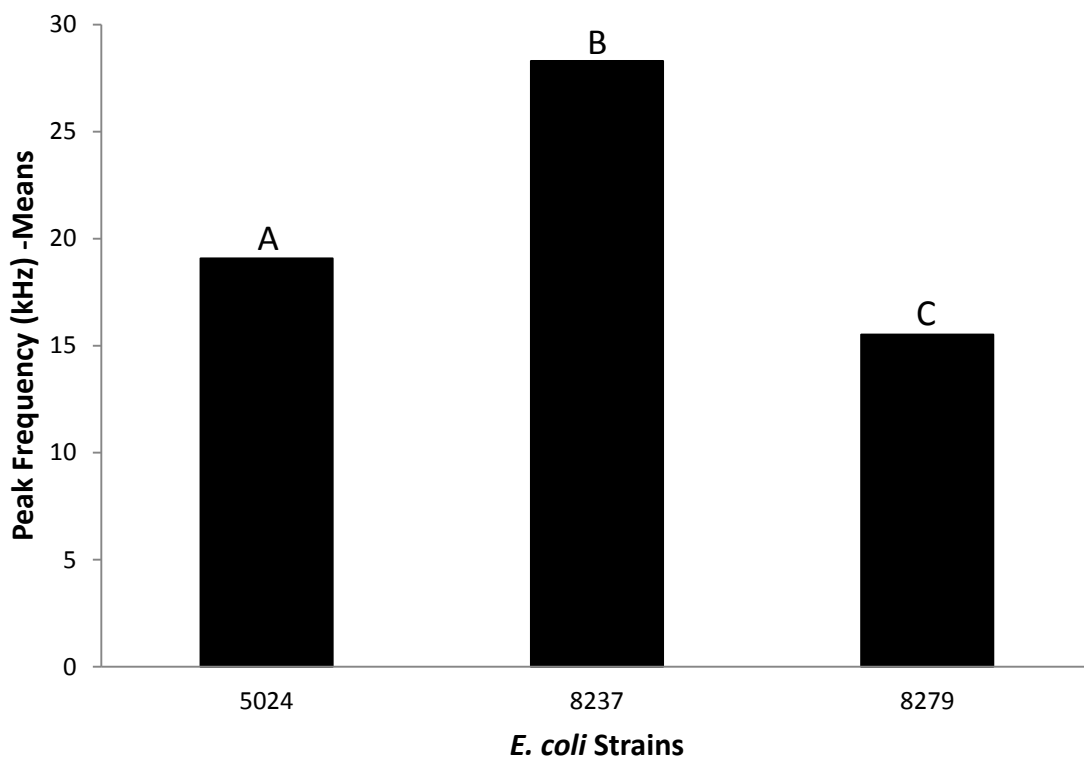
**Figure 30.** Comparison of the average PFRQ's generated by all *E. coli* strains, grown in media with and without alginate, between select periods of time from 5-100 min.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ), between each time period.

\*Standard Error = 0.4223 for 5-15 min according to the least square mean.

\*Standard Error = 0.4201 for 15-25 min according to the least square mean.

\*Standard Error = 0.4312 for 90-100 min according to the least square mean.



**Figure 31.** Comparison of the average PFRQ's between each *E. coli* strain, grown in media with and without alginate, from 5-100 min.

ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each strain.

\*Standard Error = 0.4471 for strain 5024 according to the least square mean.

\*Standard Error = 0.4283 for strain 8237 according to the least square mean.

\*Standard Error = 0.3966 for strain 8279 according to the least square mean.

strains 5024 and 8237 and would therefore be expected to possess a different growth rate and possible cellular activities.

**4.2.2 Enumeration of *E. coli* Strains 5042 and 8237.** Tables 4 and 5 list the CFU's, average CFU's and the average number of cells/mL for each dilution factor and strains 5024 and 8237 at the end of the growth period. Both strains of *E. coli* showed optimal growth, as evidenced by a population of  $10^9$  CFU/ml after 8-9 h of incubation in medium with alginate. However, the mutant strain 8237 grew slightly faster than the parent strain 5024, which was unexpected. By the time the parent strain 5024 and the mutant strain 8237 reached  $10^8$  CFU/ml, strain 5024 averaged a 0.1 log lower CFU than strain 8237. Although this was only a minor difference in the growth rate, the parent strain 5024 possesses the *rpoS396* gene that is the primary regulator of stationary phase genes, which suggests it may limit the rate of metabolism and cell division, as well as, what occurs during the stationary phase, thus accounting for the slightly slower rate of growth.

**4.2.3. Average OD at Time of ABSE Increase.** Observations revealed the average time in which ABSE increased rapidly for strains 5024 and 8237, when grown in media with and without alginate (Tables 6 and 7) occurred between 5.63 h and 5.90 h, however, in strain 8279 the average time extended from 7.04 h to 7.36 h. Note that when all three strains were grown in medium with alginate, the OD's were essentially the same at the time the ABSE began to increase, suggesting that all strains were at the same cell numbers. Average OD's at the time of the ABSE increase ranged from 0.57 to 0.59, for all *E. coli* strains grown in alginate. However, when alginate was not present, the OD's varied at the point of the ABSE increase. Strains grown without alginate had average

**Table 4.** Enumeration of *E. coli* 5024 at different dilution factors.

Dilution Factor	Number of Bacterial Colonies (CFU's)							
	5024 Ch4	5024 Ch4	Avg # CFU	Avg # cells/ml	5024 Ch6	5024 Ch6	Avg # CFU	Avg # cells/ml
1:10 <sup>-3</sup>	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
1:10 <sup>-6</sup>	TNTC	TNTC	TNTC	TNTC	7.4x10 <sup>8</sup>	8.2x10 <sup>8</sup>	7.8x10 <sup>8</sup>	7.8x10 <sup>8</sup>
1:10 <sup>-7</sup>	4.8x10 <sup>9</sup>	4.6x10 <sup>9</sup>	4.7x10 <sup>9</sup>	4.7x10 <sup>9</sup>	1.0x10 <sup>9</sup>	1.0x10 <sup>9</sup>	1.0x10 <sup>9</sup>	1.0x10 <sup>9</sup>

TNTC=Too numerous to count

CFU=Colony forming units

Ch =Channel

**Table 5.** Enumeration of *E. coli* 8237 at different dilution factors.

Dilution Factor	Number of Bacterial Colonies (CFU's)							
	8237 Ch4	8237 Ch4	Avg # CFU	Avg # cells/ml	8237 Ch6	8237 Ch6	Avg # CFU	Avg # cells/ml
1:10 <sup>-3</sup>	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
1:10 <sup>-6</sup>	TNTC	TNTC	TNTC	TNTC	6.8x10 <sup>8</sup>	4.0x10 <sup>8</sup>	5.4x10 <sup>8</sup>	5.4x10 <sup>8</sup>
1:10 <sup>-7</sup>	6.2x10 <sup>9</sup>	5.6x10 <sup>9</sup>	5.9x10 <sup>9</sup>	5.9x10 <sup>9</sup>	4.2x10 <sup>9</sup>	3.8x10 <sup>9</sup>	4.0x10 <sup>9</sup>	4.0x10 <sup>9</sup>

TNTC=Too numerous to count

CFU=Colony forming units

Ch =Channel

**Table 6.** The time and estimated OD at ABSE increase (and averages) for all *E. coli* strains grown in medium with alginate.

<i>E. coli</i> Strains (with Alginate)	Channel	Time of ABSE Increase (Hours)	Estimated OD at Time of ABSE Increase	Average Time of ABSE Increase (Hours)	Average OD at Time of ABSE Increase*
5024	4	5.27	0.61	5.64	0.57
	3	5.44	0.76		
	4	6.26	0.55		
	3	5.60	0.37		
8237	4	7.22	0.73	5.63	0.59
	3	5.08	0.27		
	4	4.07	0.43		
	6	6.15	0.93		
8279	4	7.78	0.44	7.36	0.57
	3	6.87	0.42		
	4	7.51	0.71		
	6	7.28	0.71		

\* = No significant difference



**Table 7.** The time and estimated OD at ABSE increase (and averages) for all *E. coli* strains grown in medium without alginate.

<i>E. coli</i> Strains (No Alginate)	Channel	Time of ABSE Increase (Hours)	Estimated OD at Time of ABSE Increase	Average Time of ABSE Increase (Hours)	Average OD at Time of ABSE Increase
5024	4	5.12	0.55	5.90	0.73
	6	5.11	0.72		
	4	7.22	0.97		
	6	6.13	0.67		
8237	4	6.13	0.92	5.68	0.93 <sup>†</sup>
	6	5.14	0.86		
	4	5.83	0.99		
	6	5.63	0.98		
8279	4	7.12	0.77	7.04	0.63 <sup>†</sup>
	6	6.08	0.47		
	4	7.96	0.57		
	6	6.98	0.69		

<sup>†</sup> $P < 0.05$

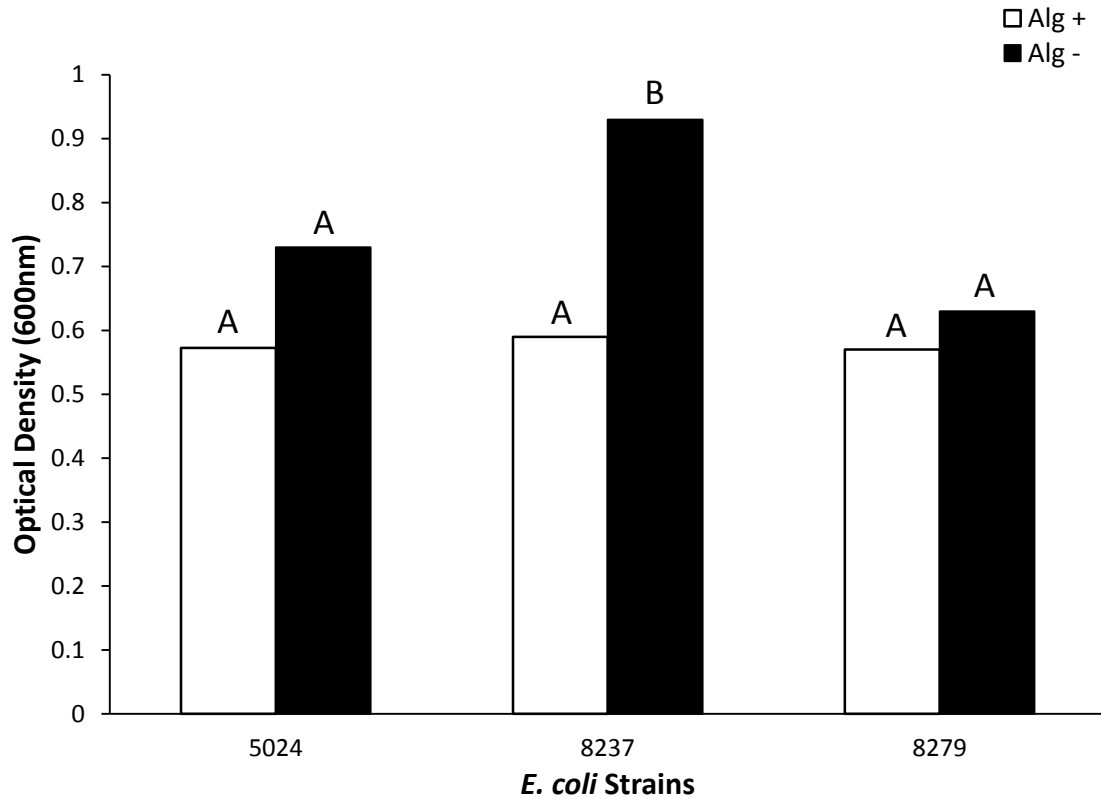
OD's ranging from 0.63 to 0.93. These findings suggest that alginate may have had a slight inhibitory effect on *E. coli* growth, even though it worked extremely well in maintaining cell suspension.

Strain 8237 was the only strain that significantly differed ( $P < 0.02$ ) between OD's at the point of the ABSE increase, in media with and without alginate, shown in **Figure 32**. In medium without alginate, strain 8279 differed significantly ( $P < 0.05$ ) from strain 8237, shown in **Figure 33**. This may be due to differences in the non-settling characteristics of strain 8279 from the other strains. It may also be indicative that the medium with alginate maintained the suspension of more cells, which prevented fewer cells from falling to the bottom and settling on the face of the acoustic sensor, thus giving a lower ABSE signal.

### 4.3. AE Observations.

**4.3.1. OD Versus Time.** OD's were obtained from growth tubes by channel, for each strain of *E. coli*, during two separate experiments. Each strain of *E. coli* was observed to have growth, as shown in **Figures 34-36**. All *E. coli* strains had a lag phase for the first 3 to 4 h. Generally, strains 5024 and 8237 reached their stationary phases by the 9<sup>th</sup> h. Strain 8279, which grew slower, was still in its log phase by the 9<sup>th</sup> h. The effect of channel on strain growth was not significant, which was expected.

**4.3.2. ABSE and PFRQ of Water and Media Versus Time.** The ABSE and PFRQ of water and media were obtained to establish baseline "noise" levels. The ABSE baseline levels in water, on channels 3 and 4 (**Figure 37**), were between 1 and 1.5 aJ's



**Figure 32.** Comparison of the average OD's at the time of the ABSE increase between media type within each *E. coli* strain.

ABC=Means with different letters are significantly different ( $P < 0.05$ ).between media type.

\*Standard Error = 0.57 for strain 5024 grown in media with alginate according to the least square mean.

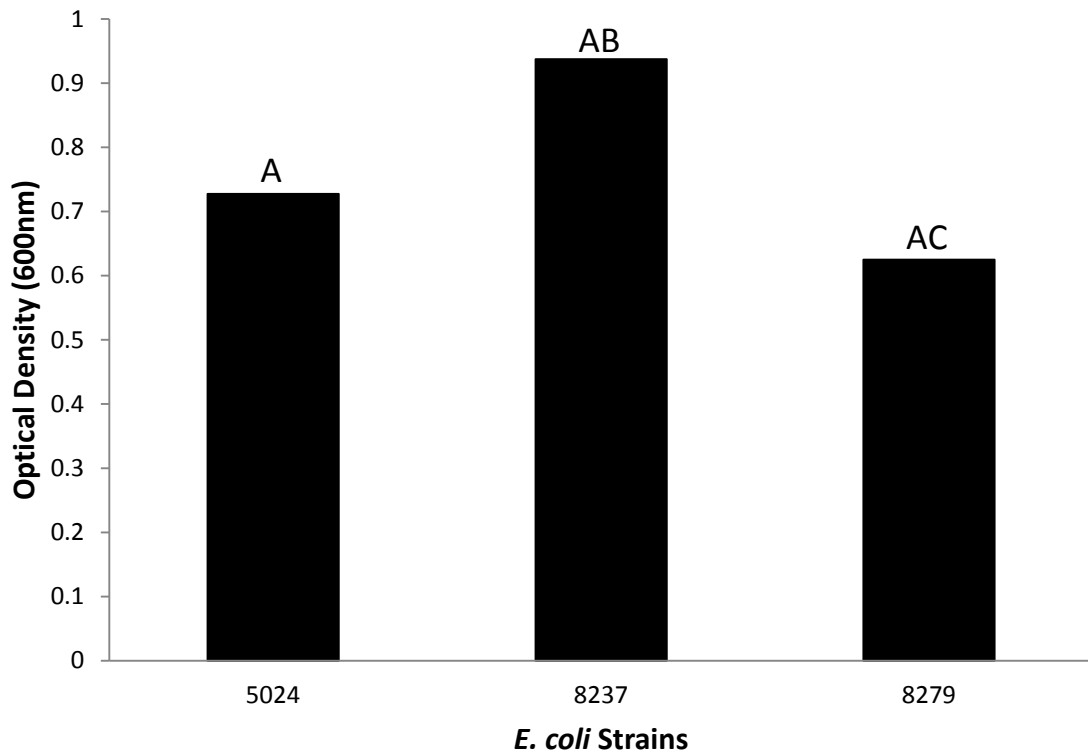
\*Standard Error = 0.73 for strain 5024 grown in media without alginate according to the least square mean.

\*Standard Error = 0.59 for strain 8237 grown in media with alginate according to the least square mean.

\*Standard Error = 0.94 for strain 8237 grown in media without alginate according to the least square mean.

\*Standard Error = 0.57 for strain 8279 grown in media with alginate according to the least square mean.

\*Standard Error = 0.63 for strain 8279 grown in media without alginate according to the least square mean.



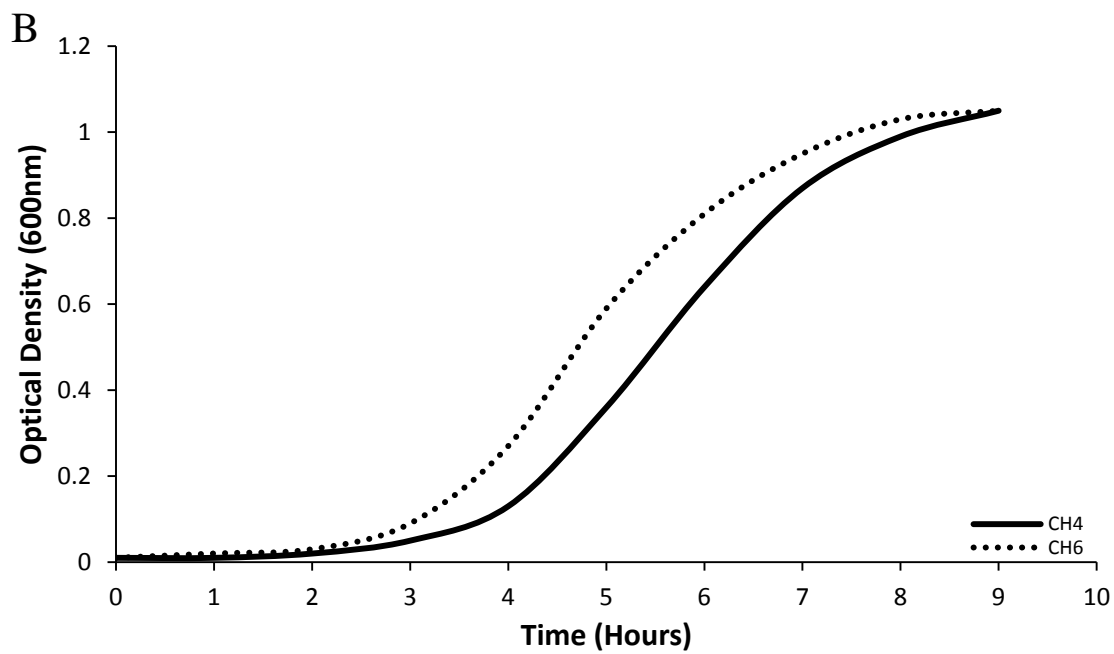
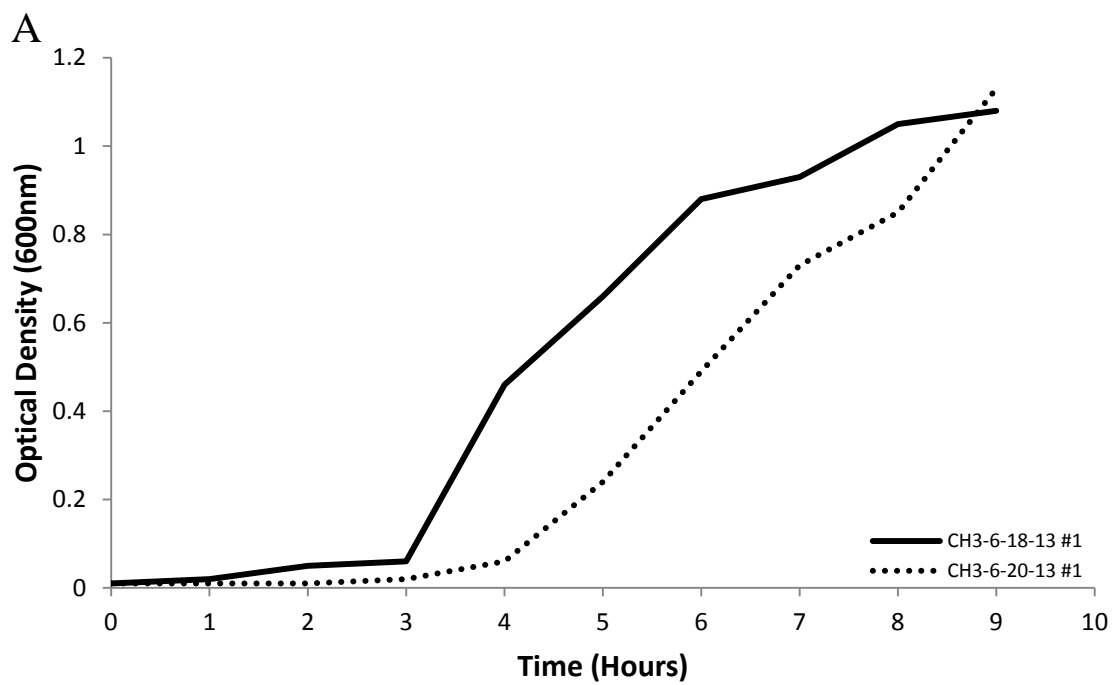
**Figure 33.** Comparison of the average OD's at the time of the ABSE increase between each *E. coli* strain grown in medium without alginate.

ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain

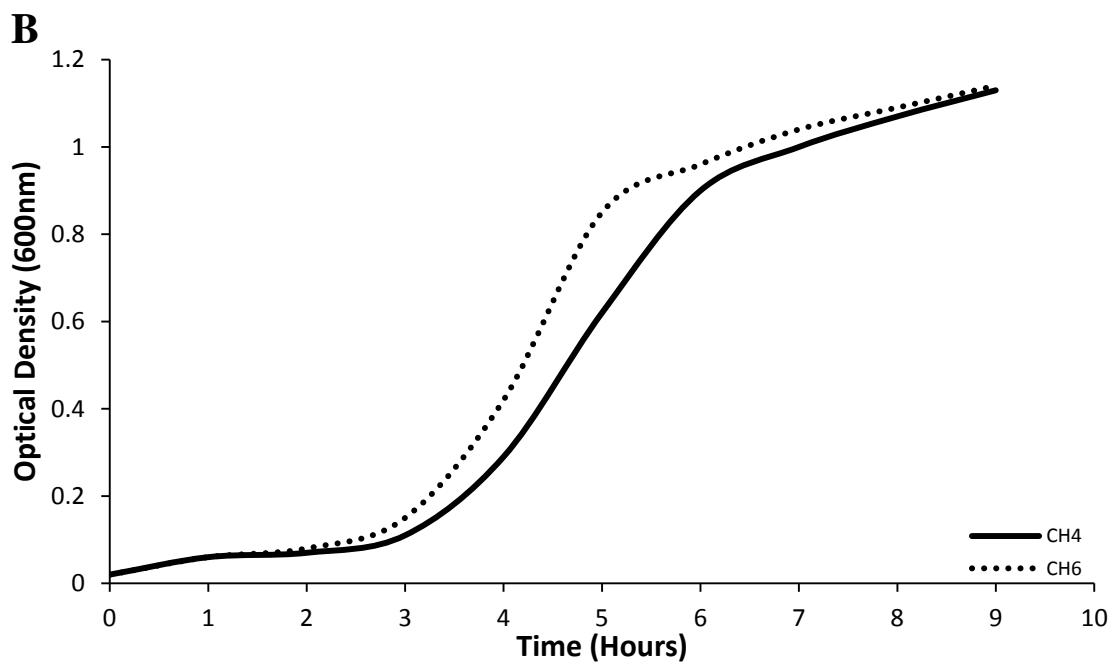
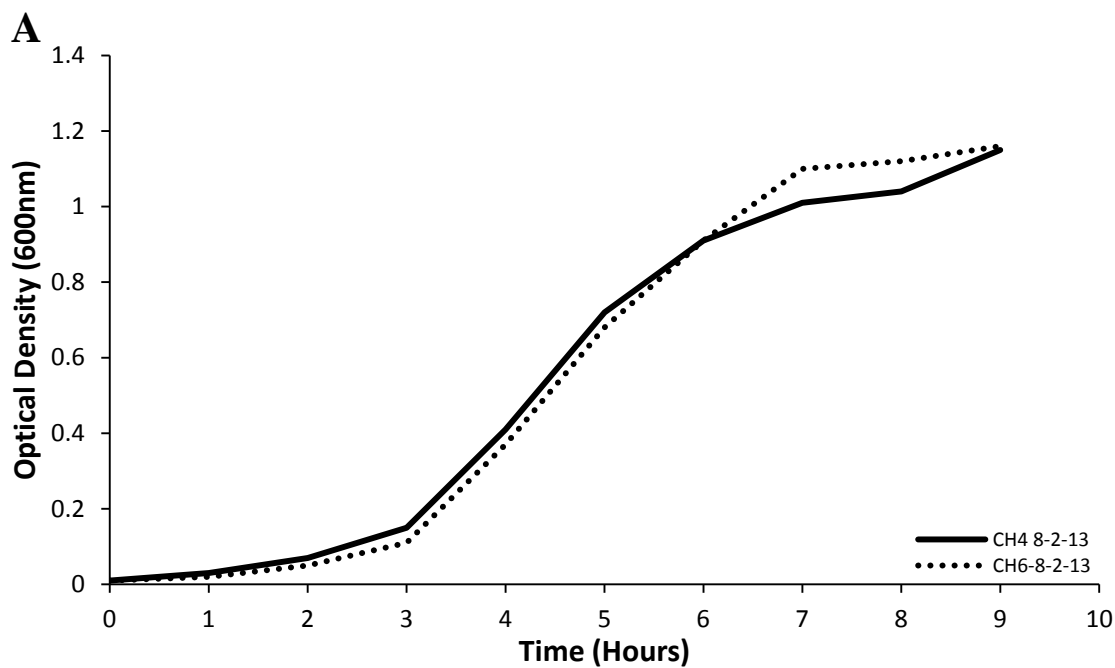
\*Standard Error = 0.73 for strain 5024 according to the least square mean.

\*Standard Error = 0.94 for strain 8237 according to the least square mean.

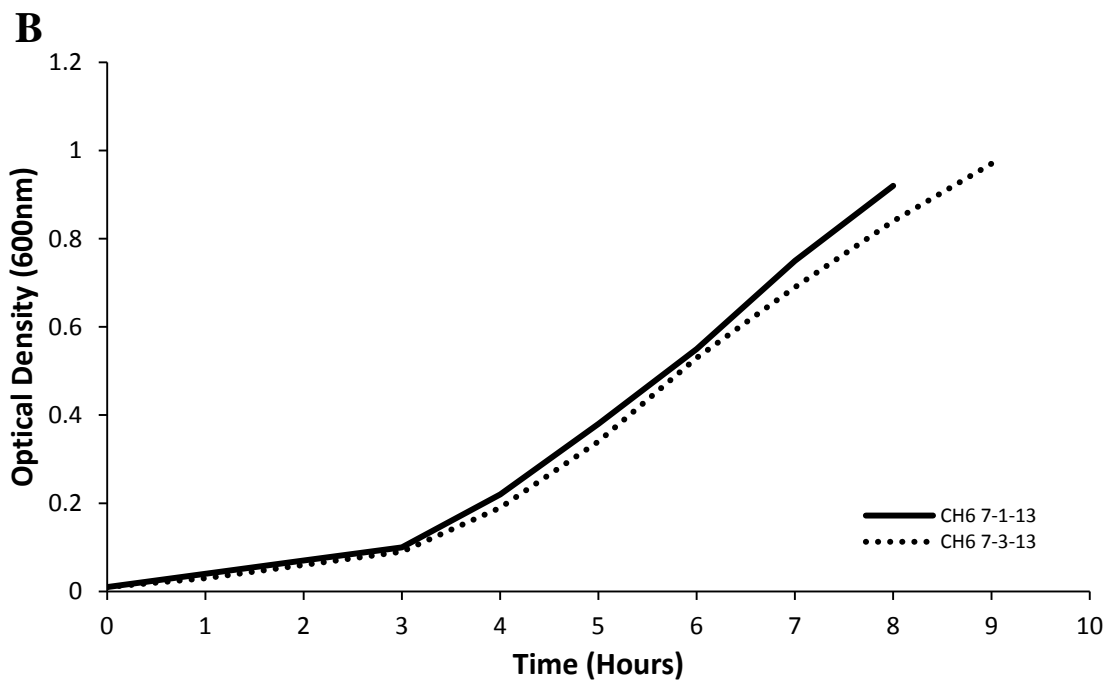
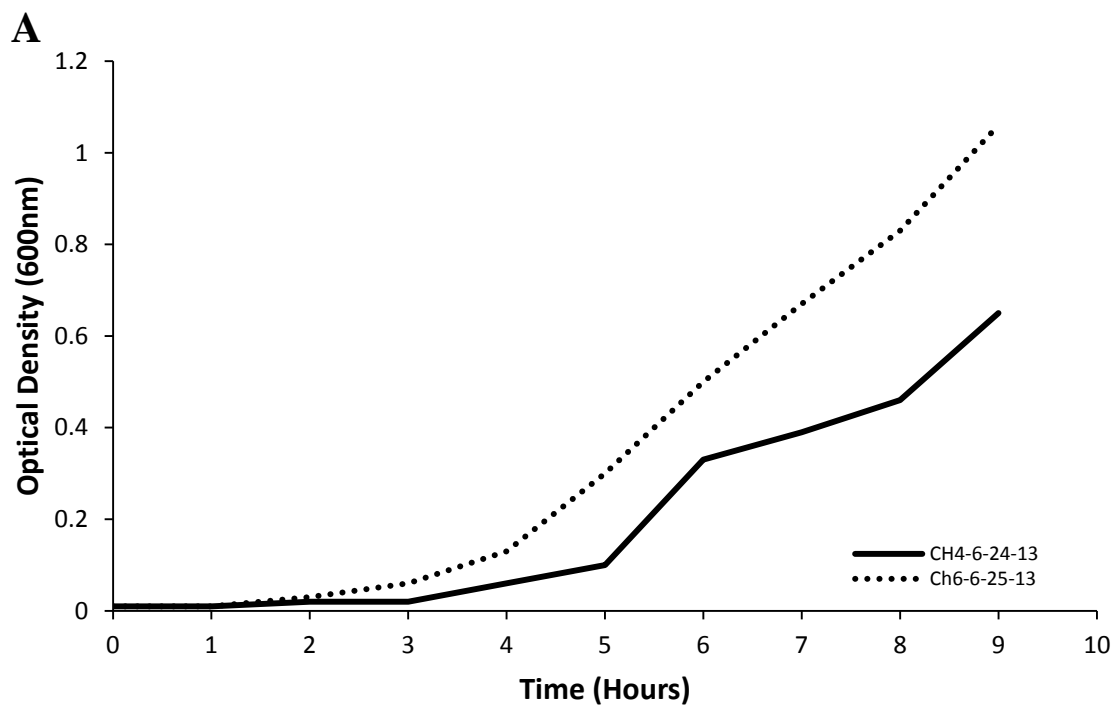
\*Standard Error = 0.63 for strain 8279 according to the least square mean.



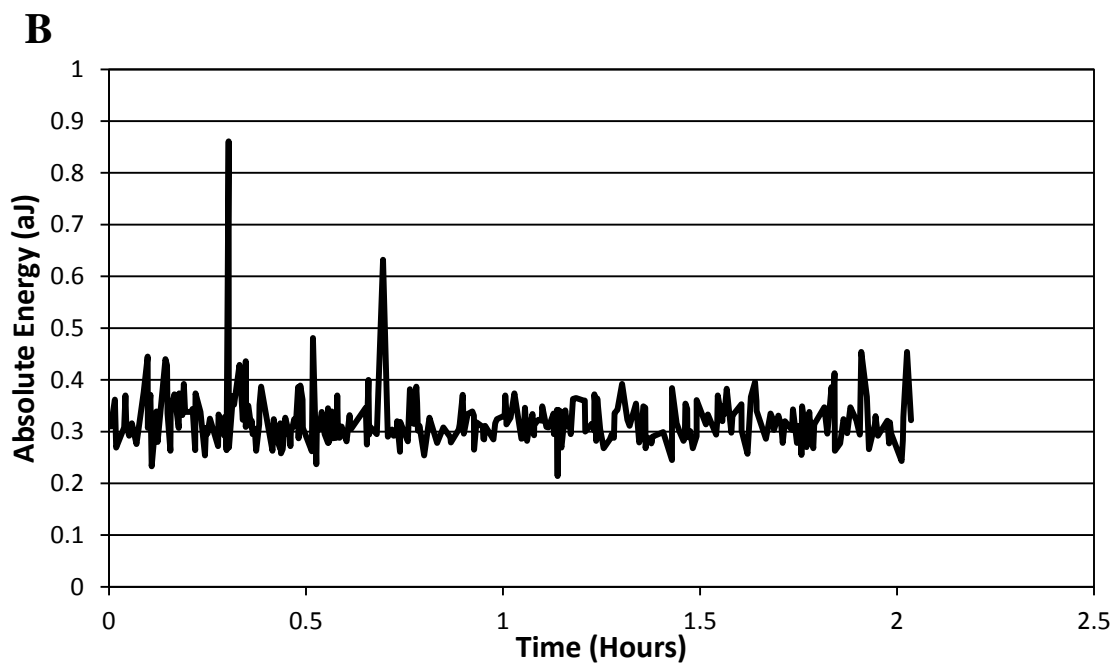
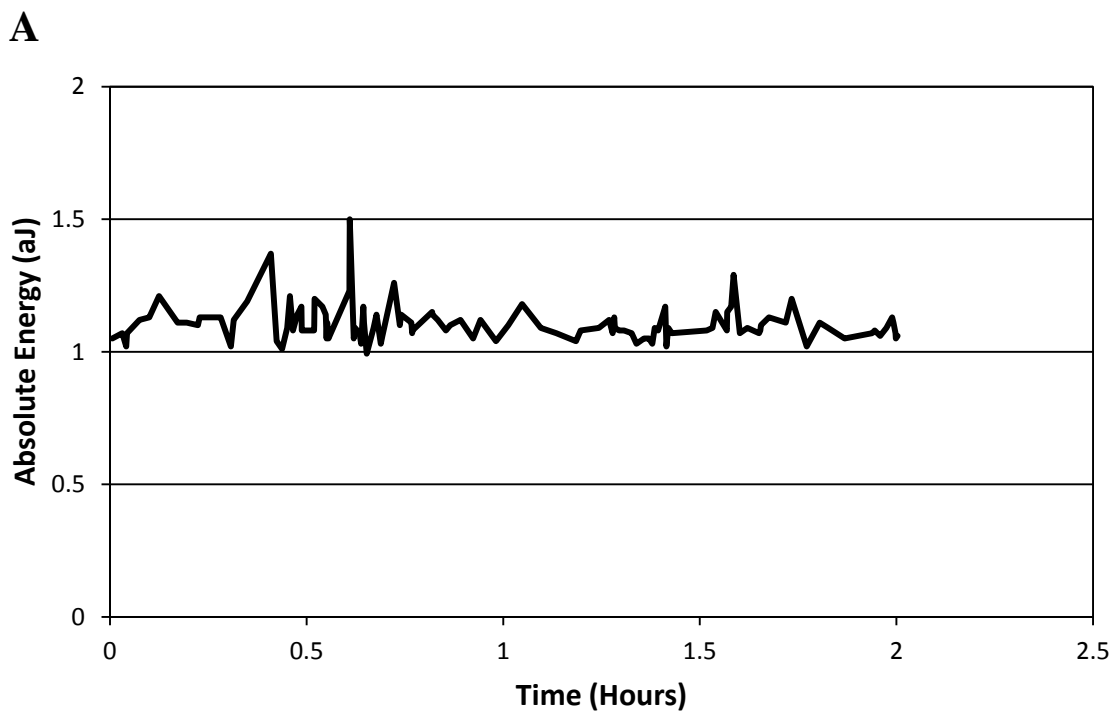
**Figure 34.** (A) OD's versus time with 5024 strains grown in media with alginate and (B) without alginate.



**Figure 35.** (A) OD's versus time with 8237 strains grown in media with alginate and (B) without alginate.



**Figure 36.** (A) OD's versus time with 8279 strains grown in media with alginate and (B) without alginate.



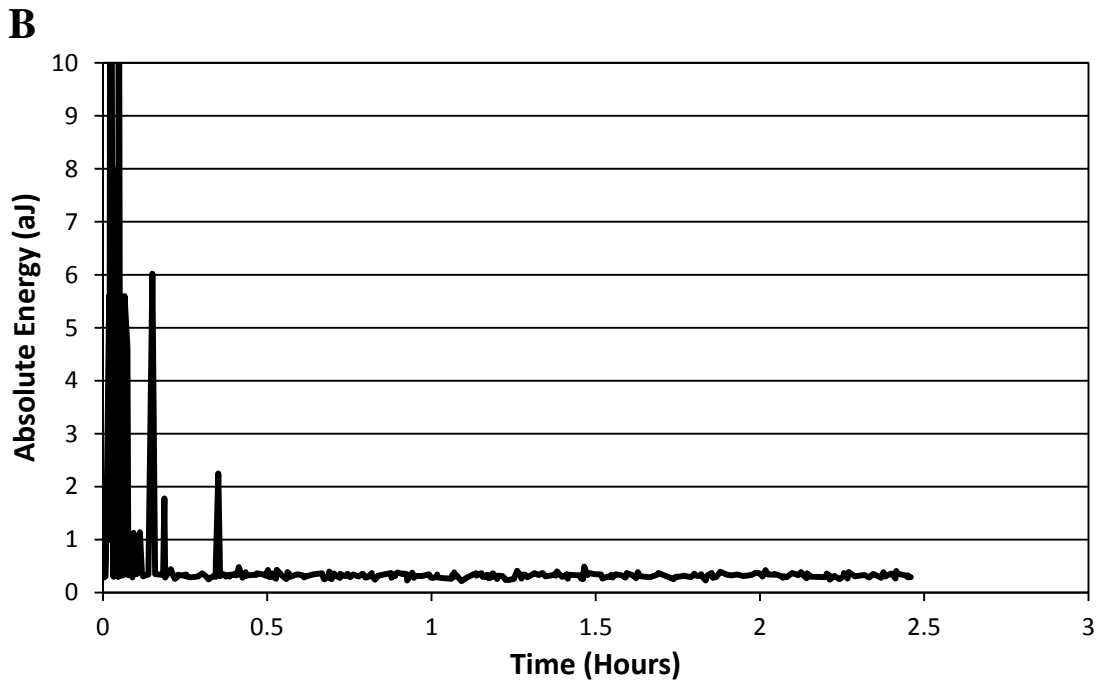
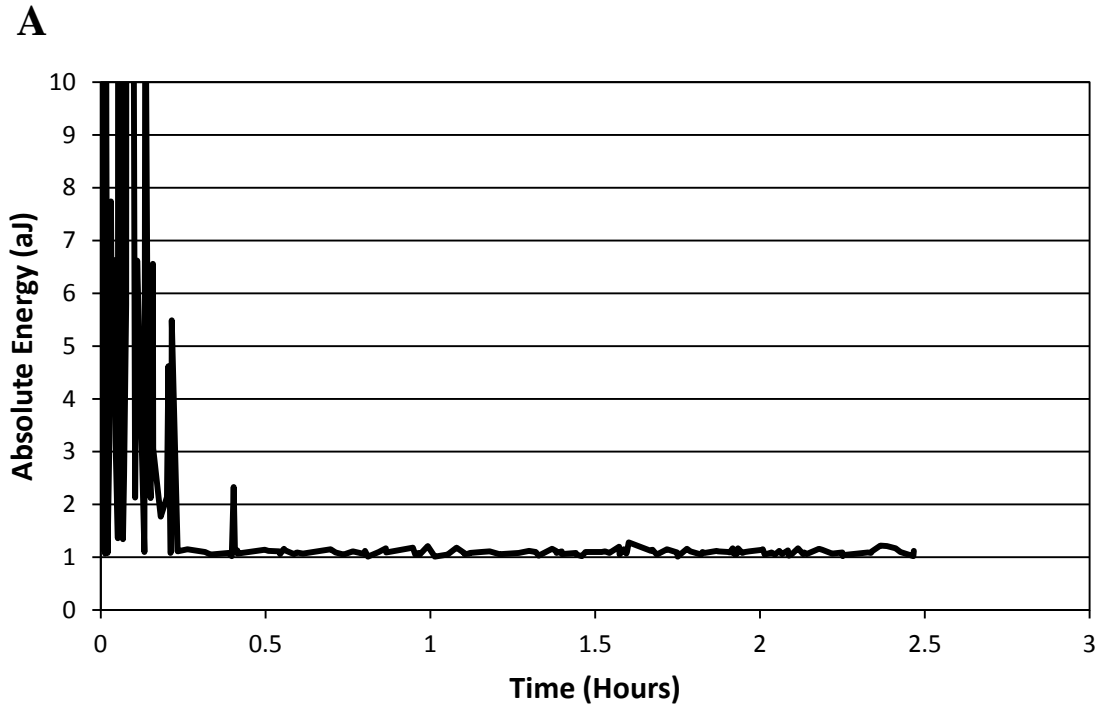
**Figure 37.** ABSE baseline noise levels in water on (A) channel 3 and (B) channel 4.



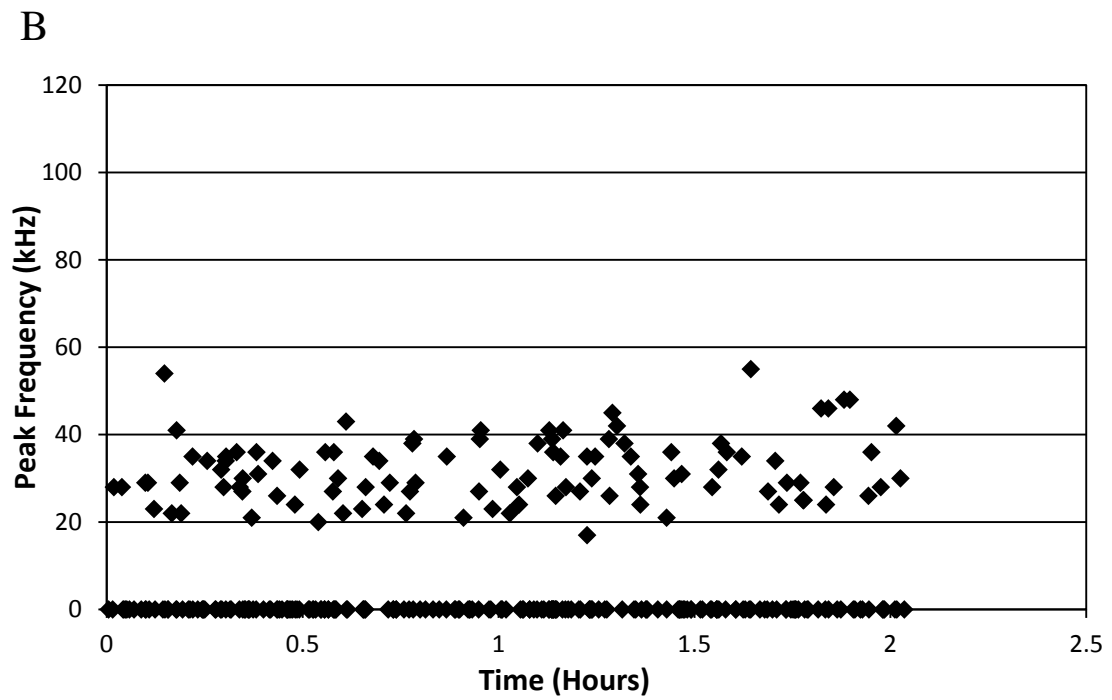
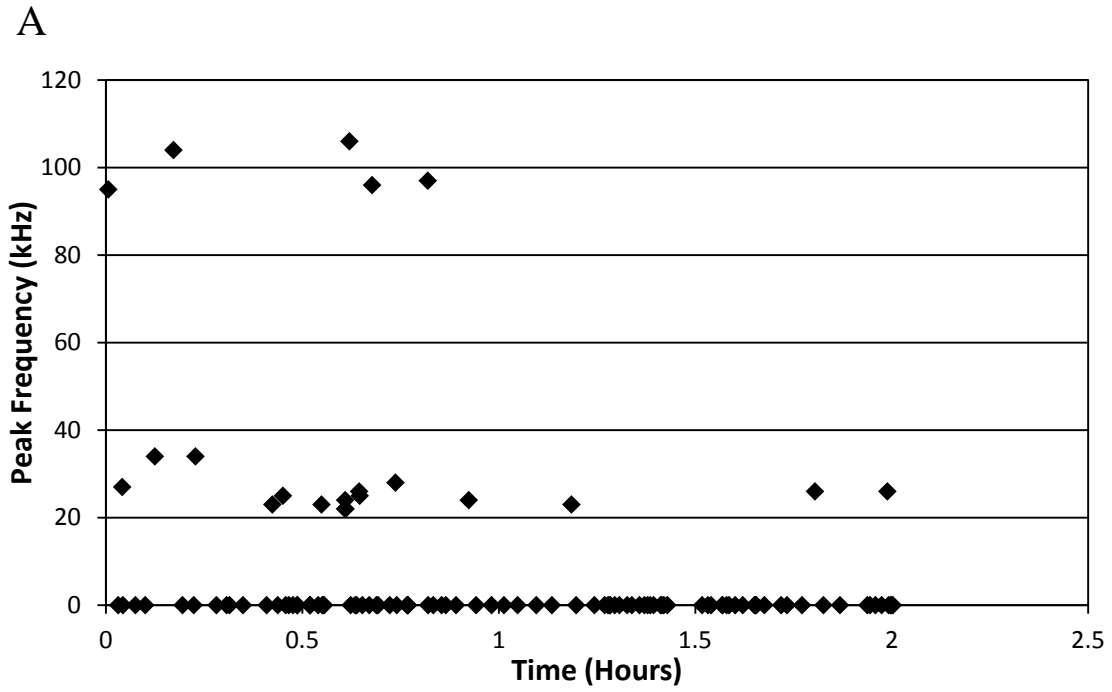
and 0.2 and 0.5 aJ's, respectively. The ABSE baseline levels in medium with fructose and alginate are shown in **Figure 38**. Unlike the ABSE with water, channels 3 and 4 both appear to have noise within the first 20 min and then becomes more quiescent, with a background of 1 and 2 aJ's for the next 2 h. While the initial ABSE is low ( $< 1.5$  aJ), the data suggest that the molecular rearrangement of alginate molecules forming a loose gel can be discerned using this technology.

Observations revealed PFRQ baseline “noise” levels in water to be present at 0 kHz in both channels. Sparse noise levels were observed between 20-40 kHz and around 100 kHz in channel 3, while channel 4 also had noise levels between 20-50 kHz, though denser, as shown in **Figure 39**. In medium with alginate, the PFRQ noise levels were similar to those in water. Both channels had noise levels at 0 kHz, while channel 3 also had levels between 20-40 kHz and 100 kHz and channel 4 levels were between 20-50 kHz, as shown in **Figure 40**. Both channels were observed to have PFRQ loading at 35 kHz for the first 15 min, which was similar to the ABSE loading that occurred during the first 15 min.

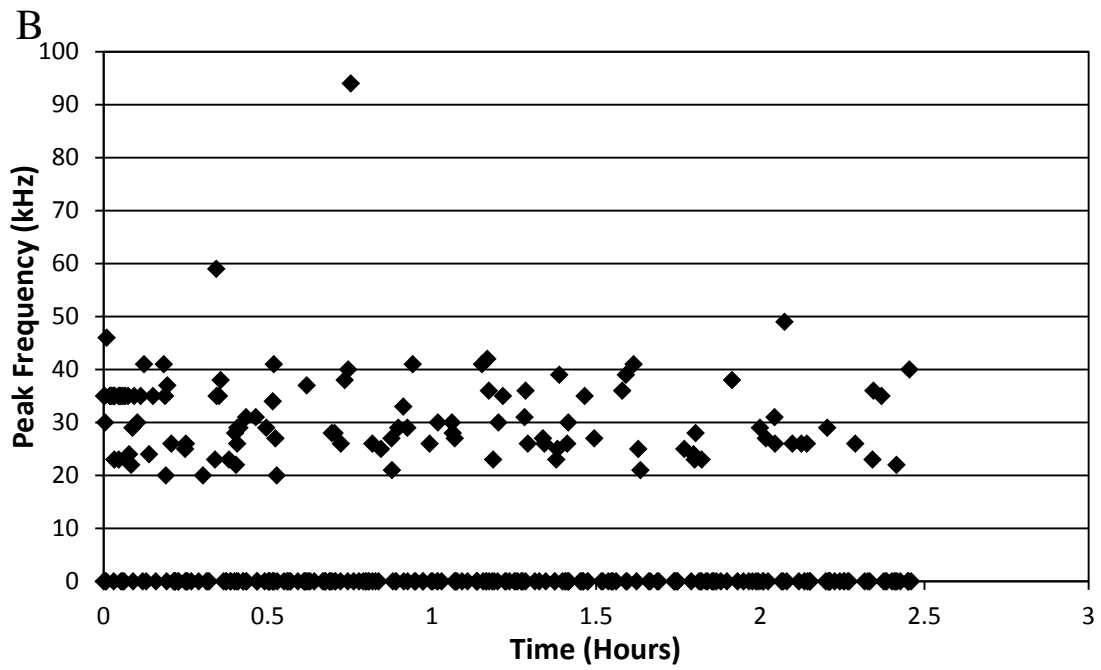
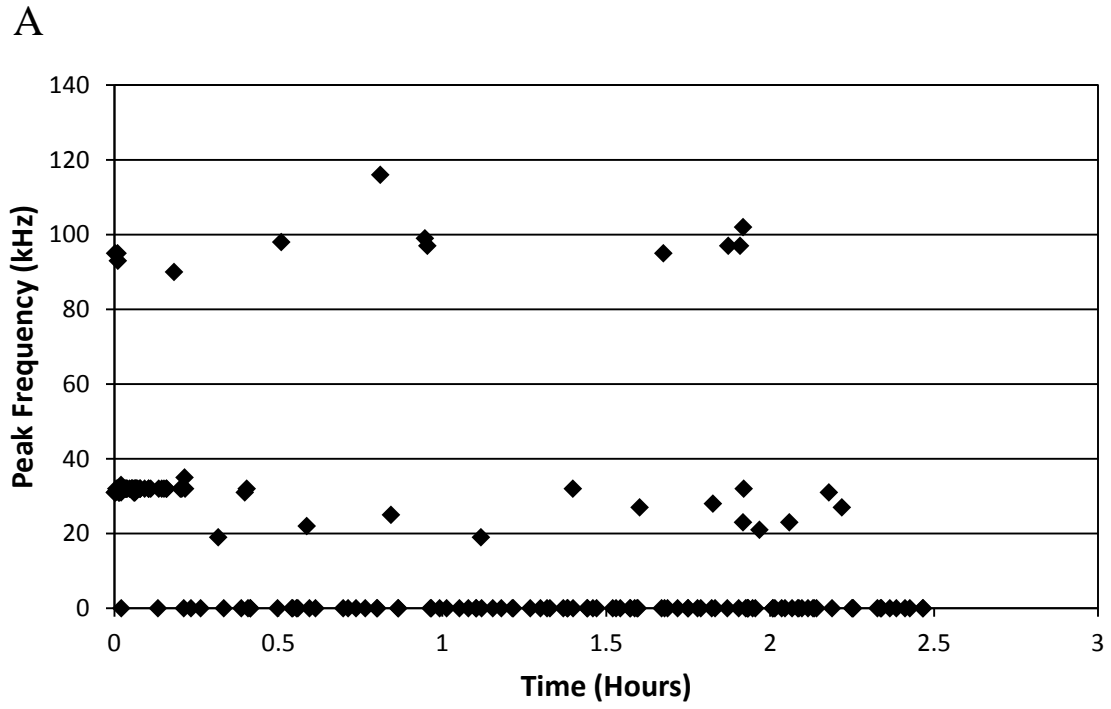
**4.3.3. Generated ABSE From *E. coli* Versus Time.** From all of the AE experiments with each strain of *E. coli*, two of the most similar ABSE means were chosen for visual observation. The 5024 strain represented in **Figure 41(A)** reveals several peak formations with intensities  $>5$  aJ and one with an intensity  $>30$  aJ, within the first hour. This same strain had two other peak formations with intensities  $>30$  aJ before and after the 3<sup>rd</sup> h. The other strain represented in **Figure 41(B)**, reveals a single peak formation  $>5$  aJ between the 1<sup>st</sup> and 2<sup>nd</sup> h. In both strains, peak formations became more frequent between the 5<sup>th</sup> and 9<sup>th</sup> h. The intensity of the ABSE appears much



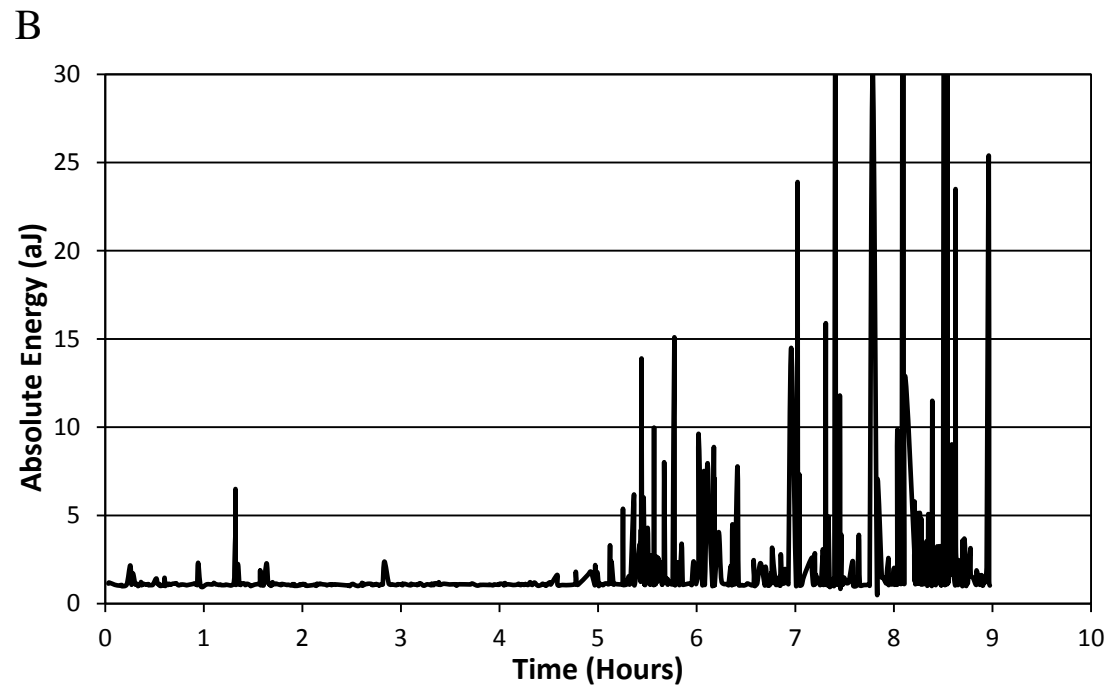
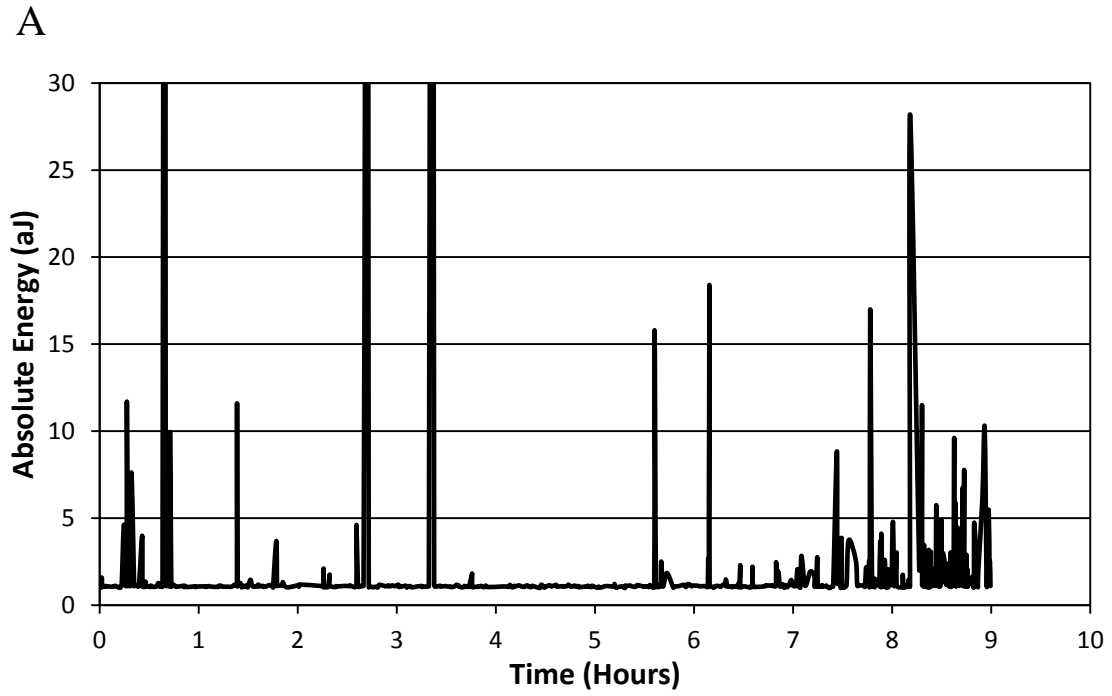
**Figure 38.** ABSE baseline noise levels in medium with alginate on (A) channel 3 and (B) channel 4.



**Figure 39.** PFRQ baseline noise levels in water on (A) channel 3 and (B) channel 4.



**Figure 40.** PFRQ baseline noise levels in medium with alginate on (A) channel 3 and (B) channel 4.

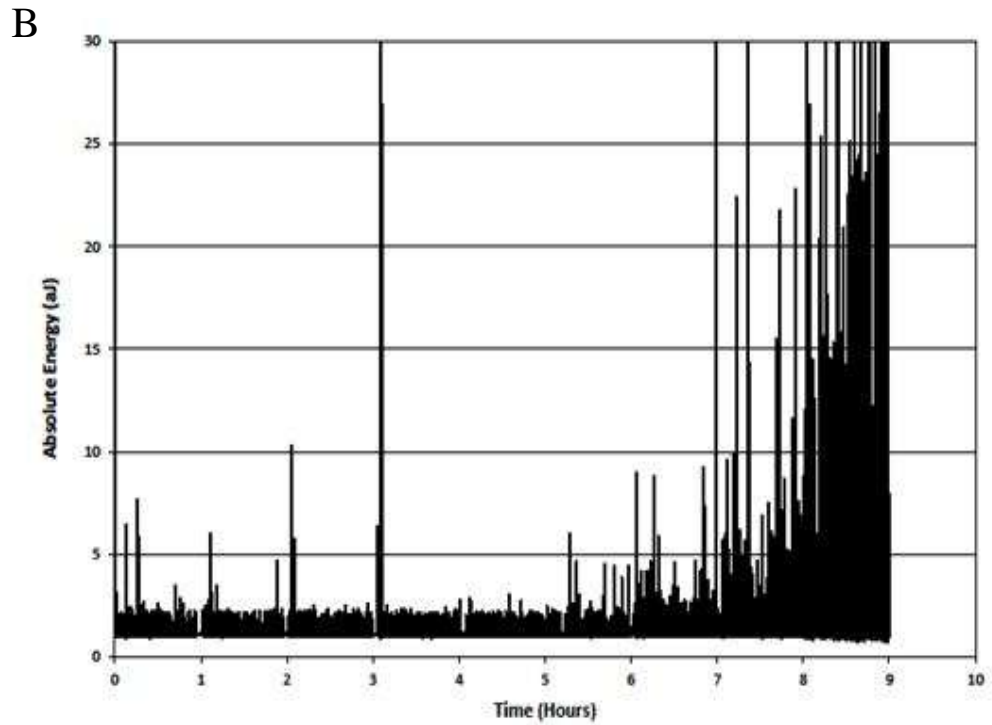
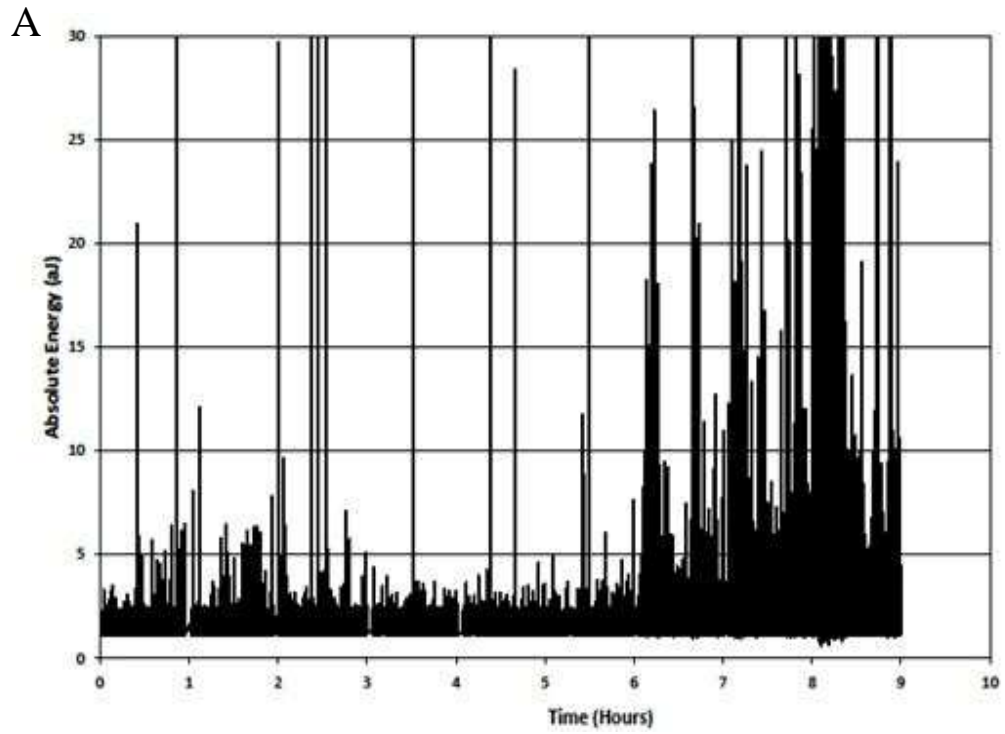


**Figure 41. (A) and (B)** ABSE generated by strains 5024 (with similar means) when grown in medium with alginate.

stronger and more frequent in medium without alginate. Both strains of 5024 revealed several peak formations with intensities >5 aJ within the first hour. Strain 5024 represented in **Figure 42(A)**, also generated peak formations with intensities >30 aJ within the first hour, as well as, within the 2<sup>nd</sup> to 5<sup>th</sup> h. The other strain of 5024 represented in **Figure 42(B)** revealed two peak formations >25 aJ just after the 3<sup>rd</sup> h. During the 6<sup>th</sup> to 9<sup>th</sup> h, the ABSE generated by both strains became more frequent and intense. Again, these findings suggest that when the cells are allowed to settle (grown in medium without alginate), a stronger signal is produced.

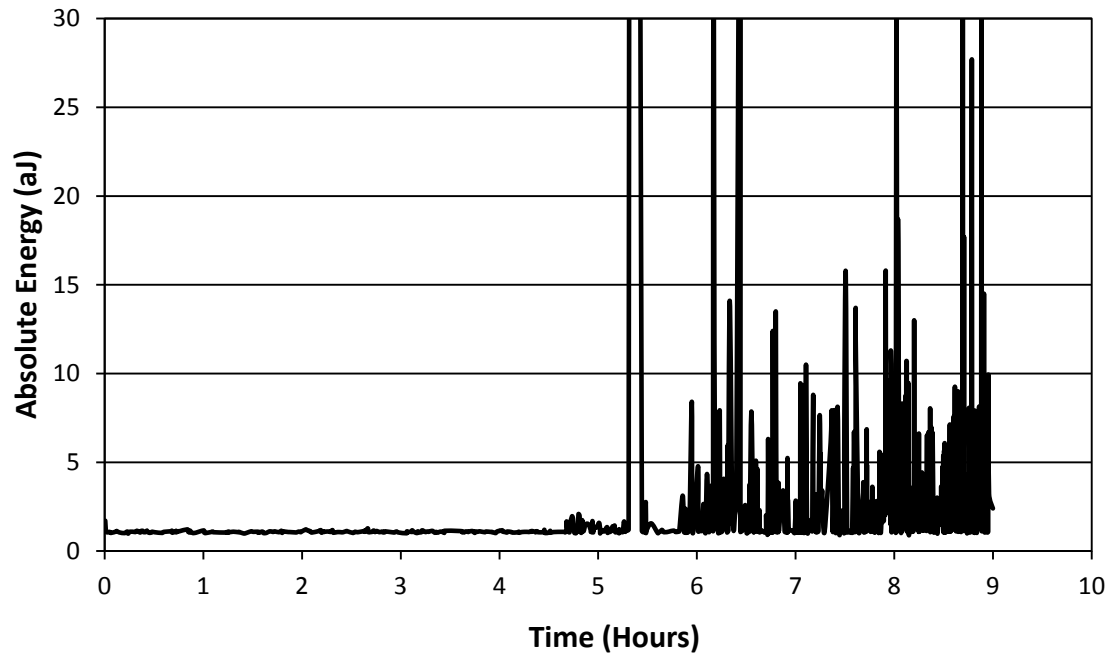
Observations of strains 8237, when grown in medium with alginate, revealed one strain represented in **Figure 43(A)** generated no ABSE activity within the first 5h, while the other strain, represented in **Figure 43(B)**, generated several peak formations >20 aJ, within the first 30 min. No ABSE activity is seen in both strains from the 2<sup>nd</sup> to 4<sup>th</sup> h, however, from the 5<sup>th</sup> to 9<sup>th</sup> h, the ABSE became more frequent and intense. The ABSE generated from strains 8237, when grown in medium without alginate, was even more intense than that with alginate. Within the first 5 min, strain 8237 represented in **Figures 44(A) and (B)**, revealed peak formations with intensities of 10 aJ and 25aJ, respectively. While little ABSE was generated during the 1<sup>st</sup> to 4<sup>th</sup> h, the intensity and frequency became much stronger during the 5<sup>th</sup> to 9<sup>th</sup> h. Overall, strain 8237 generated more intense ABSE signals than strain 5024.

In media with alginate, strains of 8279 appeared to generate little ABSE during the 1<sup>st</sup> to 8<sup>th</sup> h, compared to the other two strains of *E. coli*. Strain 8279 represented in **Figure 45(A)**, revealed two peak intensities >10 aJ within the first 10 min, as well as, one intensity > 30 aJ at the 1st h. The other strain represented in **Figure 45(B)** revealed only

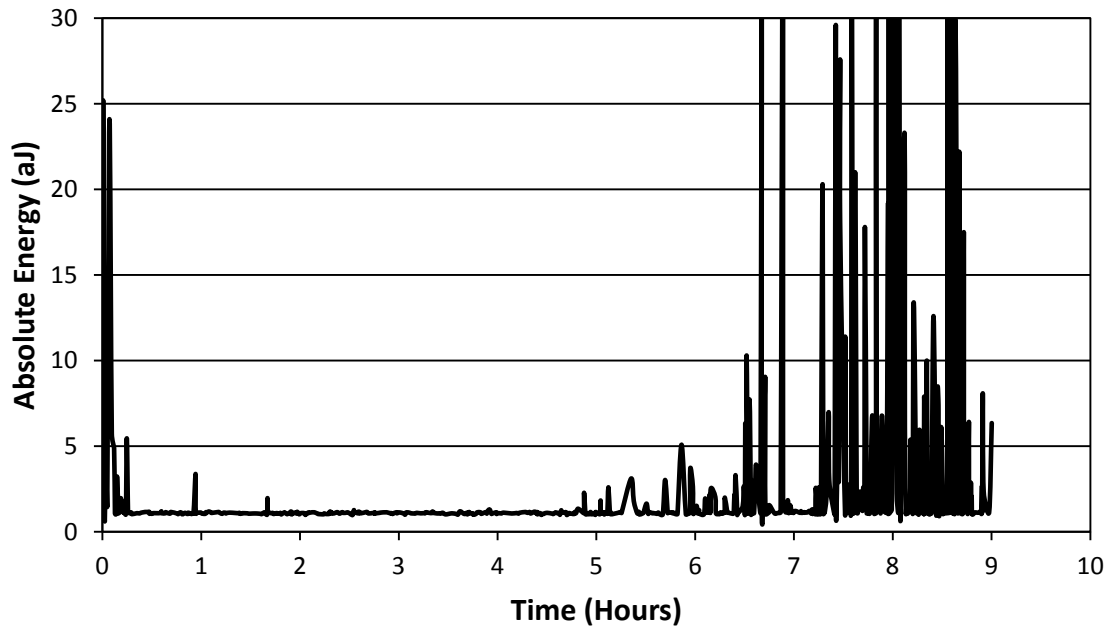


**Figure 42.** (A) and (B) ABSE generated by strains 5024 (with similar means) when grown in medium without alginate.

A

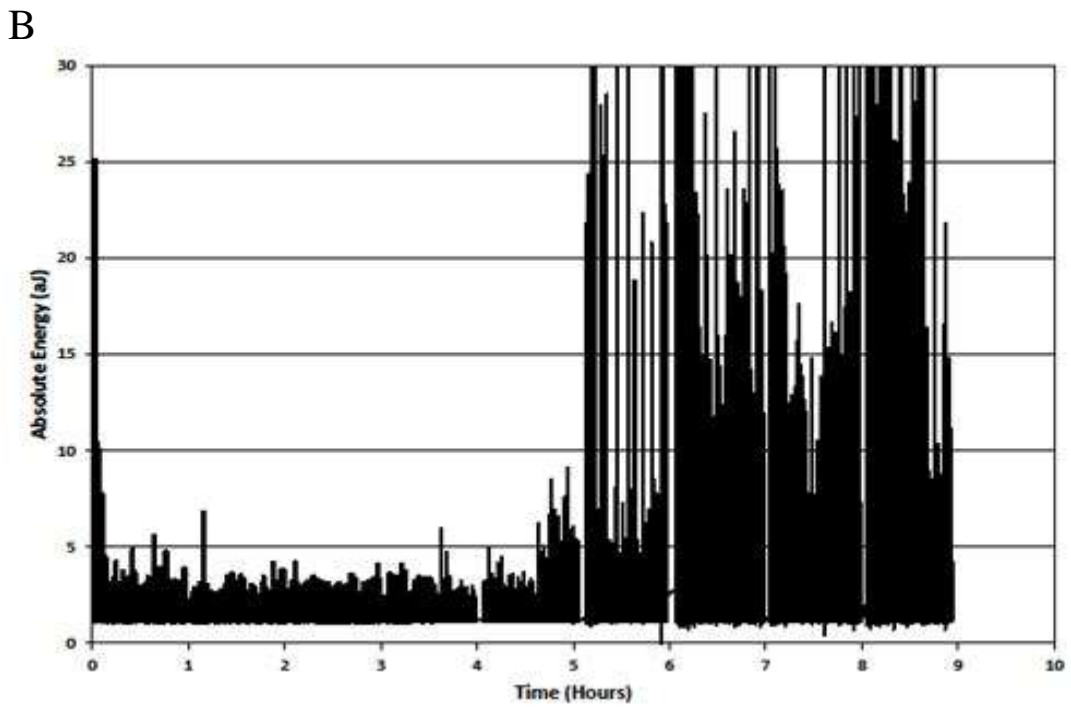
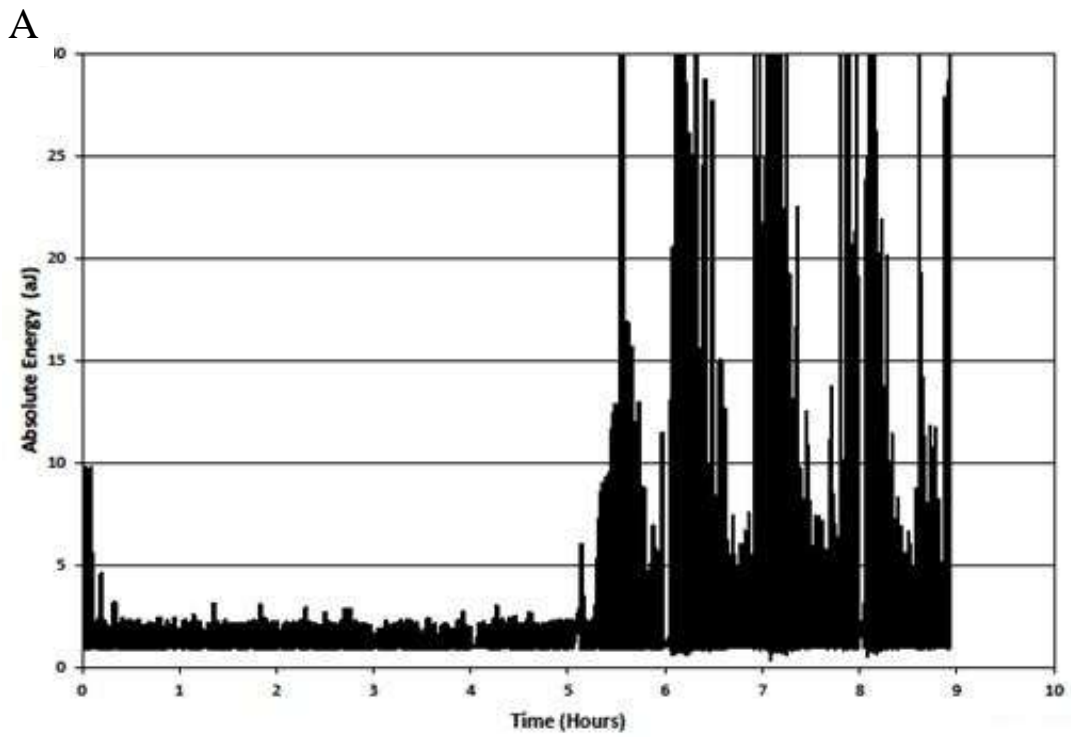


B

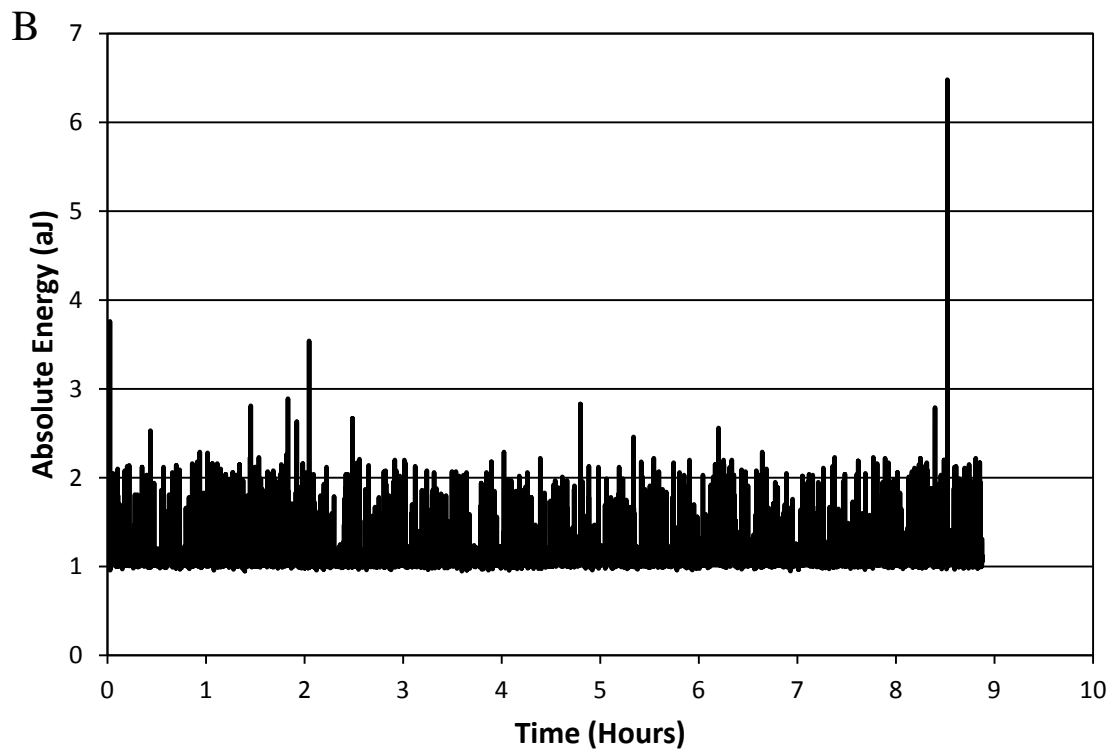
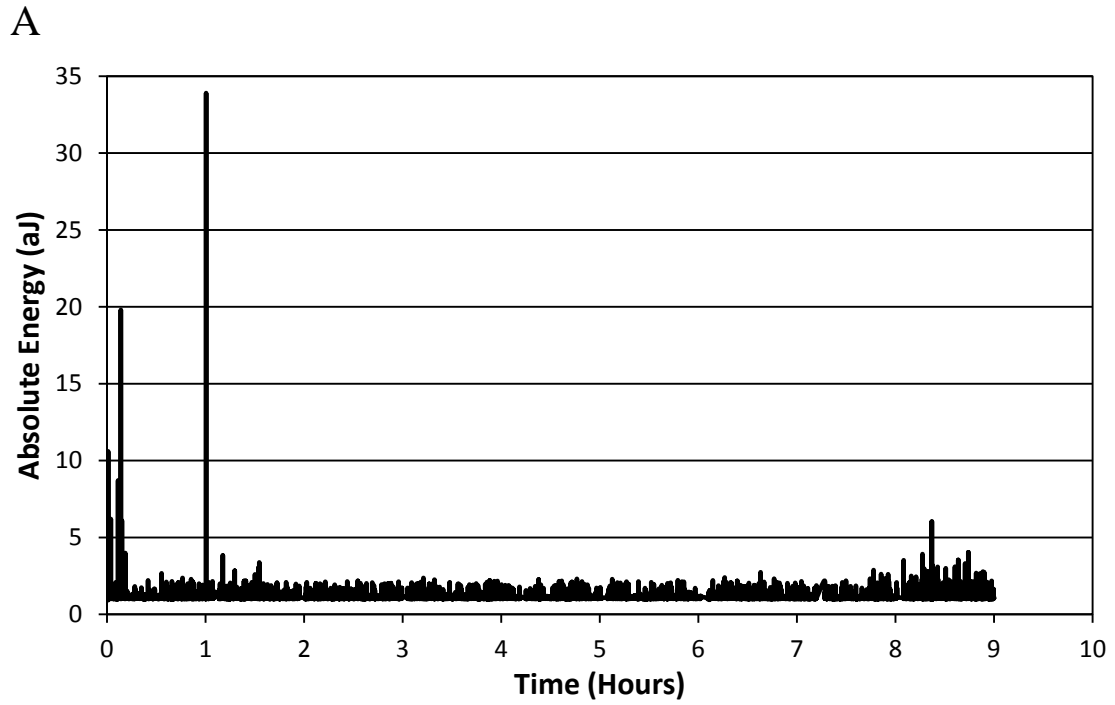


**Figure 43.** (A) and (B) ABSE generated by strains 8237 (with similar means) when grown in medium with alginate.





**Figure 44.** (A) and (B) ABSE generated by strains 8237 (with similar means) when grown in medium without alginate.

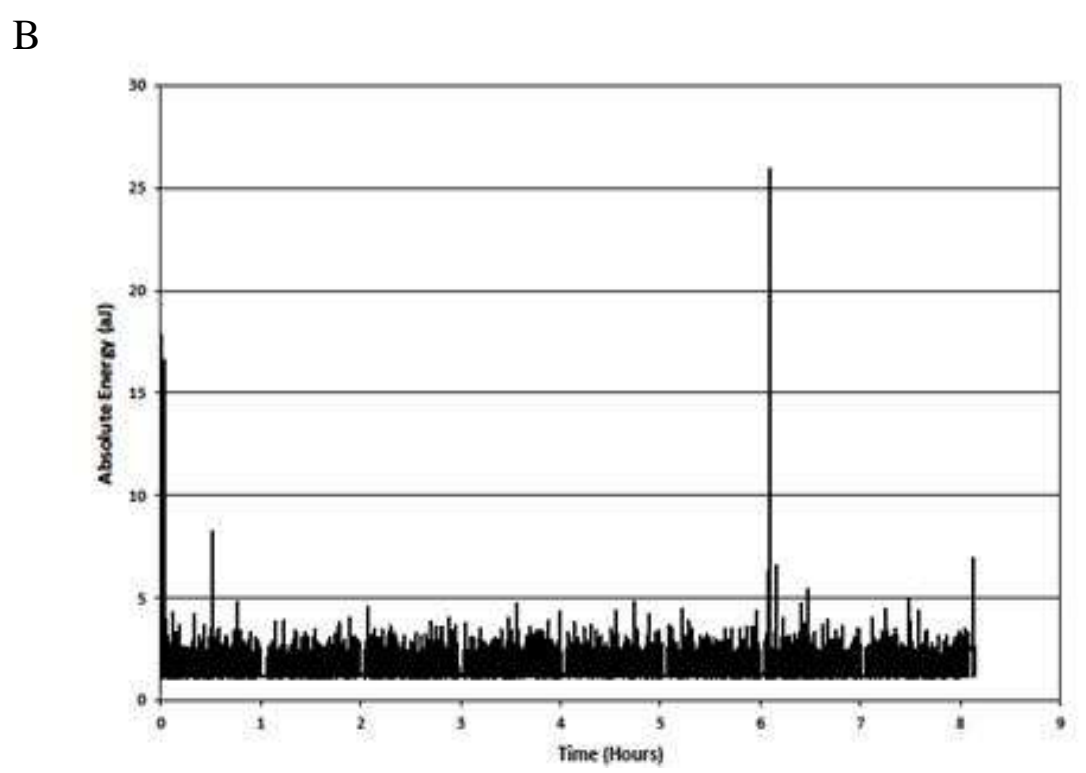
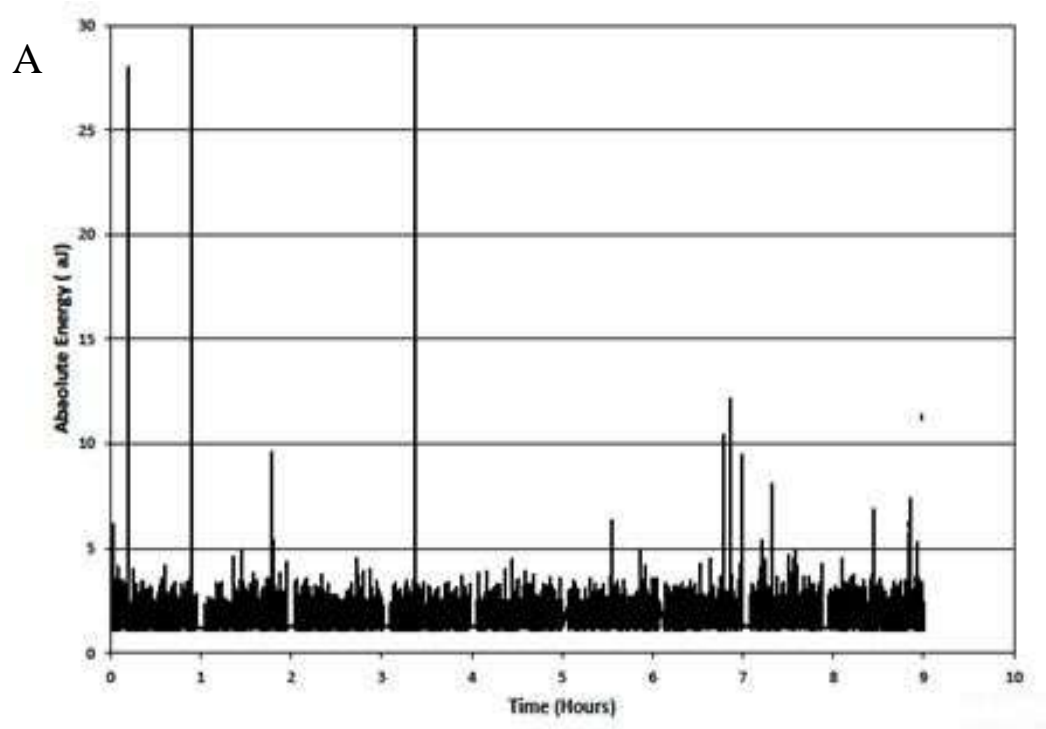


**Figure 45. (A) and (B)** ABSE generated by strains 8279 (with similar means) when grown in medium with alginate.

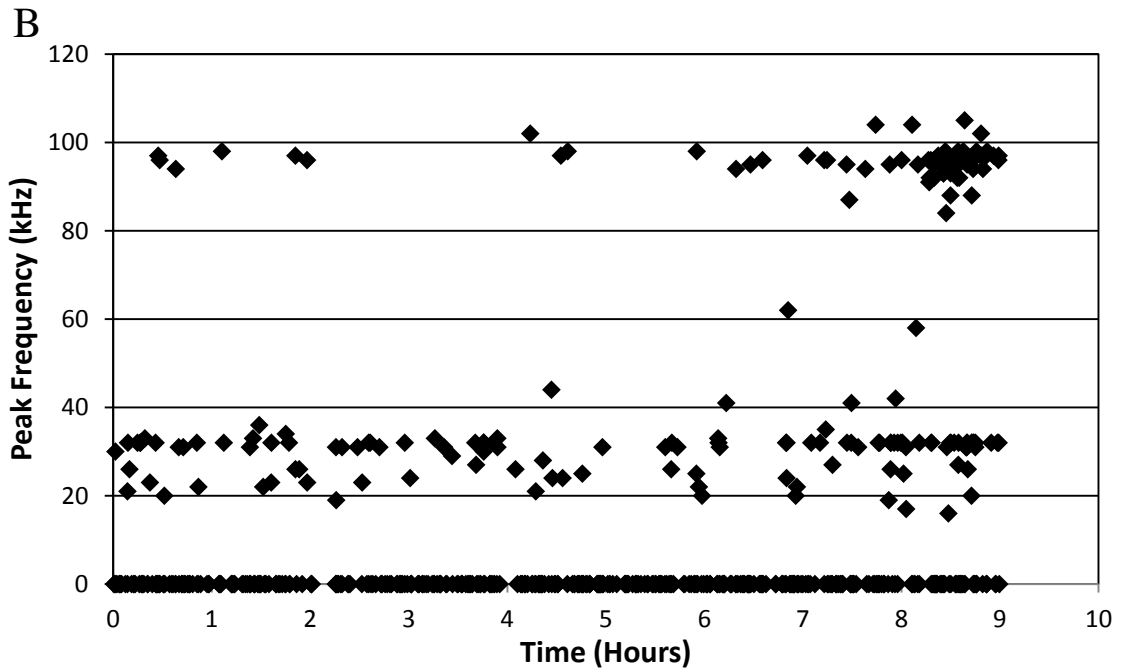
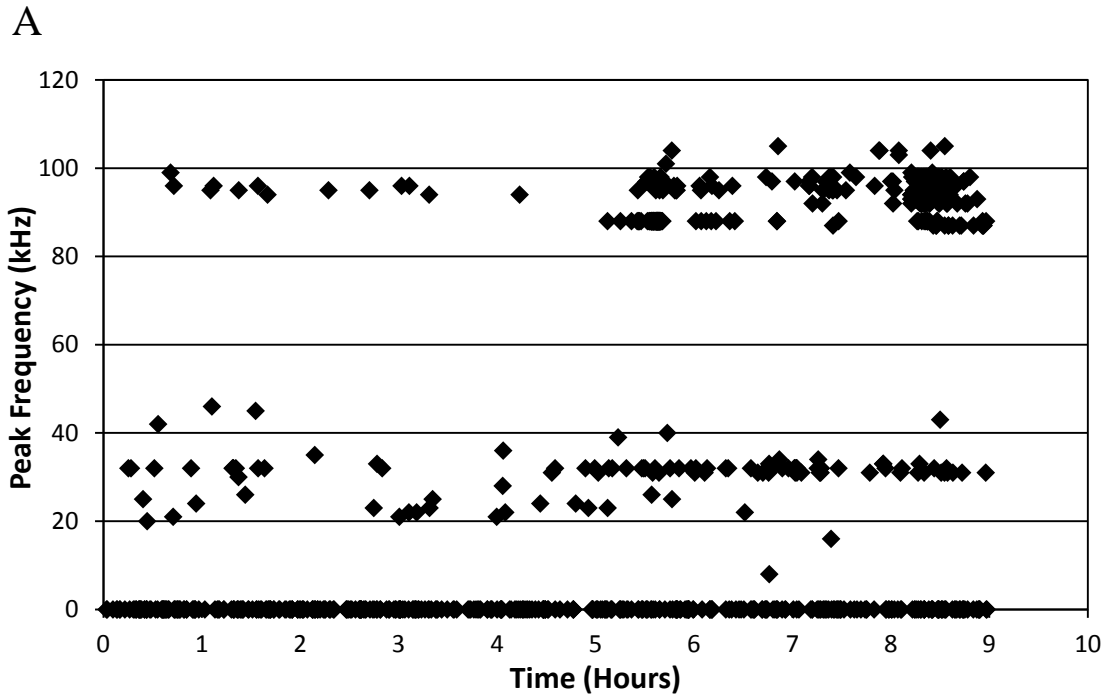
one peak intensity >6 aJ during the 8<sup>th</sup> h. In media without alginate, strain 8279 represented in **Figure 46(A)**, revealed peak formations >5aJ within 5 min, >10 aJ around the 7<sup>th</sup> h and >30 aJ within the first 4h. The other strain represented in **Figure 46(B)**, revealed several peak formations > 15 aJ within 5 min and a peak formation >25 aJ at the 6<sup>th</sup> h. Both strains showed little ABSE activity during the 4<sup>th</sup> to 6<sup>th</sup> h. Overall, strain 8279 generated the weakest ABSE signals.

**4.3.4. Generated PFRQ from *E-Coli* Versus Time.** From all of the experiments with each strain of *E-Coli*, two of the most similar PFRQ means were chosen for visual observation. In the observations of strains 5024 in medium with alginate, both represented in **Figures 47(A)** and **(B)** revealed linear and clustering of PFRQ hits between 20-40 kHz and 80-105 kHz, with the number of hits increasing during the 5<sup>th</sup> to 9<sup>th</sup> h. In medium without alginate, the generated PFRQ like the ABSE, is distinctly more intense than in medium with alginate. Strain 5024 represented in **Figures 48(A)** and **(B)**, revealed an increase in the concentration of PFRQ hits between 100-200 kHz and 100-300 kHz, respectively, during the 5<sup>th</sup> to 9<sup>th</sup> h. The PFRQ data confirms that the concentration of frequencies increases when cells settle atop the sensor's surface.

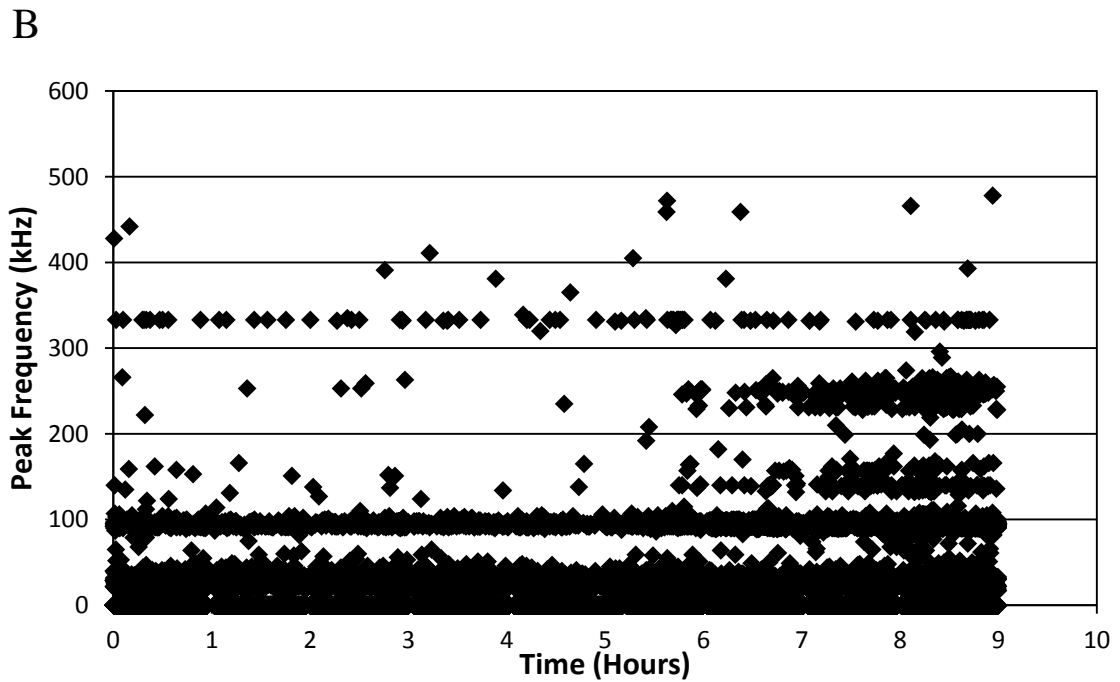
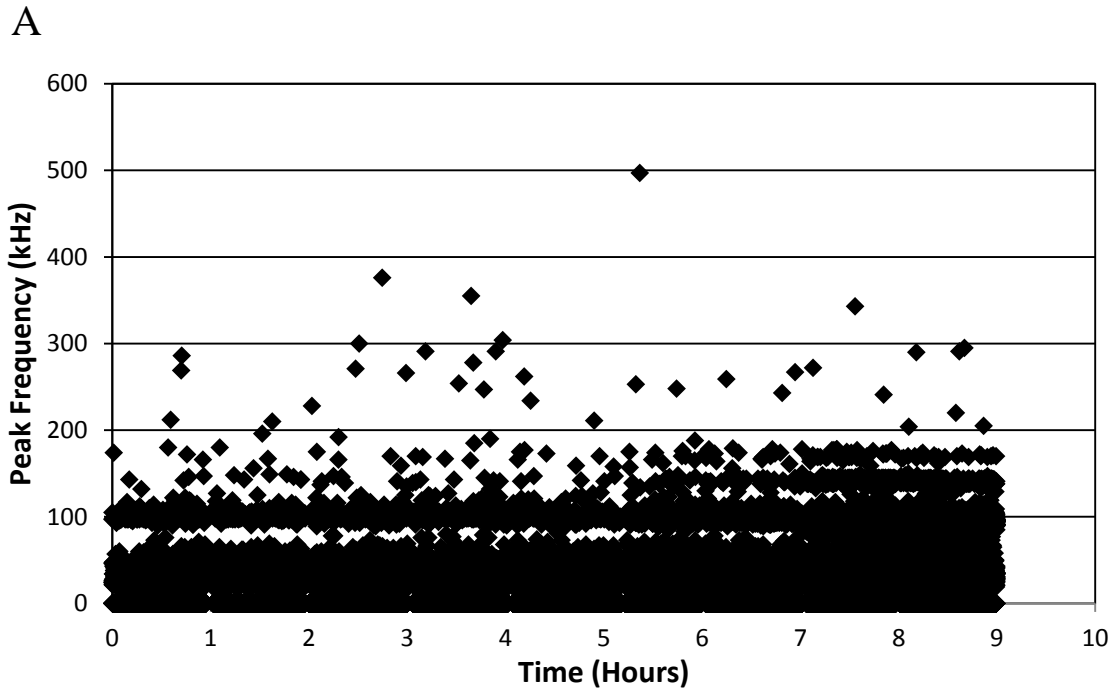
Observations for both strains of 8237 in medium with alginate, shown in **Figures 49(A)** and **(B)**, revealed similar PFRQ linear patterns at 30 kHz and cluster formations between 80-110 kHz during the 5<sup>th</sup> to 9<sup>th</sup> h. This is also seen in medium without alginate, though at higher kHz frequencies. In the 8237 strain represented in **Figures 50(A)** and **(B)**, the concentration of PFRQ hits increases between 100-400 kHz during the 5<sup>th</sup> to 9<sup>th</sup> h.



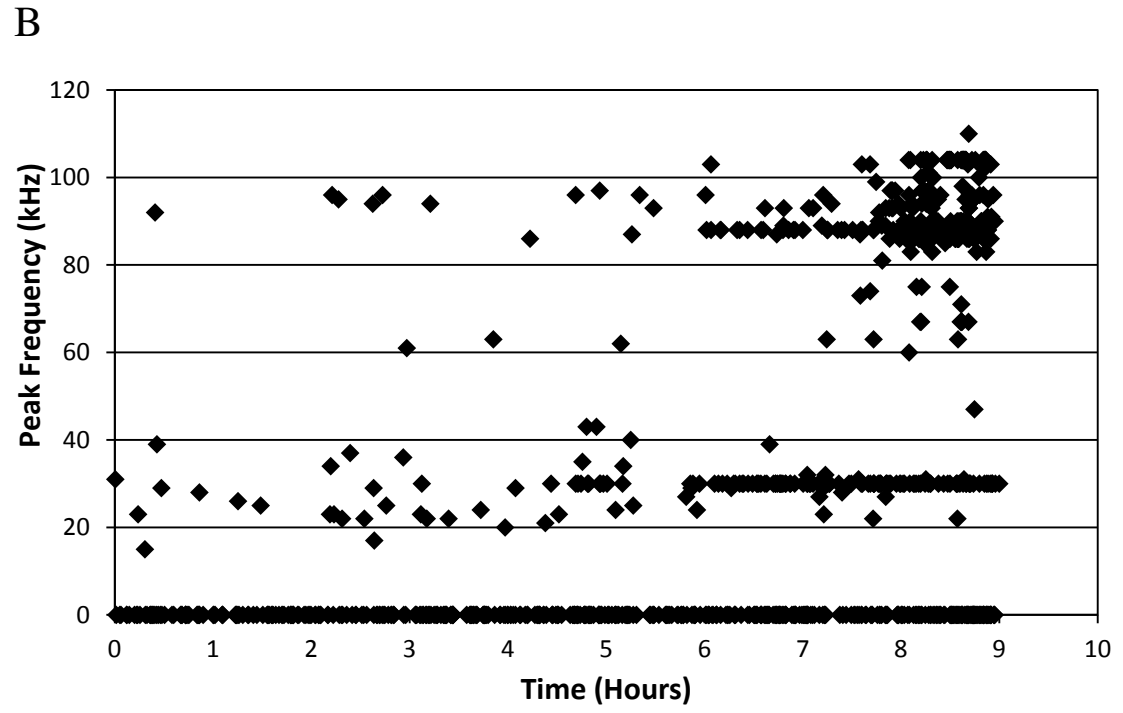
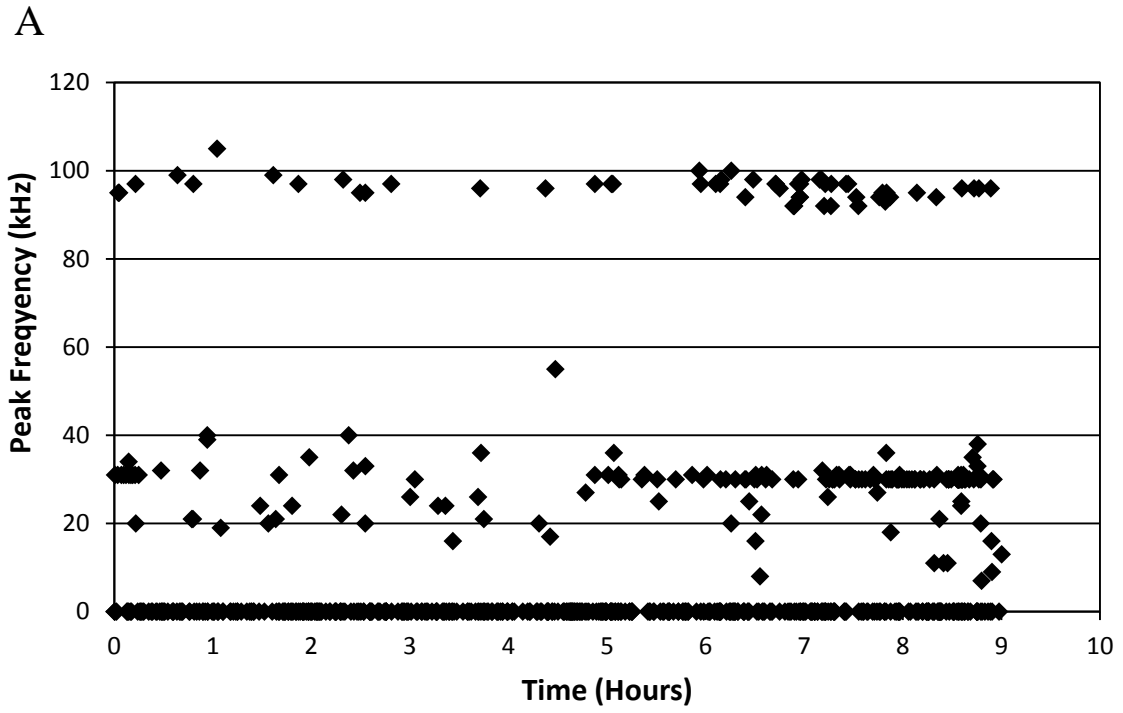
**Figure 46. (A) and (B)** ABSE generated by strains 8279 (with similar means) when grown in medium without alginate.



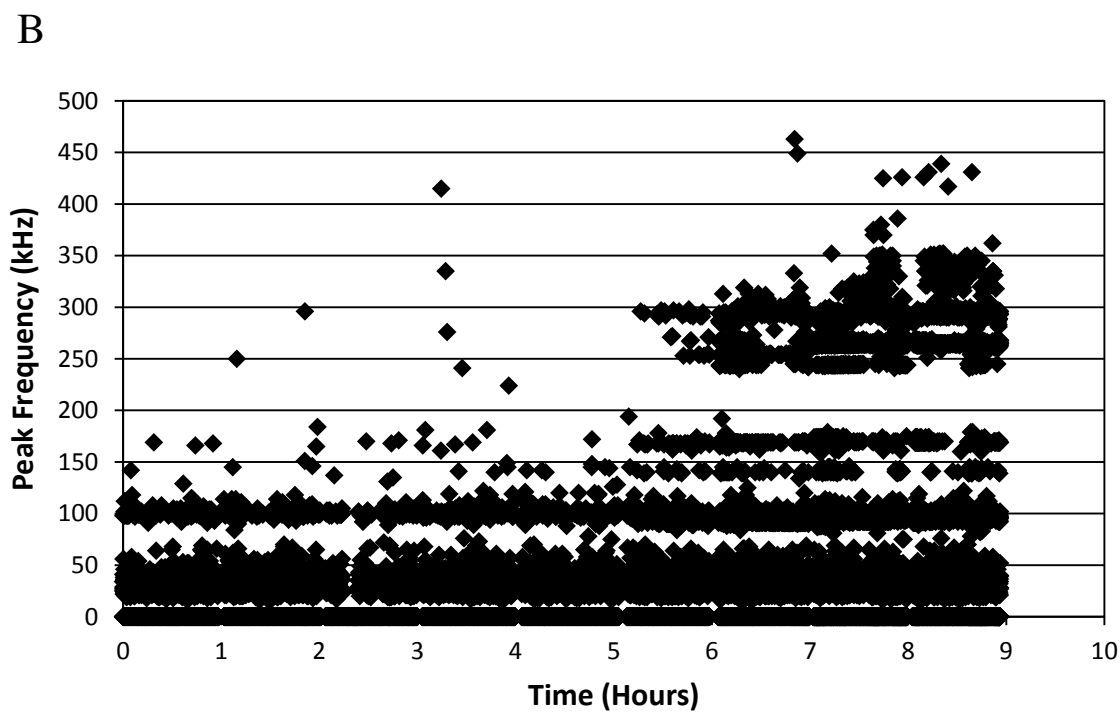
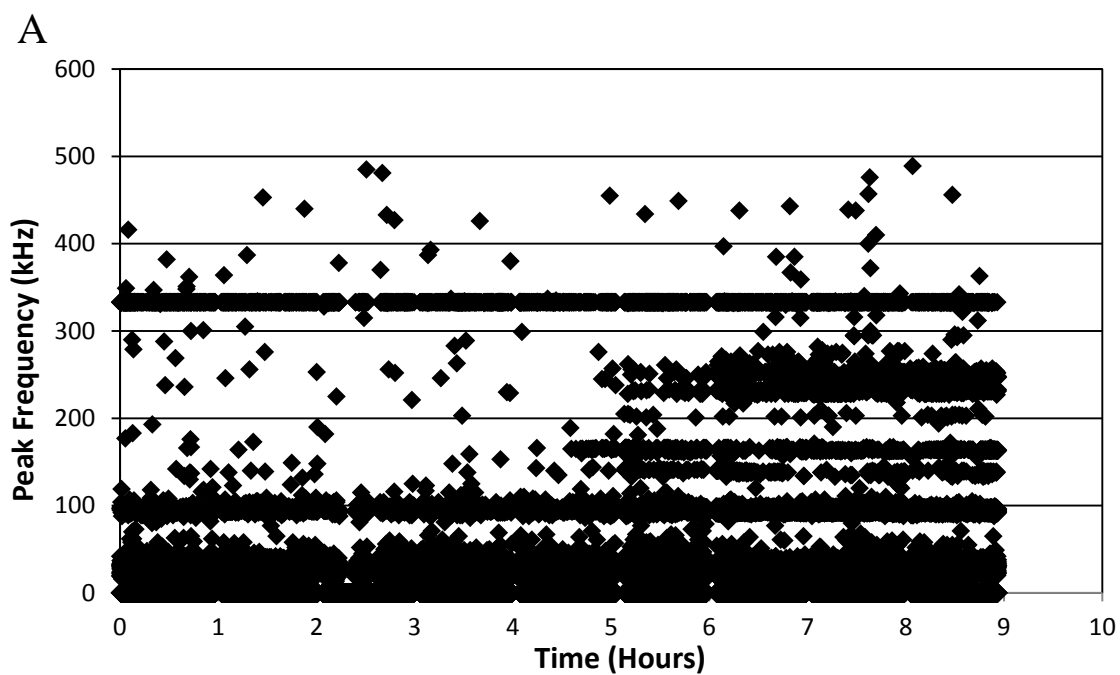
**Figure 47. (A) and (B)** PFRQ generated by strains 5024(with similar means) when grown in medium with alginate.



**Figure 48. (A) and (B)** PFRQ generated by strains 5024(with similar means) when grown in medium without alginate.



**Figure 49. (A) and (B)** PFRQ generated by strains 8237 (with similar means) when grown in medium with alginate.



**Figure 50.** (A) and (B) PFRQ generated by strains 8237 (with similar means) when grown in medium without alginate.

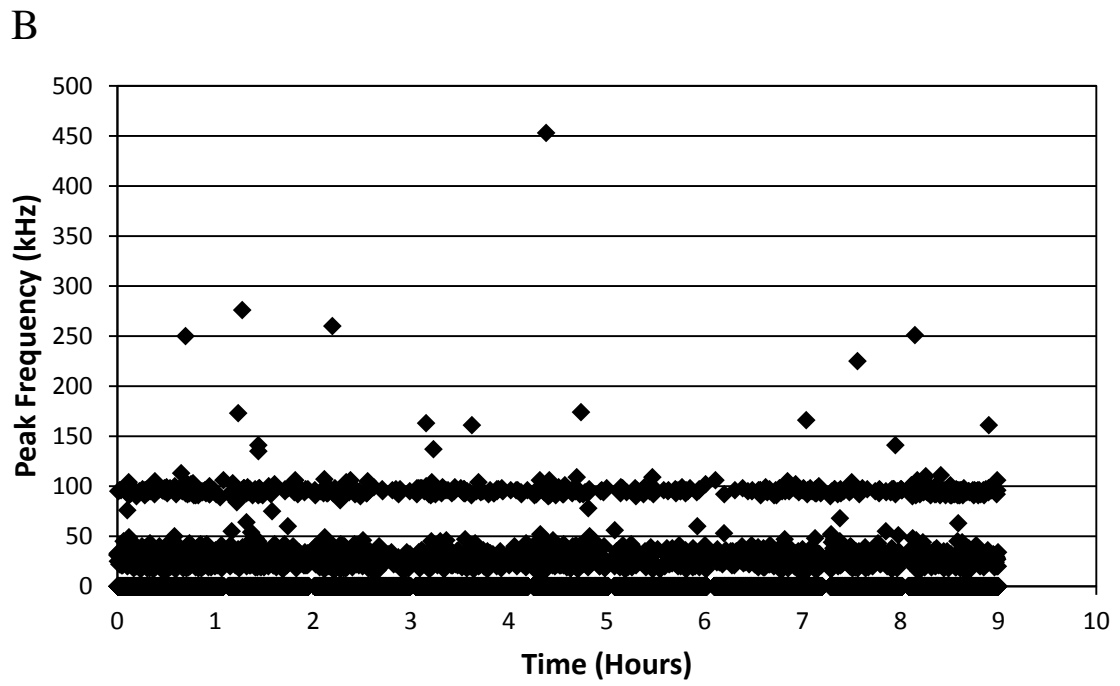
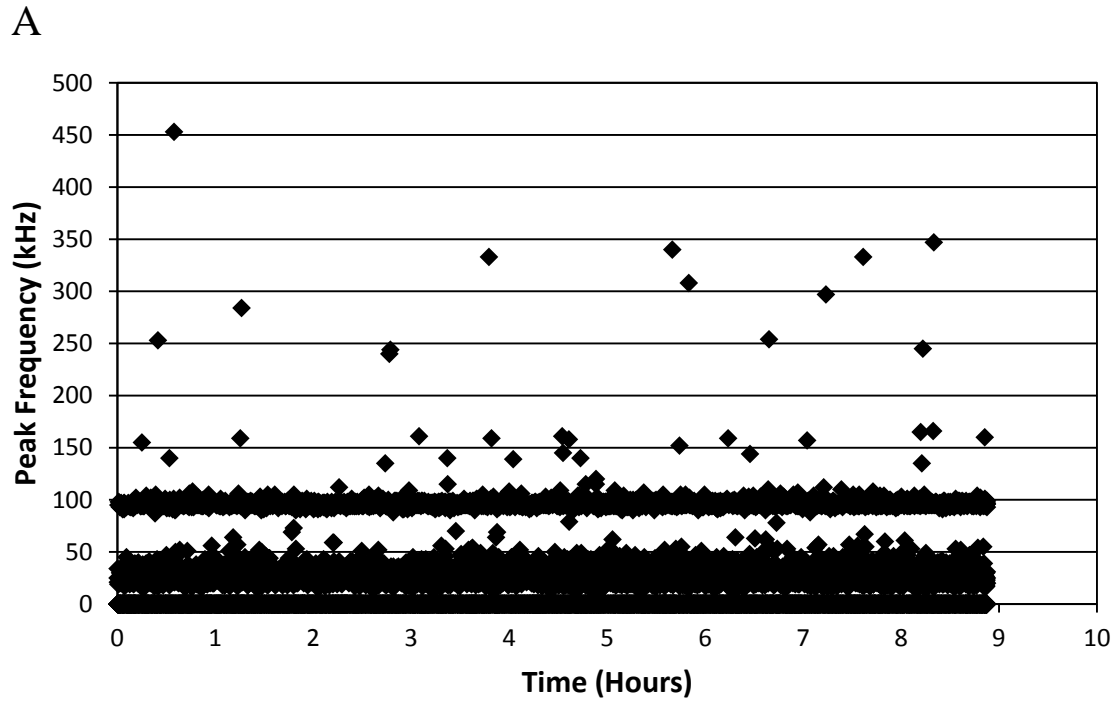


Observations for both strains of 8279, in medium with alginate, revealed a low concentration of PFRQ hits between 150-450 kHz, shown in **Figures 51(A) and (B)**. However, in medium without alginate, the concentration of PFRQ hits increased between 100-500 kHz, shown in **Figures 52(A) and (B)**.

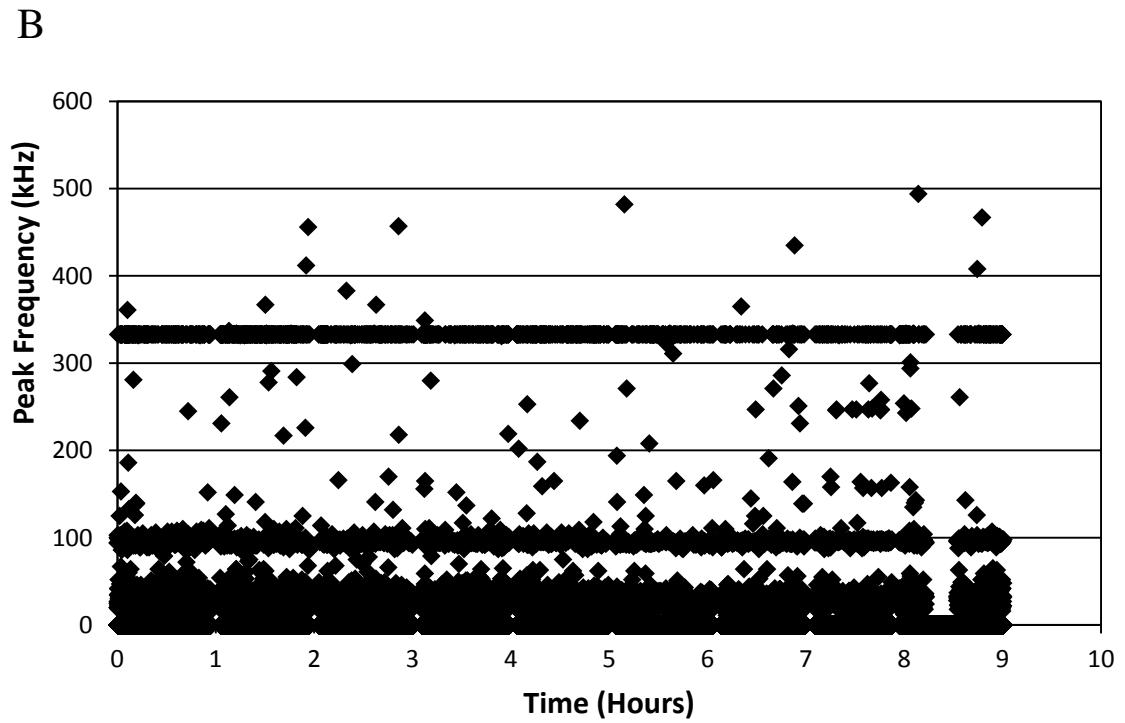
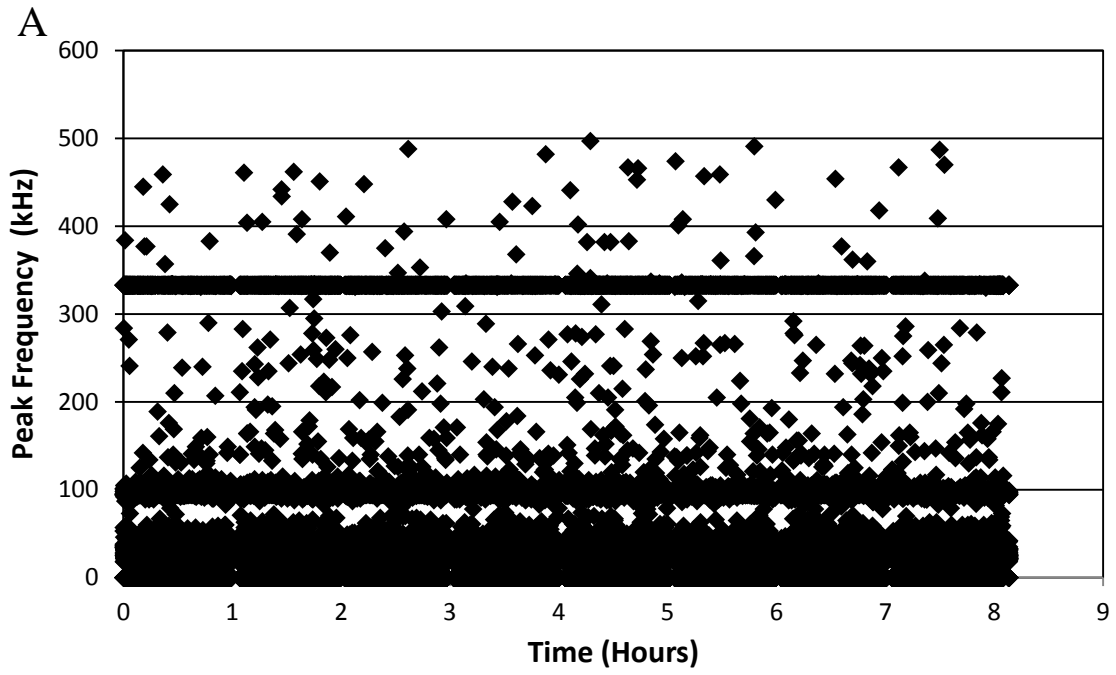
#### **4.4. The Effect of Media on ABSE and PFRQ.**

**4.4.1. Effect of Media on ABSE.** The ABSE generated from strains 5024 and 8279 were significantly different ( $P < 0.05$  and  $P < 0.0001$ , respectively) when grown in media without alginate, than with alginate. Mean differences between the media types were 0.2, 0.02 and 0.57 for strains 5024, 8237 and 8279, respectively. When grown in medium with alginate, strain 8237 generated the most ABSE, which was 13% ( $P = 0.1094$ ) and 63% ( $P < 0.0001$ ) more intense than 5024 and 8279, respectively. The ABSE of strain 5024 was also 30% ( $P < 0.0001$ ) more intense than 8279 when grown in medium with alginate. **Figure 53** shows the effect of media with and without alginate on ABSE in the *E. coli* strains. When grown in medium without alginate, strains 5024 and 8237 equally generated the most ABSE, which was 3% ( $P = 0.65$ ) more intense than strain 8279. Overall, medium without alginate generated ABSE's that were 18% stronger ( $P < 0.0001$ ) than with alginate, shown in **Figure 54**.

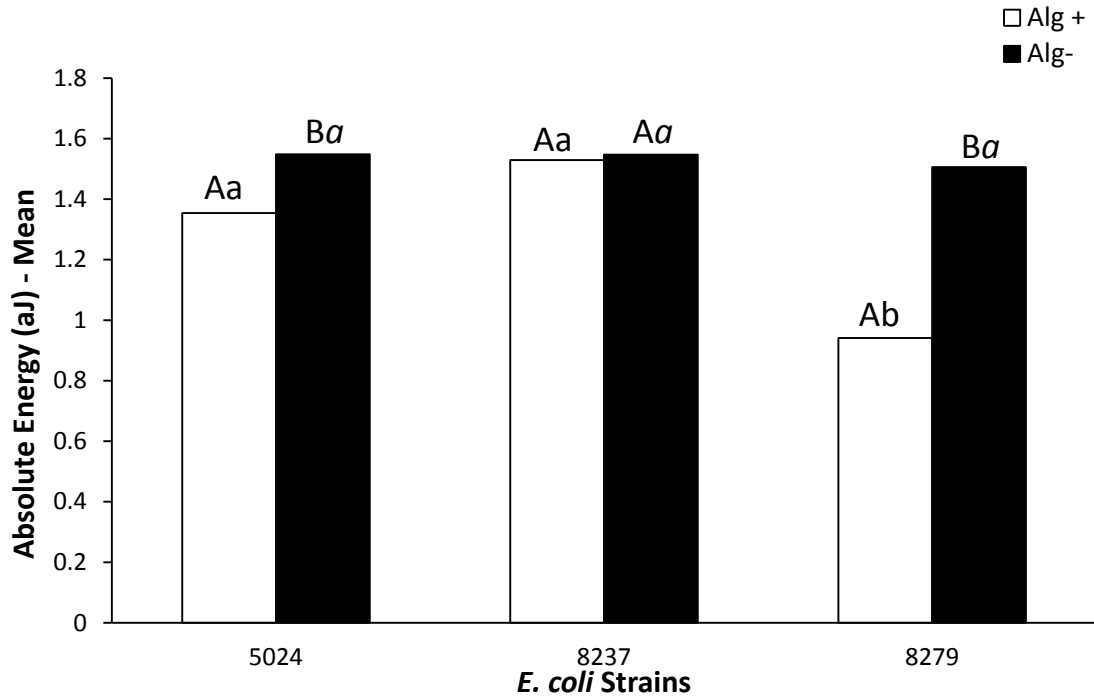
**4.4.2. Effect of Media on PFRQ.** The initial observation of the data presented a stark contrast between PFRQ and ABSE intensities, as evidenced by PFRQ means being approximately 11-35 times greater than those of ABSE. The PFRQ's generated in all strains of *E. coli*, grown in media with and without alginate, were significantly different ( $P < 0.0001$ ), as shown in **Figure 55**. Mean differences between media types were 14.46,



**Figure 51. (A) and (B)** PFRQ generated by strains 8279 (with similar means) when grown in medium with alginate.



**Figure 52.** (A) and (B) PFRQ generated by strains 8279 (with similar means) when grown in medium without alginate.



**Figure 53.** The effect of media type on the average ABSE generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between the type of media within each strain.

\*abc =Means with different letters are significantly different ( $P < 0.0001$ ).between strains grown in medium with alginate.

\*abc =Means with different letters are significantly different ( $P < 0.0001$ ).between strains grown in medium without alginate.

\*Standard Error = 0.08 for 5024 grown in medium with alginate according to the least square mean.

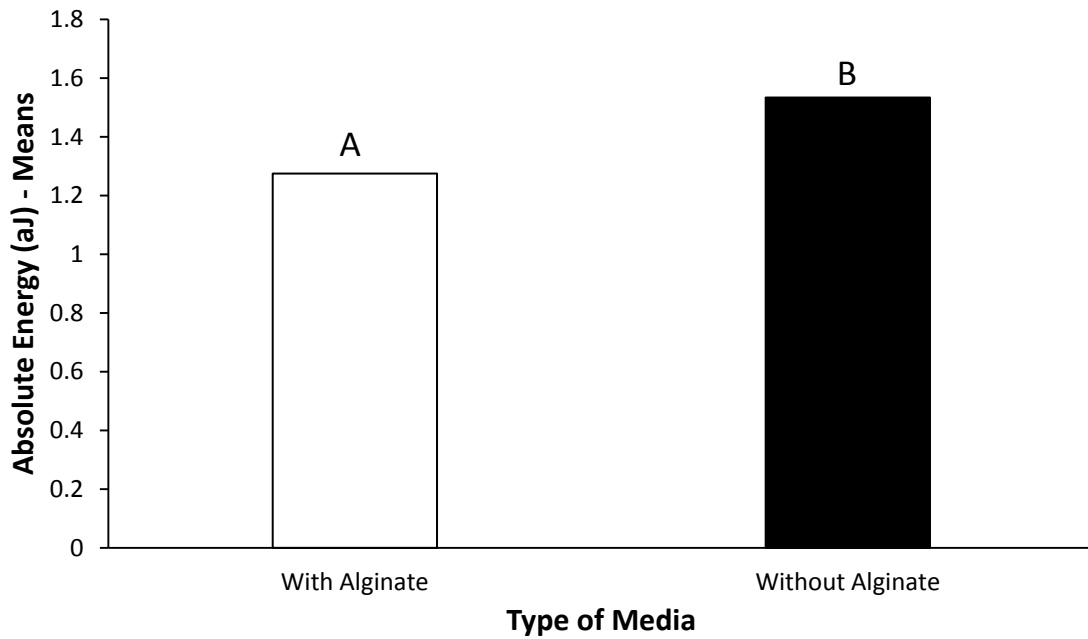
\*Standard Error = 0.06 for 5024 grown in medium without alginate according to the least square mean.

\*Standard Error = 0.08 for 8237 grown in medium with alginate according to the least square mean.

\*Standard Error = 0.06 for 8237 grown in medium without alginate according to the least square mean.

\*Standard Error = 0.06 for 8279 grown in medium with alginate according to the least square mean.

\*Standard Error = 0.07 for 8279 grown in medium without alginate according to the least square mean.

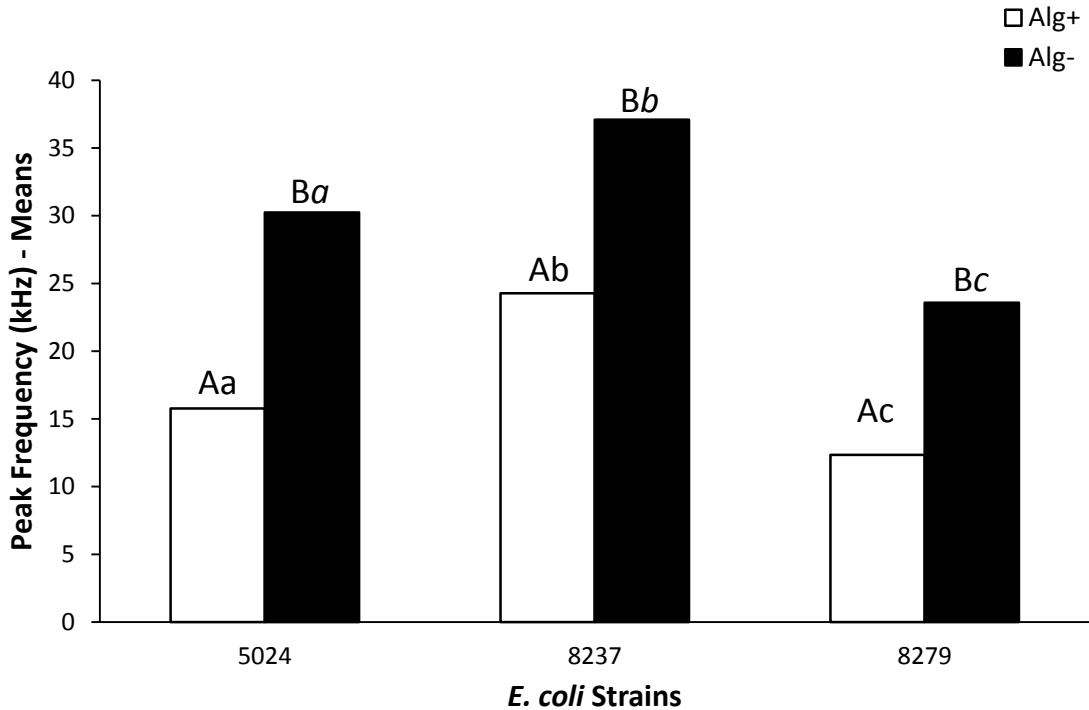


**Figure 54.** The effect of media type on the ABSE.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between the types of media.

\*Standard Error = 0.04 for medium with alginate according to the least square mean.

\*Standard Error = 0.04 for medium without alginate according to the least square mean.



**Figure 55.** The effect of media type on the average PFRQ's generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between the type of media within each strain.

\*abc=Means with different letters are significantly different ( $P < 0.0001$ ) between strains grown in medium with alginate.

\*abc=Means with different letters are significantly different ( $P < 0.0001$ ) between strains grown in medium without alginate.

\*Standard Error = 0.16 for 5024 grown in medium with alginate according to the least square mean.

\*Standard Error = 0.13 for 5024 grown in medium without alginate according to the least square mean.

\*Standard Error = 0.16 for 8237 grown in medium with alginate according to the least square mean.

\*Standard Error = 0.12 for 8237 grown in medium without alginate according to the least square mean.

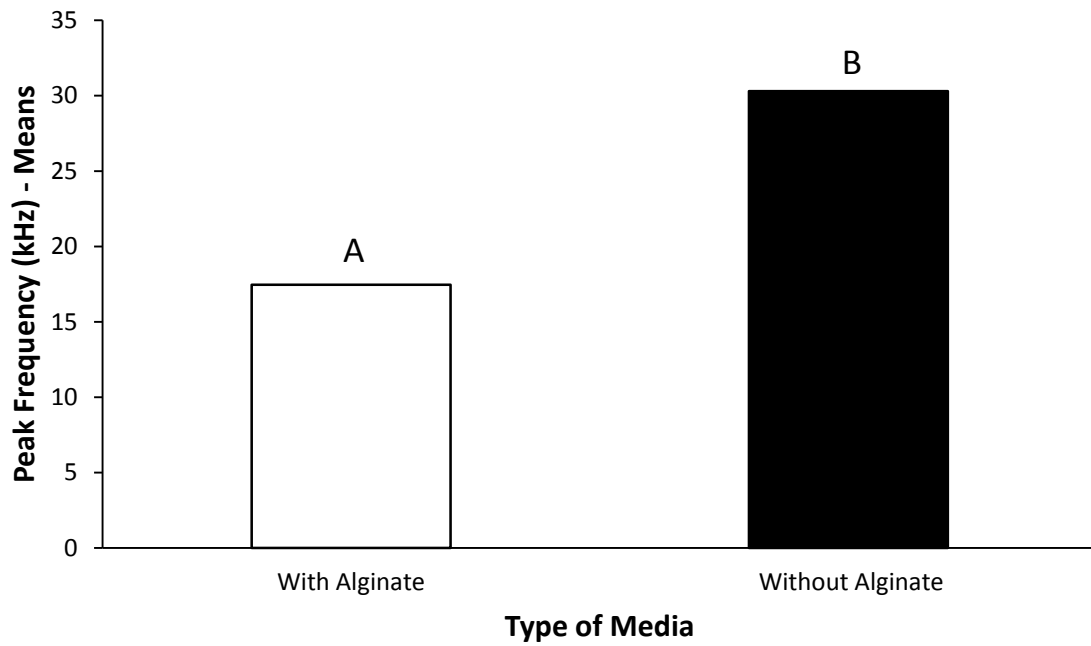
\*Standard Error = 0.13 for 8279 grown in medium with alginate according to the least square mean.

\*Standard Error = 0.14 for 8279 grown in medium without alginate according to the least square mean.

12.82 and 11.23 for strains 5024, 8237 and 8279, respectively. In medium with alginate, strain 8237 generated PFRQ's which were 54% and 96% higher than strains 5024 and 8279, respectively. The PFRQ generated by strain 5024 was also 28% ( $P < 0.0001$ ) higher than strain 8279, when grown in medium with alginate. The same was true in medium without alginate, as the generated PFRQ's from strain 8237 were 23% and 57% higher than strains 5024 and 8279, respectively. Overall, the generated PFRQ's were 74% higher ( $P < 0.0001$ ) when strains were grown in media without alginate than with alginate, shown in **Figure 56**. In comparison to the generated ABSE, the PFRQ's were 12% and 19% higher in media with and without alginate, respectively, shown in **Figure 57**.

**4.4.3. Summary of Media Effect on ABSE and PFRQ.** These findings indicated that cells were indeed settling atop the sensor, as evidenced by significant differences in the generated ABSE and PFRQ's, by strains 5024 and 8279, when grown in media with and without alginate. The settling of cells can also be supported by the fact that the overall intensity of the ABSE and concentration of PFRQ's increased 18% and 74%, respectively, in medium without alginate.

Analysis of the PFRQ data further supported these findings, which identified the generated PFRQ's to be 12-19% higher than the ABSE in media with and without alginate, respectively. The higher concentration of PFRQ's not only proved significant differences in media with and without alginate, but also the mean differences became more distinguishable between strains 5024 and 8237. In particular, the ABSE generated by strain 8237, when grown in medium without alginate, was similar to that of strain 5024, however, the generated PFRQ identified strain 8237 to be 23% higher than strain



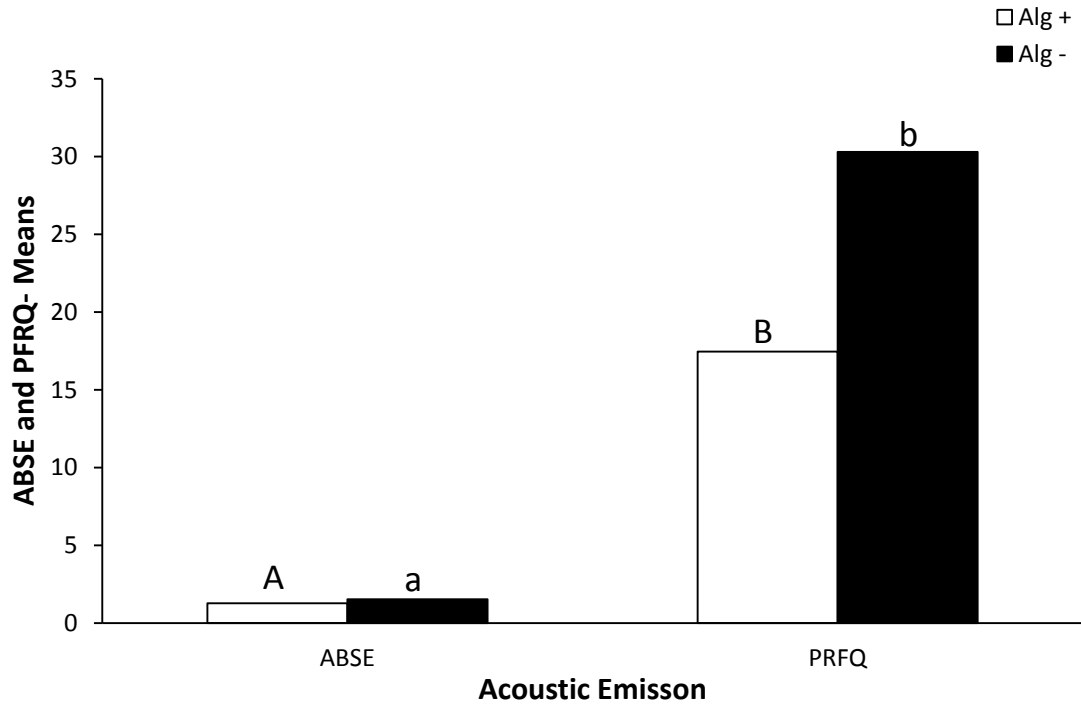
**Figure 56.** The effect of media type on the PFRQ.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between the types of media.

\*Standard Error = 0.09 for medium with alginate according to the least square mean.

\*Standard Error = 0.08 for medium without alginate according to the least square mean.





**Figure 57.** Comparison of ABSE and PRFQ mean intensities between the media type.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between the type of media.

\*abc=Means with different letters are significantly different ( $P < 0.0001$ ) between the type of media.

\*Standard Error = 0.04 for ABSE in medium with alginate according to the least square mean.

\*Standard Error = 0.09 for PRFQ in medium with alginate according to the least square mean.

\*Standard Error = 0.04 for ABSE in medium without alginate according to the least square mean.

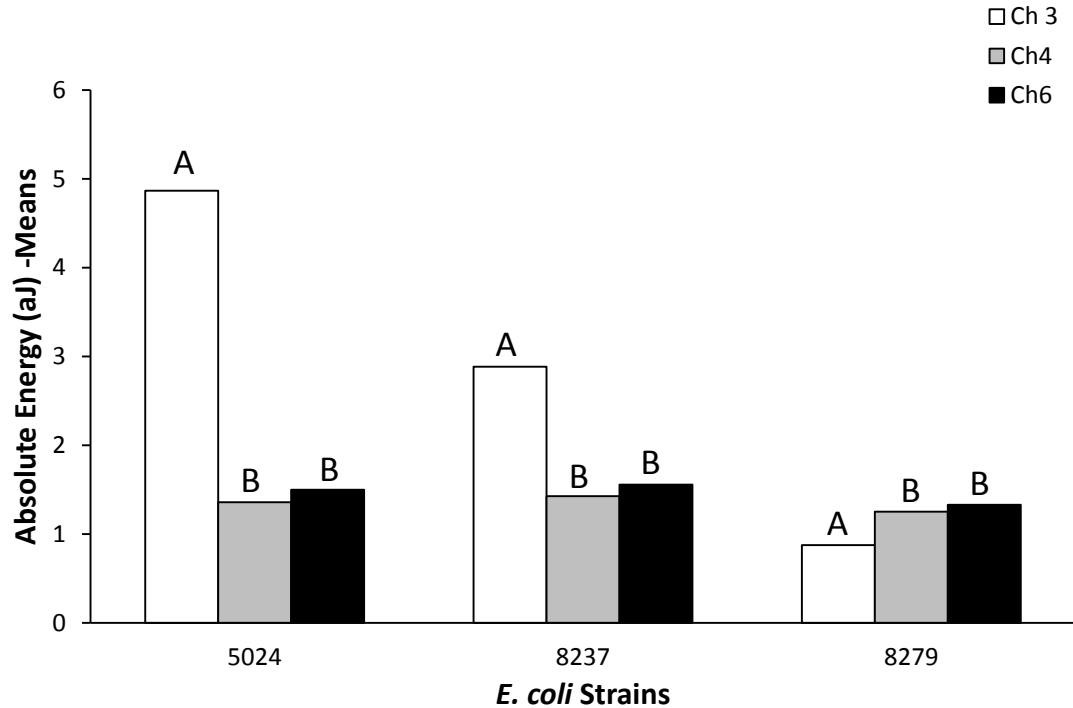
\*Standard Error = 0.07 for PRFQ in medium without alginate according to the least square mean.

5024. These differences in generated PFRQ's may be indicative of the overall cell populations at the AE sensors surface, as strain 8237 was 71% greater than 5024, thus increasing the acoustic emissions. Another indication may suggest that the parent strain 5024, which possesses the *rpoS396* gene, differs in cellular functions, metabolism and replication, than the mutant strain 8237.

#### **4.5. The Effect of Channel on ABSE and PFRQ.**

**4.5.1. Effect of Channel on ABSE.** Significant differences ( $P < 0.0001$ ) in ABSE's were observed within all strains of *E. coli*, between channels 3 and 4 and channels 3 and 6, shown in **Figure 58**. Overall, strain 5024 generated the strongest ABSE ( $P < 0.05$ ), with intensities 32% and 126% greater than strains 8237 and 8279, respectively. ABSE means collected from channels 3, 4 and 6 were 2.88, 1.33 and 1.46 aJ, respectively. **Figure 59** shows ABSE collected on all channels differed significantly ( $P < 0.0001$ ), as the ABSE on channel 3 was 116% and 97% more intense than on channels 4 and 6, respectively and the ABSE collected on channel 6 was 10% stronger ( $P < 0.05$ ) than channel 4.

**4.5.2. Effect of Channel on PFRQ.** PRFQ observations revealed significant differences ( $P < 0.0001$ ) on all channels within and between all strains of *E. coli*, as shown in **Figure 60**. In contrast to the ABSE data, the highest PFRQ's were generated by strain 8237 on all channels. PFRQ mean differences were 27.76, 15.68 and 31.62, for channels 3, 4 and 6, respectively. **Figure 61** shows PFRQ's collected on all channels differed significantly ( $P < 0.0001$ ), as the frequencies collected on channel 6 were 14%



**Figure 58.** The effect of channel on the average ABSE generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each channel within each strain.

\*Standard Error = 0.56 for Ch3 with 5024 according to the least square mean.

\*Standard Error = 0.08 for Ch4 with 5024 according to the least square mean.

\*Standard Error = 0.06 for Ch6 with 5024 according to the least square mean.

\*Standard Error = 0.31 for Ch3 with 8237 according to the least square mean.

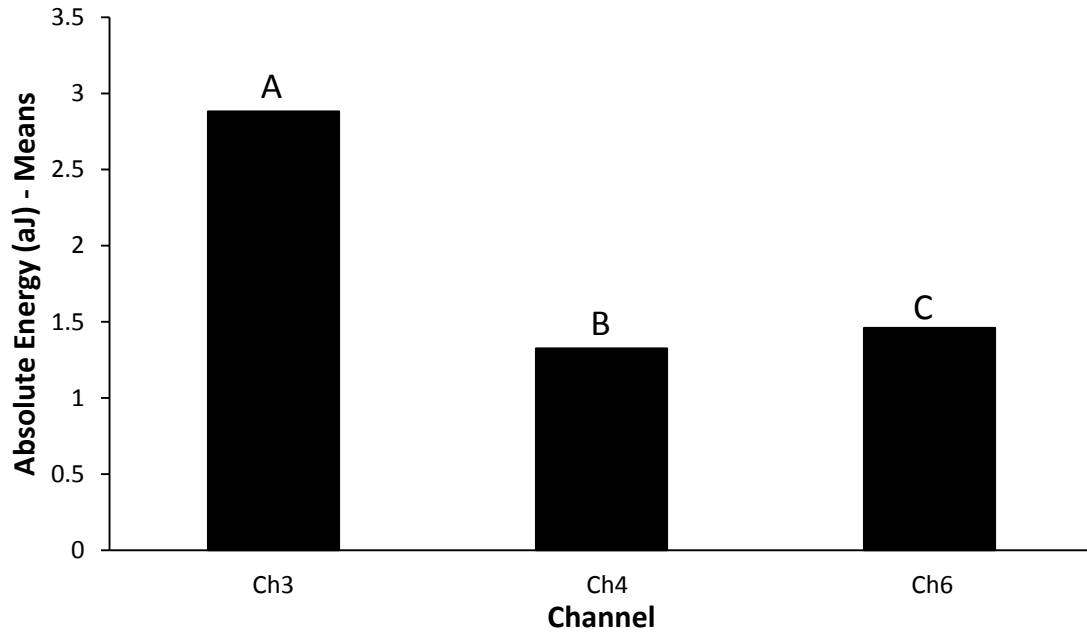
\*Standard Error = 0.08 for Ch4 with 8237 according to the least square mean.

\*Standard Error = 0.06 for Ch6 with 8237 according to the least square mean.

\*Standard Error = 0.12 for Ch3 with 8279 according to the least square mean.

\*Standard Error = 0.07 for Ch4 with 8279 according to the least square mean.

\*Standard Error = 0.07 for Ch6 with 8279 according to the least square mean.



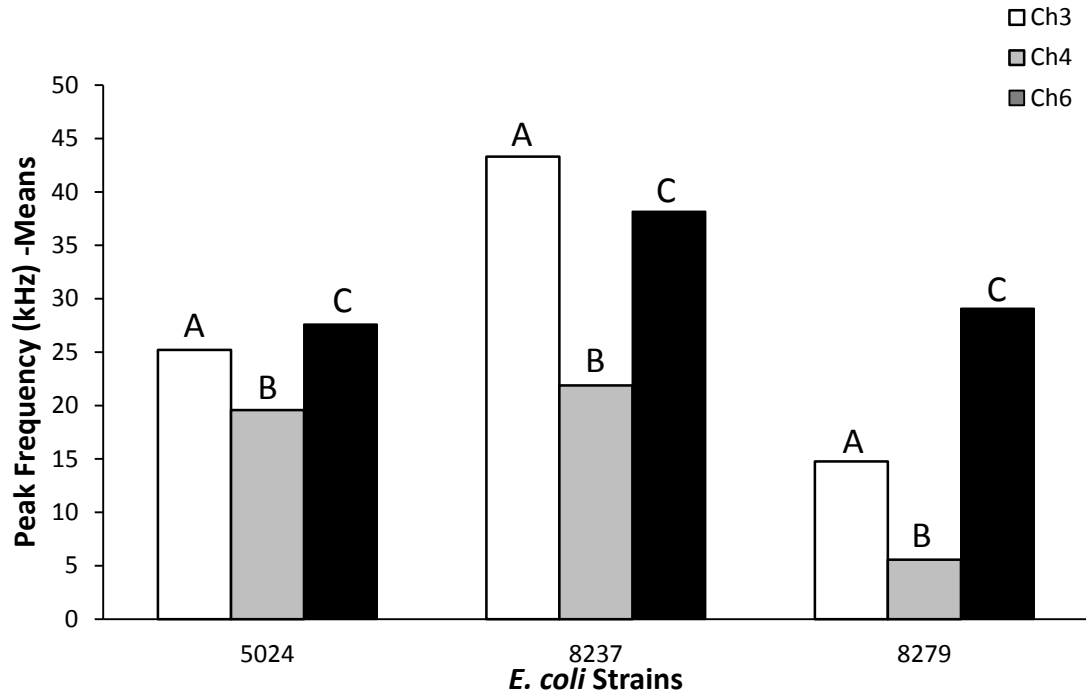
**Figure 59.** The effect of channel on the ABSE.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each channels.

\*Standard Error = 0.22 for Ch3 according to the least square mean.

\*Standard Error = 0.04 for Ch4 according to the least square mean.

\*Standard Error = 0.04 for Ch6 according to the least square mean.



**Figure 60.** The effect of channel on the average PFRQ's generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) within each strain.

\*Standard Error = 1.14 for Ch3 with 5024 according to the least square mean.

\*Standard Error = 0.16 for Ch4 with 5024 according to the least square mean.

\*Standard Error = 0.13 for Ch6 with 5024 according to the least square mean.

\*Standard Error = 0.63 for Ch3 with 8237 according to the least square mean.

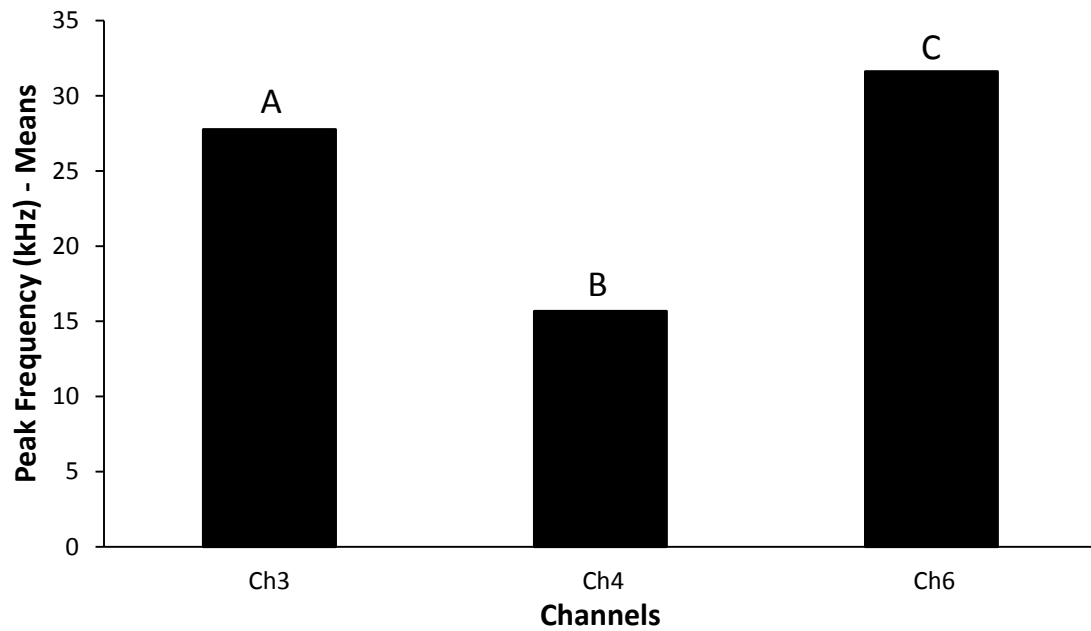
\*Standard Error = 0.16 for Ch4 with 8237 according to the least square mean.

\*Standard Error = 0.12 for Ch6 with 8237 according to the least square mean.

\*Standard Error = 0.24 for Ch3 with 8279 according to the least square mean.

\*Standard Error = 0.15 for Ch4 with 8279 according to the least square mean.

\*Standard Error = 0.14 for Ch6 with 8279 according to the least square mean.



**Figure 61.** The effect of channel on the PFRQ.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each channel.

\*Standard Error = 0.44 for Ch3 according to the least square mean.

\*Standard Error = 0.09 for Ch4 according to the least square mean.

\*Standard Error = 0.07 for Ch6 according to the least square mean.

and 102% higher than those on channels 3 and 4, respectively and those collected on channel 3 were 10% higher than those on channel 4.

**4.5.3. Summary of Channel Effect on ABSE and PFRQ.** Significant differences were observed between all channels when collecting AE's generated from different strains of *E. coli*. Overall, channels 3 and 6 were most dominate in the collection of ABSE and PFRQ's, respectively. The notable difference in channels was initially thought to be attributable to the dB levels that were ascribed to each acoustic sensor, prior to each experiment. Once the acoustic sensor is assigned to a channel, dB levels are ascribed and verified by monitoring the number of hits per second in order to eliminate unwanted background noise. A lower dB level allows the acoustic sensor to become more sensitive, thus enabling more hits per second to be collected, although many of the hits could be background noise. Channels 3 and 6 were ascribed  $\alpha$ R6 acoustic sensors 54 with dB levels of 26 and 25, respectively, while channel 4 was ascribed  $\alpha$ R6 acoustic sensors 52 with a dB level of 21. It would be anticipated that the results from channel 4, having the lowest dB level, would provide more sensitive data. However, this was not the case and therefore, the difference may lie within the acoustic sensor itself. Since each acoustic sensor is handcrafted, this may allow differences in their sensitivities. This may explain why sensor 54 was more sensitive and collected more ABSE signals and PFRQ's on channels 3 and 6, than with sensor 52 on channel 4.

Once again, differences between strains 5024 and 8237 were also observed in the channel analysis, as evidenced by strain 5024 generating a 69% stronger ABSE signal than strain 8237 on channel 3. PFRQ findings continued to support the difference

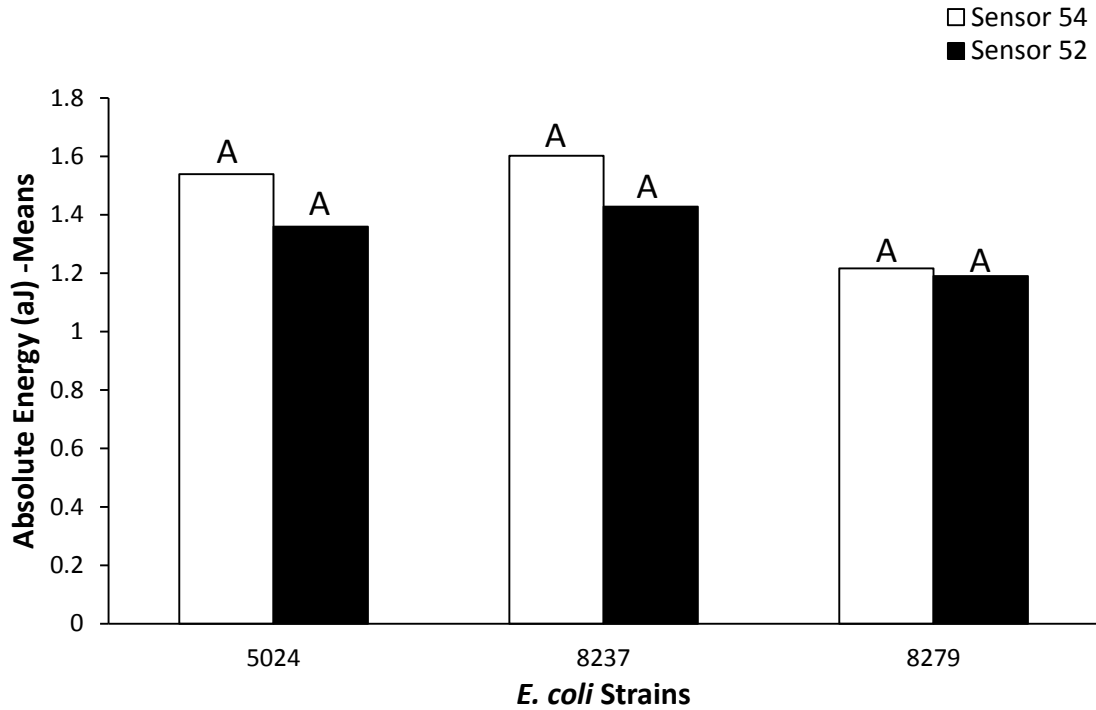
between the two strains, as the PFRQ generated from strain 8237 differed significantly from strain 5024 on all channels.

#### **4.6. The Effect of Sensor on ABSE and PFRQ.**

**4.6.1. Effect of Sensor on ABSE.** No significant differences in sensitivities, between the two R6 $\alpha$  acoustic sensors, were observed within each strain of *E. coli*, as shown in **Figure 62**. The ABSE mean differences between sensors 54 and 52 were 0.18, 0.17 and 0.03 for strains 5024, 8237 and 8279, respectively. Sensor 54 revealed significant differences ( $P < 0.0001$ ) in ABSE between strains 5024 and 8279, and strains 8237 and 8279. The intensity of ABSE generated from strain 8237 on sensor 54 was 4% ( $P < 0.45$ ) and 31% ( $P < 0.0001$ ) stronger than strains 5024 and 8279, respectively. On sensor 52, strain 8237 also generated ABSE intensities 5% ( $P < 0.54$ ) and 20% ( $P < 0.03$ ) greater than strains 5024 and 8279, respectively. **Figure 63** shows the generated ABSE from *E. coli* strains on each sensor. Sensor 54 was more sensitive than sensor 52, as evidenced by a 9% ( $P < 0.023$ ) greater collection of ABSE, as shown in **Figure 64**.

**4.6.2. Effect of Sensor on PFRQ.** Observations revealed significant differences ( $P < 0.0001$ ) in PFRQ's between both  $\alpha$ R6 acoustic sensors, within and between all strains of *E-coli* shown in **Figure 65**. Mean differences in sensor PFRQ's were 8.01, 16.46 and 19.92, for strains 5024, 8237 and 8279, respectively. On sensor 54, strain 8237 generated PFRQ's which were 39% and 50% higher than strains 5024 and 8279, respectively. The PFRQ's generated from strain 8237, on sensor 52, were also 12% and 293% higher than strains 5024 and 8279, respectively. **Figure 66** shows the generated PFRQ from *E. coli* strains on each sensor. Sensor 54 was also found to be the most





**Figure 62.** The effect of sensor on the average ABSE generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each sensor within each strain.

\*Standard Error = 0.06 for sensor 54 with 5024 according to the least square mean.

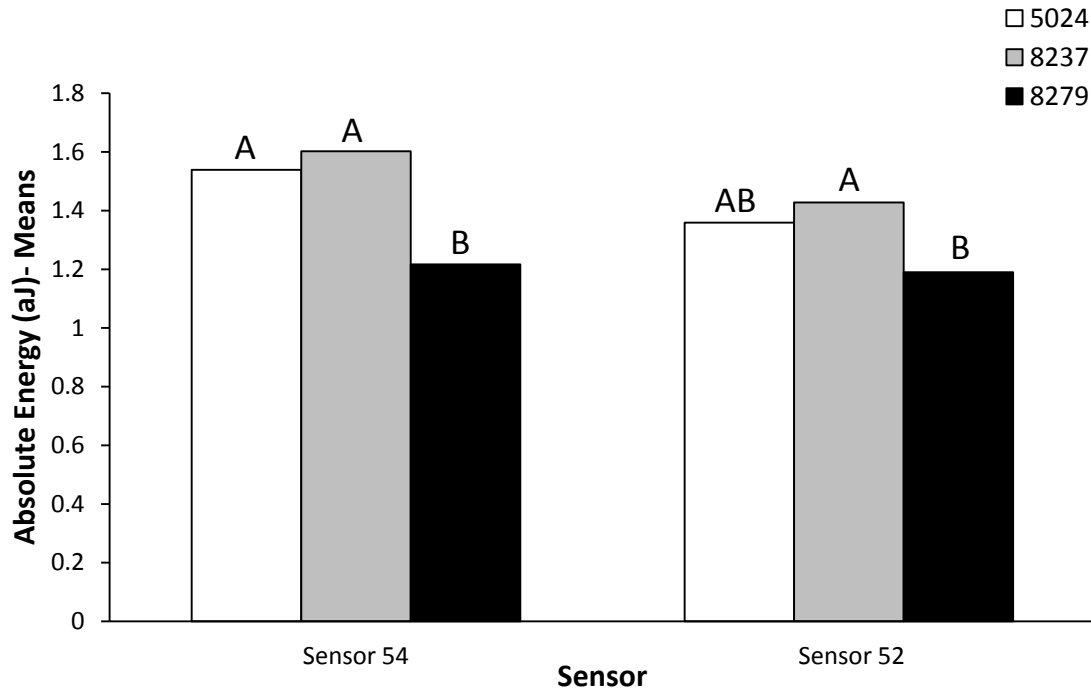
\*Standard Error = 0.08 for sensor 52 with 5024 according to the least square mean.

\*Standard Error = 0.05 for sensor 54 with 8237 according to the least square mean.

\*Standard Error = 0.08 for sensor 52 with 8237 according to the least square mean.

\*Standard Error = 0.58 for sensor 54 with 8279 according to the least square mean.

\*Standard Error = 0.07 for sensor 52 with 8279 according to the least square mean.



**Figure 63.** The average ABSE generated by *E. coli* strains on different sensors.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain within each sensor.

\*Standard Error = 0.06 for sensor 54 with 5024 according to the least square mean.

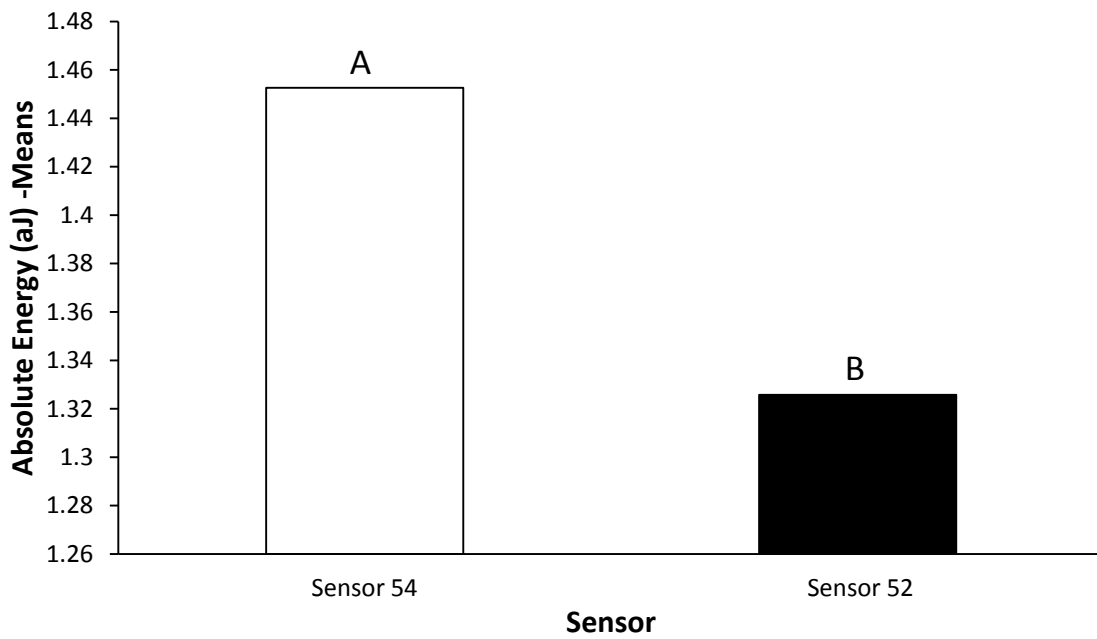
\*Standard Error = 0.05 for sensor 54 with 8237 according to the least square mean.

\*Standard Error = 0.58 for sensor 54 with 8279 according to the least square mean.

\*Standard Error = 0.08 for sensor 52 with 5024 according to the least square mean.

\*Standard Error = 0.08 for sensor 52 with 8237 according to the least square mean.

\*Standard Error = 0.07 for sensor 52 with 8279 according to the least square mean.

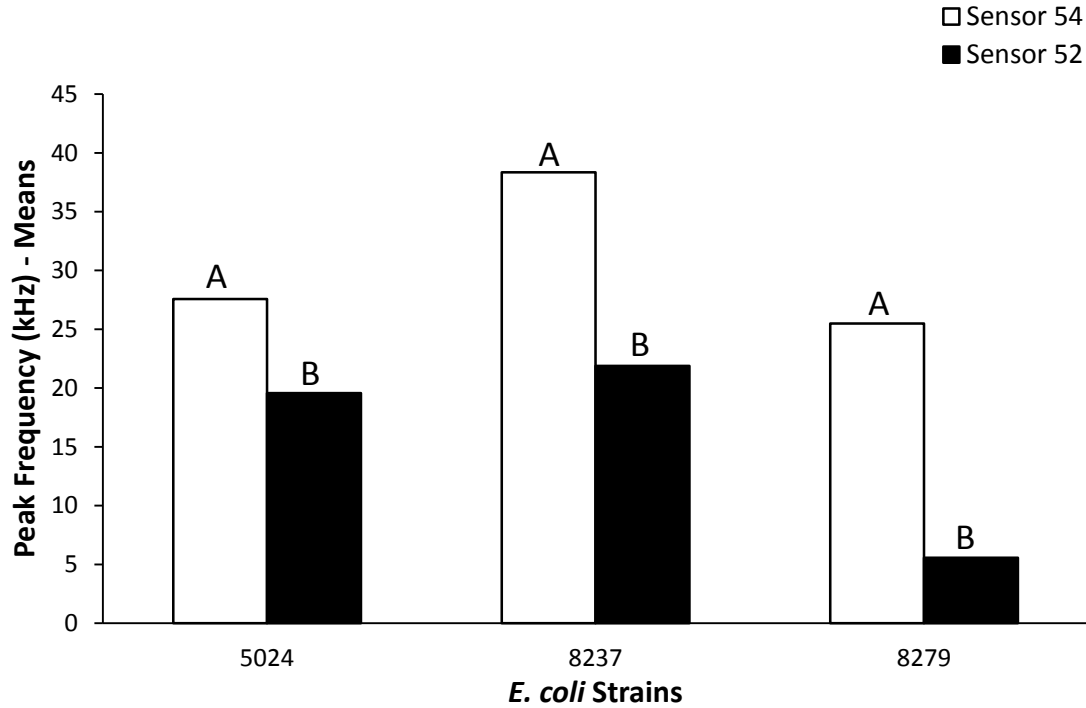


**Figure 64.** Comparison of the average ABSE between different sensors.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each sensor.

\*Standard Error = 0.03 for sensor 54 according to the least square mean.

\*Standard Error = 0.04 for sensor 52 according to the least square mean.



**Figure 65.** The average PFRQ generated by *E. coli* strains on different sensors.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each strain.

\*Standard Error = 0.12 for sensor 54 with 5024 according to the least square mean.

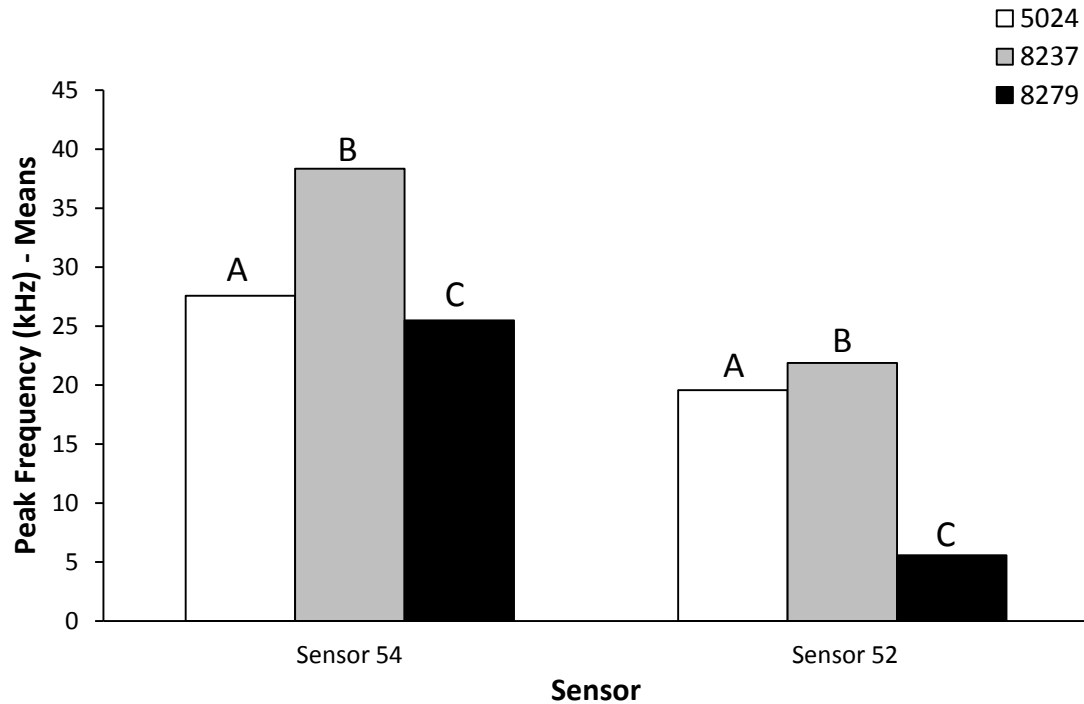
\*Standard Error = 0.16 for sensor 52 with 5024 according to the least square mean.

\*Standard Error = 0.12 for sensor 54 with 8237 according to the least square mean.

\*Standard Error = 0.16 for sensor 52 with 8237 according to the least square mean.

\*Standard Error = 0.12 for sensor 54 with 8279 according to the least square mean.

\*Standard Error = 0.15 for sensor 52 with 8279 according to the least square mean.



**Figure 66.** The effect of sensor on the average PFRQ's generated by *E. coli* strains.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each strain within each sensor.

\*Standard Error = 0.12 for sensor 54 with 5024 according to the least square mean.

\*Standard Error = 0.12 for sensor 54 with 8237 according to the least square mean.

\*Standard Error = 0.12 for sensor 54 with 8279 according to the least square mean.

\*Standard Error = 0.16 for sensor 52 with 5024 according to the least square mean.

\*Standard Error = 0.16 for sensor 52 with 8237 according to the least square mean.

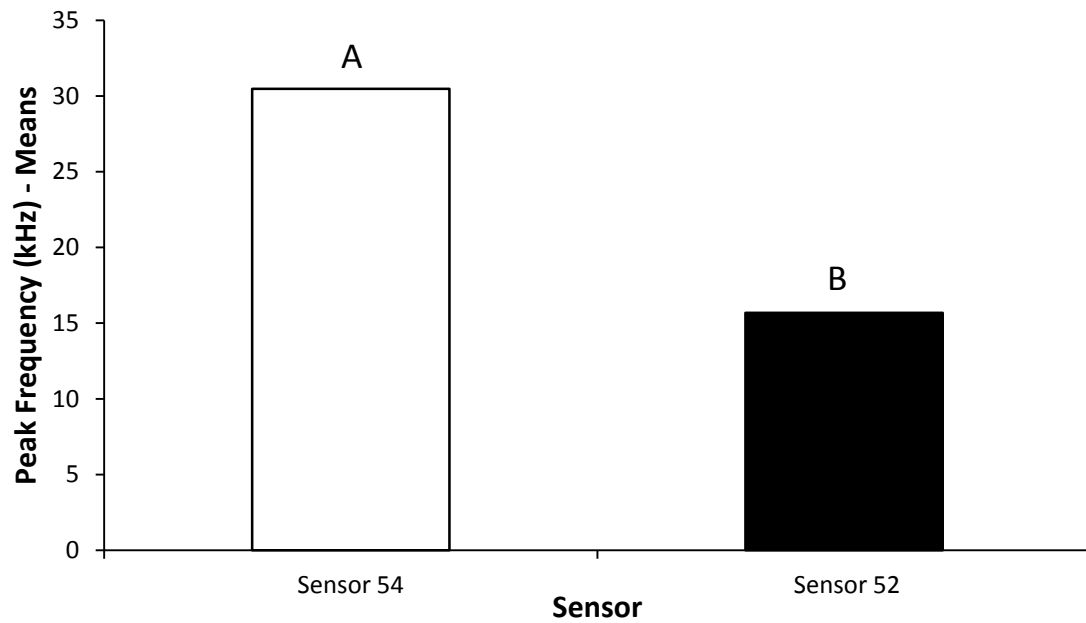
\*Standard Error = 0.15 for sensor 52 with 8279 according to the least square mean.

sensitive, as evidenced by a 94% ( $P < 0.0001$ ) greater collection of PFRQ, than that of sensor 52, shown in **Figure 67**.

**4.6.3. Summary of Sensor Effect on ABSE and PFRQ.** These observations not only revealed significant differences in the sensitivities between the R6 $\alpha$  acoustic sensors when monitoring AE's, but also distinct differences between strains 5024 and 8237. Sensor 54 was definitely more sensitive than sensor 52, as evidenced by a 9% and 94% greater collection of ABSE and PFRQ's, respectively. These findings correspond to those presented in the effect of channel summary. As previously mentioned, differences in the sensitivities of the sensors may be attributable to manufacturing of the sensors, as each  $\alpha$ R6 acoustic sensor is handcrafted, thus allowing differences in their sensitivities. Strain 8237 significantly differed from strain 5024 in generated PFRQ's. Sensor 54 revealed strain 8237 generated 4% and 39% more ABSE and PFRQ's, respectively, than strain 5024. On sensor 52, strain 8237 also generated 5% and 12% more ABSE and PFRQ's, respectively, than strain 5024.

#### **4.7. The Effect of Channel and Sensor on ABSE and PFRQ.**

**4.7.1. Effect of Channel and Sensor on ABSE.** The following are channel and sensor combinations utilized in this experiment: channel 3 with sensor 54, channel 4 with sensor 52 and channel 6 with sensor 54. The only significant difference ( $P < 0.007$ ) in ABSE was observed between channels 4 and 6, as shown in **Figure 68**. The observation revealed the ABSE intensity was strongest on channel 6 with sensor 54, which was 15% and 11% more intense than channel 3 with sensor 54 and channel 4 with sensor 52, respectively.

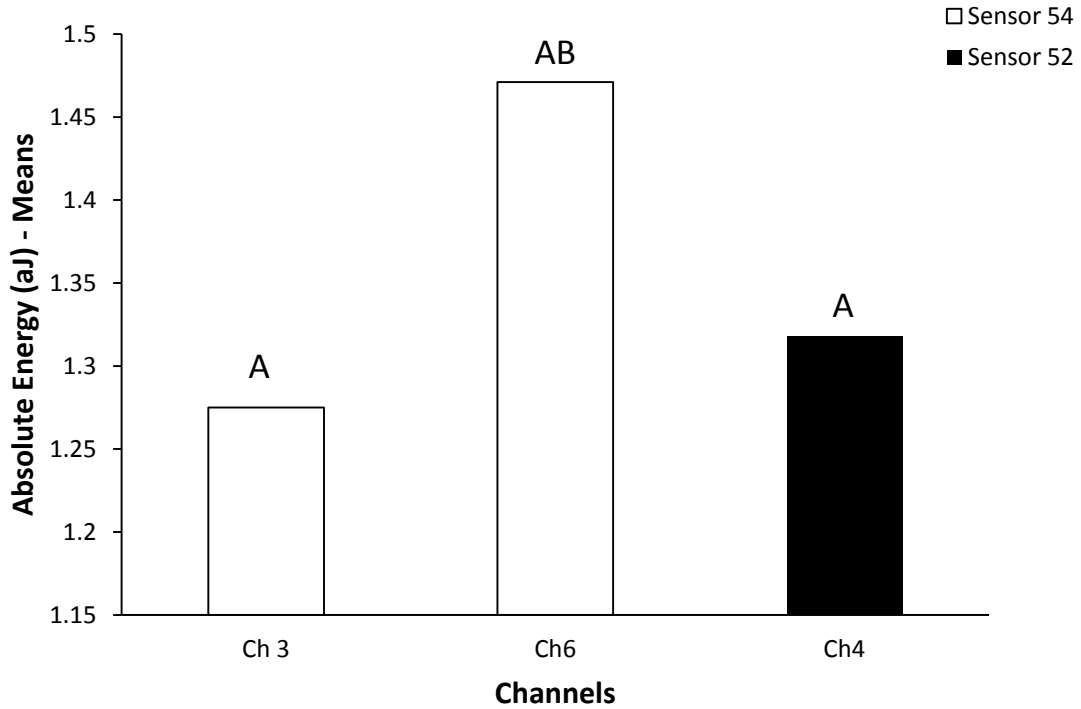


**Figure 67.** Comparison of the average PFRQ's between different sensors.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each sensor.

\*Standard Error = 0.07 for sensor 54 according to the least square mean.

\*Standard Error = 0.09 for sensor 52 according to the least square mean.



**Figure 68.** The effect of sensor on the average ABSE with different channels. Channel combinations utilized in study: Channel 3 with sensor 54, Channel 4 with sensor 52 and Channel 6 with sensor 54.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each channel.

\*Standard Error = 0.16 for Ch3 with sensor 54 according to the least square mean.

\*Standard Error = 0.04 for Ch6 with sensor 54 according to the least square mean.

\*Standard Error = 0.04 for Ch4 with sensor 52 according to the least square mean.



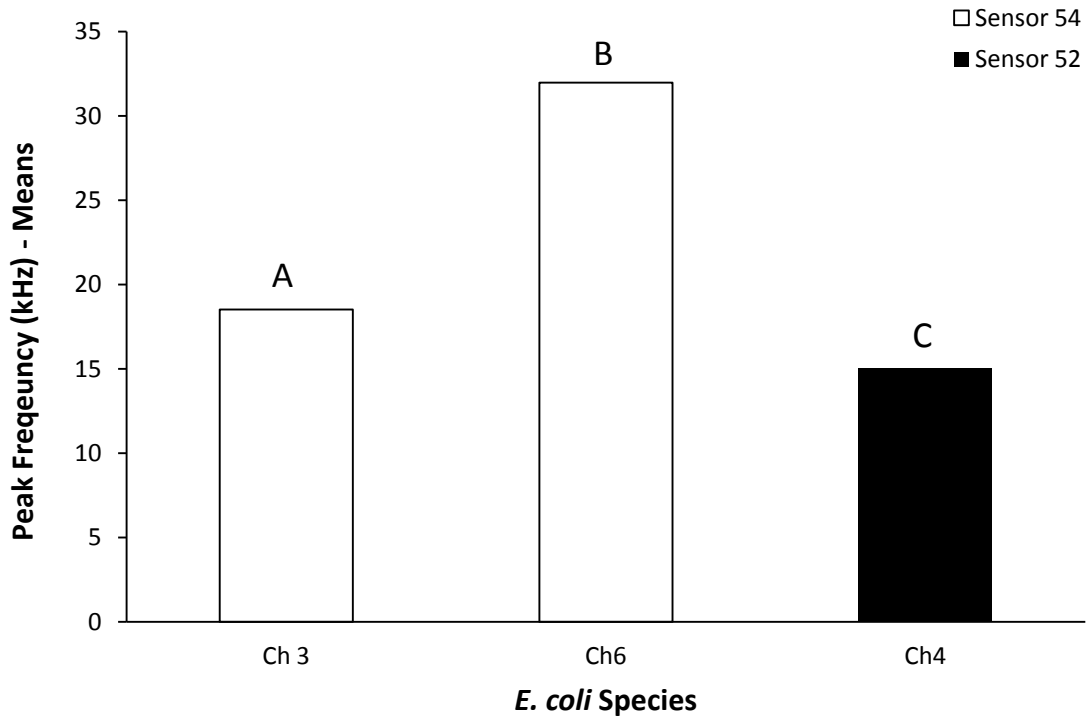
**4.7.2. Effect of Channel and Sensor on PFRQ.** In contrast to the ABSE, significant differences ( $P < 0.0001$ ) in PFRQ's were observed between both sensors on and between all three channels, as shown in **Figure 69**. Channel 6 with sensor 54 had the highest collection of generated PFRQ's, 113% and 73% more than channel 4 with sensor 52 and channel 3 with sensor 54, respectively.

**4.7.3. Summary of Channel and Sensor Effect on ABSE and PFRQ.** Results from the observations indicate sensitivities of the sensors vary on different channels and appear to be more significant with PFRQ intensities. These results correspond with results of individual channel and sensor observations and conclude that the AE differences in the channel and sensors is ultimately attributable to the handcrafting of each R6 $\alpha$  acoustic sensor, thus allowing the sensitivity to vary between them.

#### **4.8. Comparison of *E. coli* Strains 5024 and 8237 without Alginate.**

Observations continued to focus on *E. coli* 5024 (parent strain) and 8237 (mutant strain) in order to determine if any further significant differences existed. The following observations investigated differences in the generated ABSE and PFRQ, within different time periods, as well as, various kHz ranges, between *E. coli* strains 5024 and 8237, grown in medium without alginate

**4.8.1. Comparison of ABSE at Select Times.** Initially, three time periods were observed (0-2h, 2-6h and 6-9h) for ABSE differences, revealing a significant difference ( $P < 0.0001$ ) only within 2-6 h, between strains 5024 and 8237, as shown in **Figure 70**. Hourly comparisons were then analyzed and revealed significant differences ( $P = 0.03$ ) in the ABSE between 1-2 h and every hour in between 3-9 h, as shown in **Figure 71**. Strain



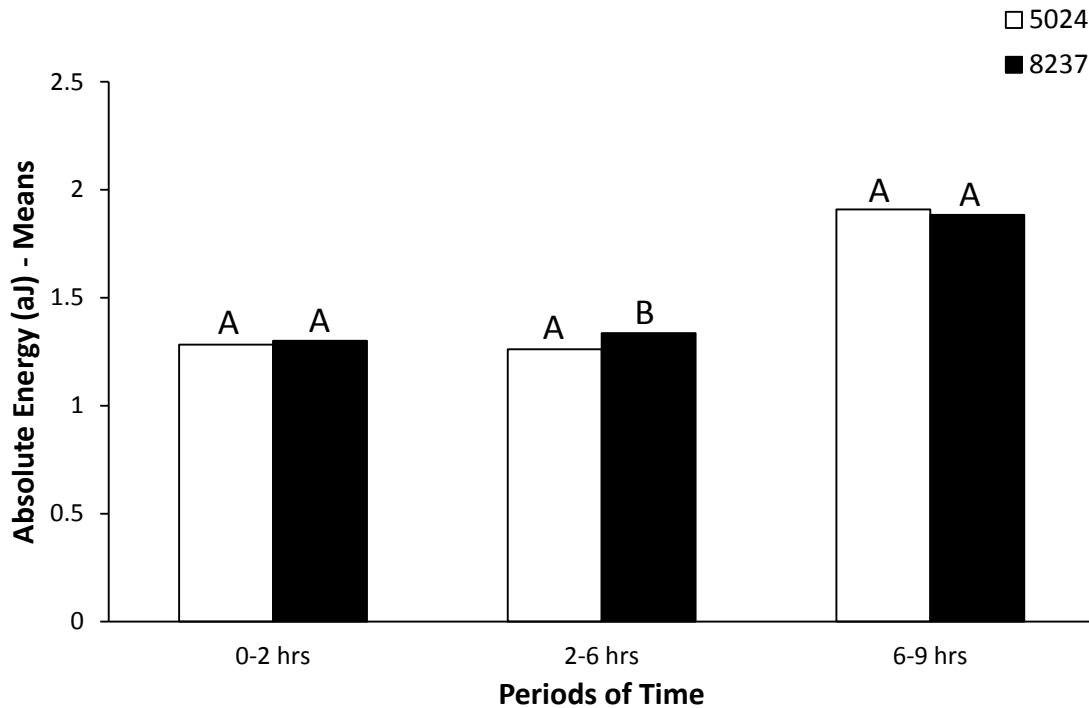
**Figure 69.** The effect of sensor on the average PFRQ's with different channels. Channel combinations utilized in study: Channel 3 with sensor 54, Channel 4 with sensor 52 and Channel 6 with sensor 54.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each channel.

\*Standard Error = 0.22 for Ch3 with sensor 54 according to the least square mean.

\*Standard Error = 0.07 for Ch6 with sensor 54 according to the least square mean.

\*Standard Error = 0.09 for Ch4 with sensor 52 according to the least square mean.

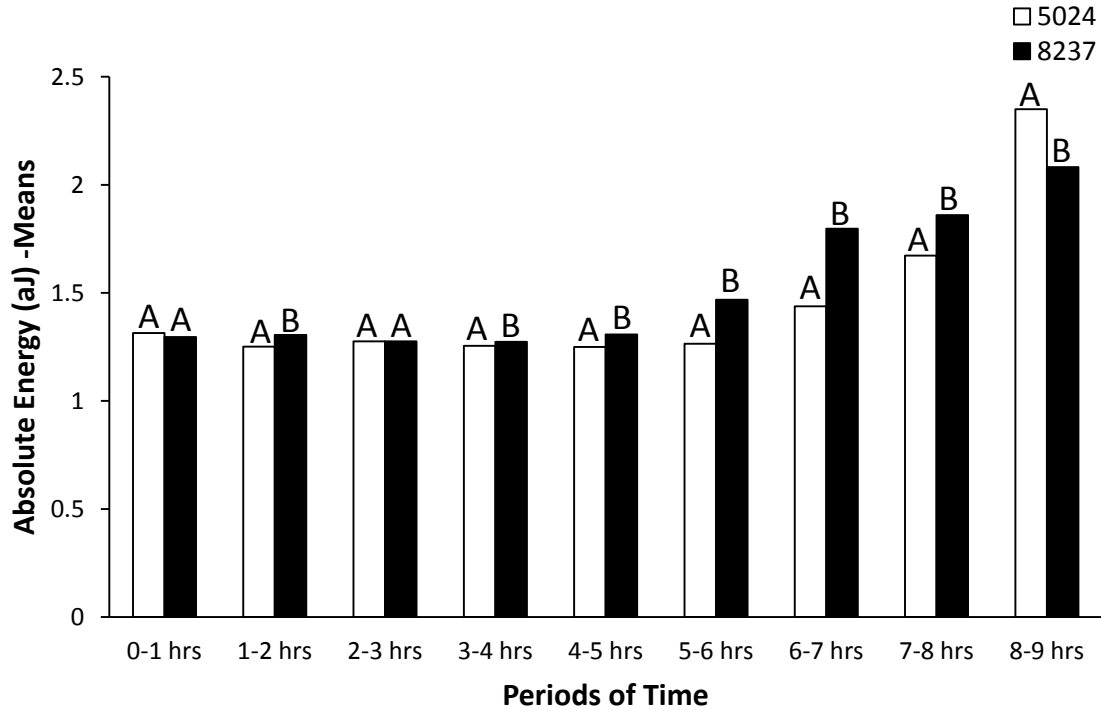


**Figure 70.** Comparison of the average ABSE between *E. coli* strains 5024 and 8237, when grown in medium without alginate, within select periods of time .

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each time period.

\*Standard Error = 0.005 for 5024 during 2-6h according to the least square mean.

\*Standard Error = 0.004 for 8237 during 2-6h according to the least square mean.



**Figure 71.** Hourly comparison of the average ABSE generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.018 for 5024 during 1-2h according to the least square mean.

\*Standard Error = 0.017 for 8237 during 1-2h according to the least square mean.

\*Standard Error = 0.005 for 5024 during 3-4h according to the least square mean.

\*Standard Error = 0.004 for 8237 during 3-4h according to the least square mean.

\*Standard Error = 0.003 for 5024 during 4-5h according to the least square mean.

\*Standard Error = 0.003 for 8237 during 4-5h according to the least square mean.

\*Standard Error = 0.015 for 5024 during 5-6h according to the least square mean.

\*Standard Error = 0.013 for 8237 during 5-6h according to the least square mean.

\*Standard Error = 0.030 for 5024 during 6-7h according to the least square mean.

\*Standard Error = 0.024 for 8237 during 6-7h according to the least square mean.

\*Standard Error = 0.029 for 5024 during 7-8h according to the least square mean.

\*Standard Error = 0.025 for 8237 during 7-8h according to the least square mean.

\*Standard Error = 0.032 for 5024 during 8-9h according to the least square mean.

\*Standard Error = 0.045 for 8237 during 8-9h according to the least square mean.

8237 generated ABSE intensities that were overall 12% stronger than strain 5024 from the 3<sup>rd</sup> -8<sup>th</sup> h, while strain 5024 was 15% stronger than strain 8237 during the final hour of growth. **Table 8** shows the ABSE means for strains 5024 and 8237 and the number of observations that occurred during each period of time.

**4.8.2. Comparison of PFRQ at Select Times.** Significant differences ( $P < 0.0001$ ) in the generated PFRQ's were revealed in all three initial time periods between strains 5024 and 8237, as shown in **Figure 72**. Each hourly observation, from 0-9 h, revealed significant differences ( $P = 0.0002$ ) in the generated PFRQ'S between both strains, as shown in **Figure 73**. Overall, the PFRQ generated by strain 8237 was 51% higher than strain 5024 from 0-8 h. However, strain 5024 was 31% higher than 8237 from 8-9 h. These findings correspond to those of ABSE, though the generated PFRQ's were much higher. The PFRQ means and significant differences for each strain of *E. coli*, at select time periods, along with the number of observations are listed in **Table 9**.

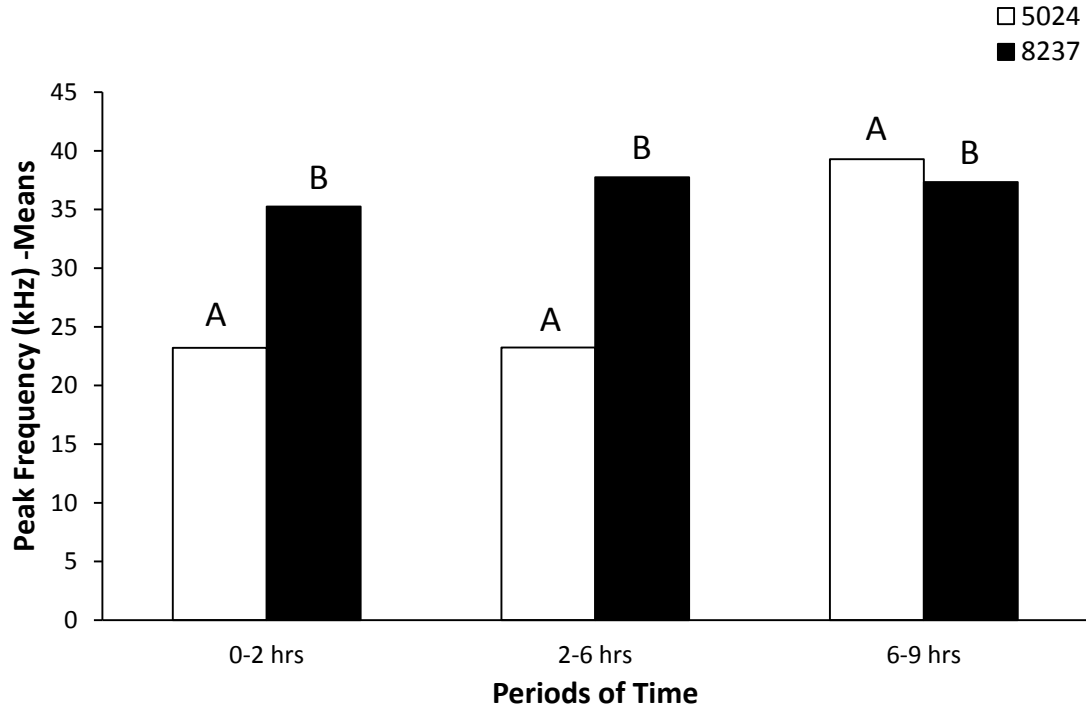
**4.8.3. Summary of ABSE and PFRQ at Select Times.** Initially, the ABSE generated from strains 5024 and 8237 only differed within the observed 2-6 h period, however, upon further investigation significant differences ( $P = 0.03$ ) were observed hourly from 1-2 h and 3-9 h. Observations of PFRQ's further justified distinguishable differences between strains 5024 and 8237, as evidenced by significant differences ( $P = 0.0002$ ) in all time periods. AE's were more dominant coming from strain 8237 during 0-8 h, while strain 5024 generated more intensity during the final hour. These differences may be attributable to possible differences in cellular activity and metabolism of each strain.

**Table 8.** Average ABSE for *E. coli* strains 5024 and 8237 at different time periods.

Period of Time (Hours)	Average ABSE for <i>E. coli</i> Strains without Alginate		Number of Observations
	5024	8237	
0-2	1.28	1.30	105420
2-6 <sup>††</sup>	1.26	1.34	215975
6-9	1.91	1.88	229081
0-1	1.31	1.30	53016
1-2 <sup>†</sup>	1.25	1.30	52404
2-3	1.28	1.28	52588
3-4 <sup>†</sup>	1.26	1.27	52674
4-5 <sup>††</sup>	1.25	1.31	53147
5-6 <sup>††</sup>	1.26	1.47	57566
6-7 <sup>††</sup>	1.44	1.80	71307
7-8 <sup>††</sup>	1.67	1.86	84268
8-9 <sup>††</sup>	2.35	2.08	73504

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$



**Figure 72.** Comparison of the average PFRQ's between *E. coli* strains 5024 and 8237, when grown in medium without alginate, within select periods of time.

\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each strain.

\*Standard Error = 0.35 for 5024 during 0-2h according to the least square mean.

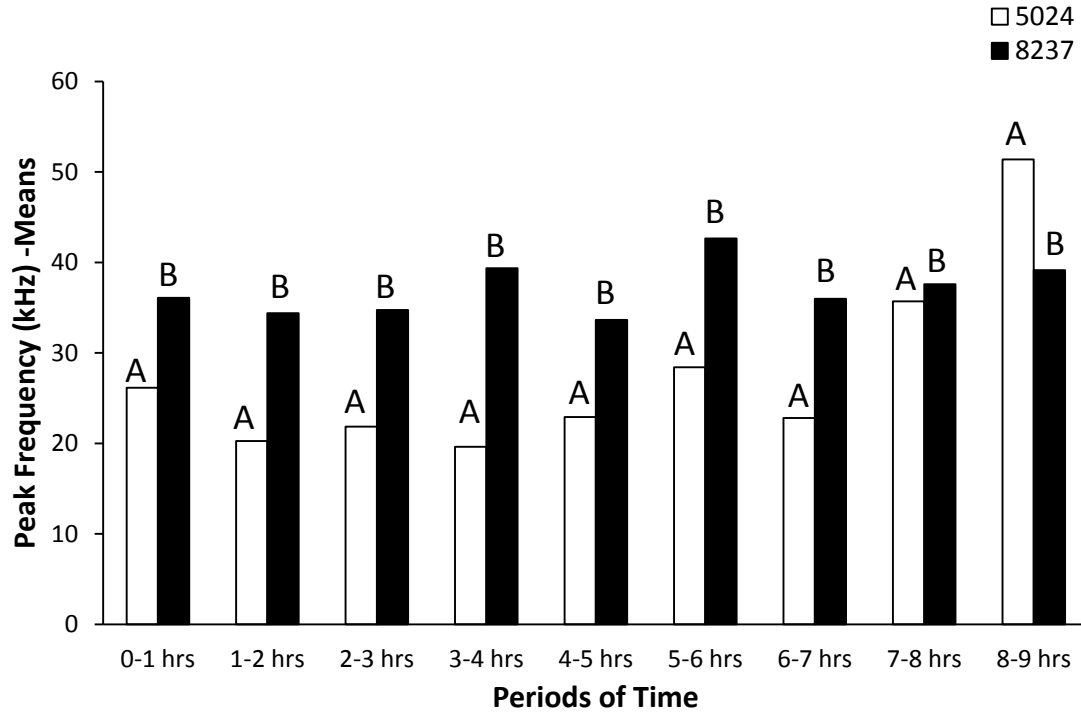
\*Standard Error = 0.32 for 8237 during 0-2h according to the least square mean.

\*Standard Error = 0.25 for 5024 during 2-6h according to the least square mean.

\*Standard Error = 0.23 for 8237 during 2-6h according to the least square mean.

\*Standard Error = 0.21 for 5024 during 6-9h according to the least square mean.

\*Standard Error = 0.21 for 8237 during 6-9h according to the least square mean.



**Figure 73.** Hourly comparison of the average PFRQ's generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.51 for 5024 during 0-1h according to the least square mean.

\*Standard Error = 0.47 for 8237 during 0-1h according to the least square mean.

\*Standard Error = 0.49 for 5024 during 1-2h according to the least square mean.

\*Standard Error = 0.44 for 8237 during 1-2h according to the least square mean.

\*Standard Error = 0.50 for 5024 during 2-3h according to the least square mean.

\*Standard Error = 0.45 for 8237 during 2-3h according to the least square mean.

\*Standard Error = 0.50 for 5024 during 3-4h according to the least square mean.

\*Standard Error = 0.46 for 8237 during 3-4h according to the least square mean.

\*Standard Error = 0.49 for 5024 during 4-5h according to the least square mean.

\*Standard Error = 0.44 for 8237 during 4-5h according to the least square mean.

\*Standard Error = 0.53 for 5024 during 5-6h according to the least square mean.

\*Standard Error = 0.46 for 8237 during 5-6h according to the least square mean.

\*Standard Error = 0.42 for 5024 during 6-7h according to the least square mean.

\*Standard Error = 0.34 for 8237 during 6-7h according to the least square mean.

\*Standard Error = 0.39 for 8237 during 7-8h according to the least square mean.

\*Standard Error = 0.33 for 8237 during 7-8h according to the least square mean.

\*Standard Error = 0.31 for 8237 during 8-9h according to the least square mean.

\*Standard Error = 0.44 for 8237 during 8-9h according to the least square mean.



**Table 9.** Average PFRQ for *E. coli* strains 5024 and 8237 at different time periods.

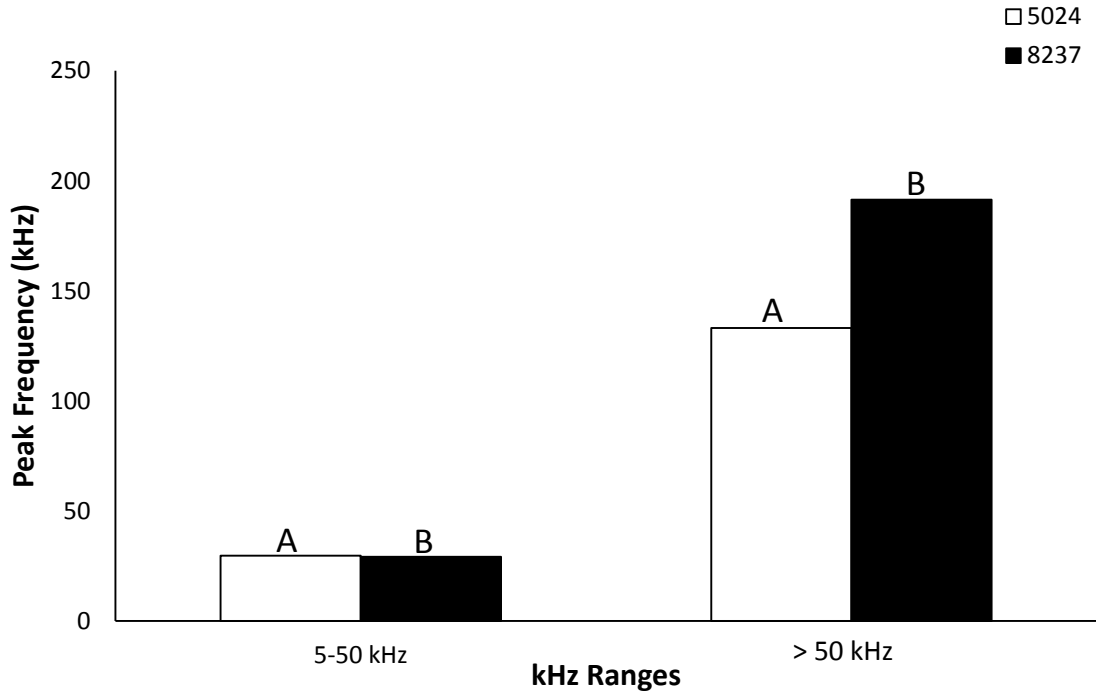
Period of Time (Hours)	Average PFRQ for <i>E. coli</i> Strains without Alginate		Number of Observations
	5024	8237	
0-2 <sup>††</sup>	23.21	35.25	105420
2-6 <sup>††</sup>	23.22	37.75	215975
6-9 <sup>††</sup>	39.28	37.33	229081
0-1 <sup>††</sup>	26.14	36.08	53016
1-2 <sup>††</sup>	20.27	34.40	52404
2-3 <sup>††</sup>	21.85	34.74	52588
3-4 <sup>††</sup>	19.62	39.36	52674
4-5 <sup>††</sup>	22.92	33.5	53147
5-6 <sup>†</sup>	28.41	42.65	57566
6-7 <sup>††</sup>	22.82	35.99	71307
7-8 <sup>†</sup>	35.71	37.58	84268
8-9 <sup>††</sup>	51.38	39.15	73504

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$

**4.8.4. Comparison of PFRQ Ranges.** Initial observations of the frequency range between 5-50 kHz, revealed the generated PFRQ's from strain 5024 were 2% ( $P < 0.0001$ ) higher than strain 8237. However, beyond the 50 kHz range, strain 8237 generated frequencies 44% ( $P < 0.0001$ ) higher than strain 5024, as shown in **Figure 74**. In order to determine where strain differences occurred beyond 50 kHz, further investigations revealed neither strain generated detectable PFRQ's beyond 500 kHz and therefore, the range from 100 to 500 kHz was established and analyzed. Observations from 100-500 kHz, revealed the PFRQ's generated by strain 8237 were 23% ( $P < 0.0001$ ) higher than strain 5024, shown in **Figure 75**. When the frequency range was analyzed by 100 kHz increments, the generated PFRQ's of strain 8237 were 15% and 5% higher ( $P < 0.0001$ ) than strain 5024, in the ranges of 100-200 kHz and 200-300 kHz, respectively. In the 300-400 kHz range, strain 5024 was 0.06% higher ( $P < 0.0002$ ) than strain 8237. Though PFRQ's were generated by both strains in the 400-500 kHz range, no significant differences were observed. **Table 10** shows all of the means and significant differences for both strains of *E. coli*, for each kHz range and the number of observations.

The 100 kHz increments were then analyzed by every 5 kHz, to determine if any distinct differences were present between strains 5024 and 8237. Observations revealed both strains differed significantly in their generated PFRQ's, as strain 8237 generated higher frequencies in the 100-300 kHz range, while strain 5024 did so in the 300-400 kHz range. From 100-200 kHz, strain 8237 generated higher ( $P < 0.05$ ) frequencies in the ranges of 105-110 kHz and 155 to 170 kHz, while strain 5024 was significantly higher ( $P < 0.0001$ ) in the 140-145 kHz range, shown in **Figure 76**. In the 200-300 kHz



**Figure 74.** Comparison of the average PFRQ's between 5 to 50 kHz and beyond 50 kHz generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

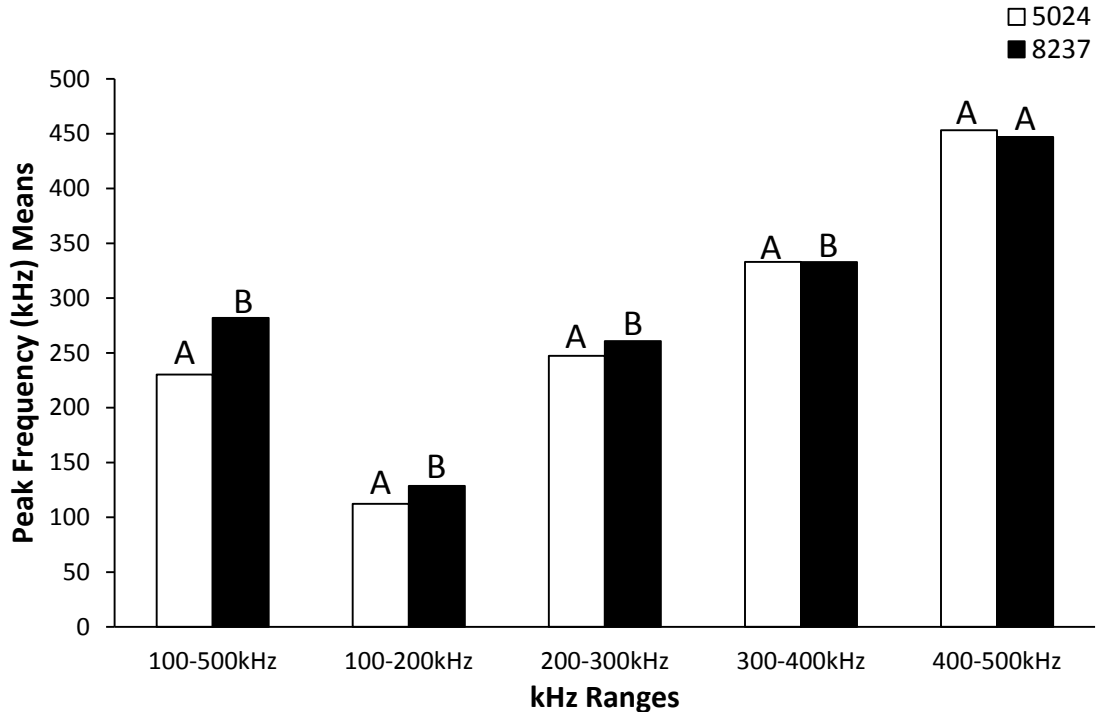
\*ABC=Means with different letters are significantly different ( $P < 0.0001$ ) between each strain.

\*Standard Error = 0.03 for 5024 within 5-50 kHz according to the least square mean.

\*Standard Error = 0.03 for 8237 within 5-50 kHz according to the least square mean.

\*Standard Error = 0.45 for 5024 beyond 50 kHz according to the least square mean.

\*Standard Error = 0.44 for 8237 beyond 50 kHz according to the least square mean.



**Figure 75.** Comparison of the average PFRQ's from 100 to 500 kHz generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.76 for 5024 within 100-500 kHz according to the least square mean.

\*Standard Error = 0.56 for 8237 within 100-500 kHz according to the least square mean.

\*Standard Error = 0.31 for 5024 within 100-200 kHz according to the least square mean.

\*Standard Error = 0.36 for 8237 within 100-200 kHz according to the least square mean.

\*Standard Error = 0.68 for 5024 within 200-300 kHz according to the least square mean.

\*Standard Error = 0.25 for 8237 within 200-300 kHz according to the least square mean.

\*Standard Error = 0.04 for 5024 within 300-400 kHz according to the least square mean.

\*Standard Error = 0.03 for 8237 within 300-400 kHz according to the least square mean.

\*Standard Error = 4.00 for 5024 within 400-500 kHz according to the least square mean.

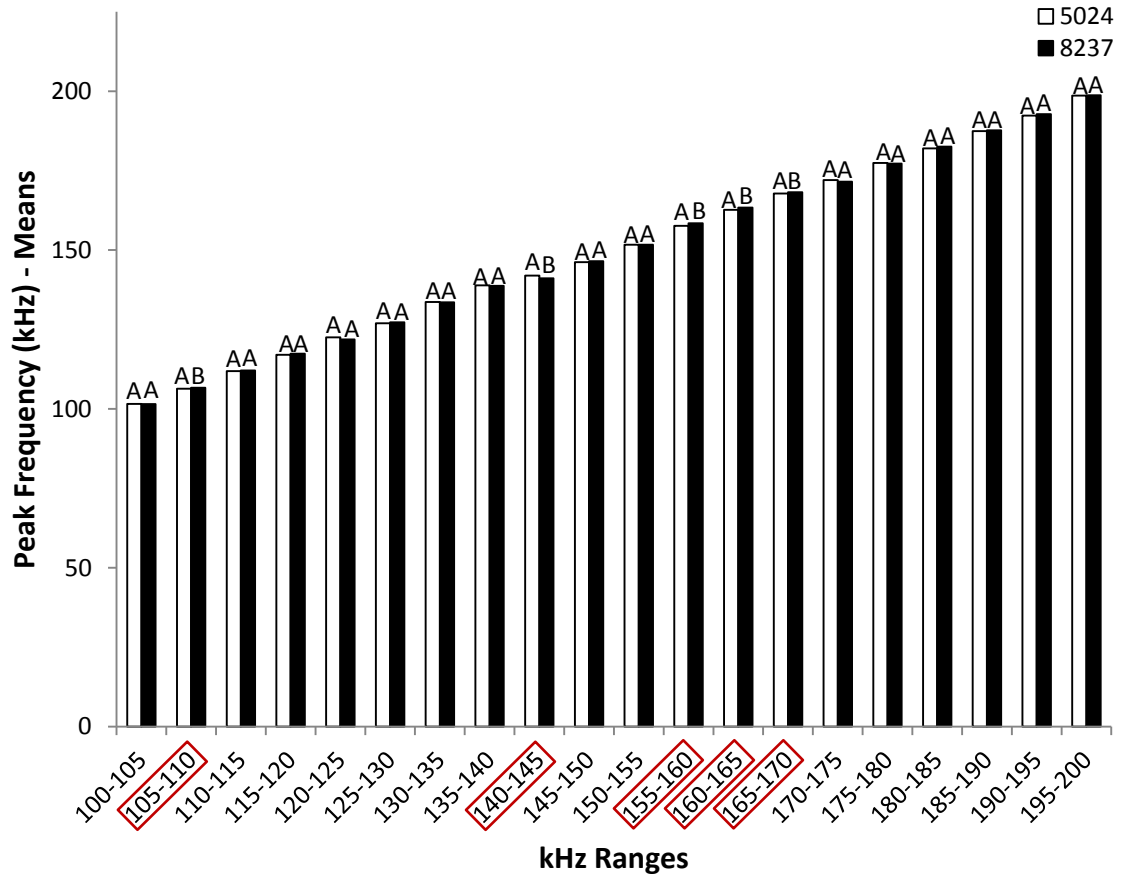
\*Standard Error = 3.08 for 8237 within 400-500 kHz according to the least square mean.

**Table 10.** Average PFRQ for *E. coli* strains 5024 and 8237 at different kHz ranges.

Peak Frequency (kHz)	Average PFRQ for <i>E. coli</i> Strains without Alginate		Number of Observations
	5024	8237	
5-50 <sup>†</sup>	29.79	29.15	86471
>50 <sup>††</sup>	133.13	191.44	99065
100-500 <sup>††</sup>	230.22	281.95	40538
100-200 <sup>††</sup>	112.40	128.73	10970
200-300 <sup>††</sup>	247.43	260.86	6457
300-400 <sup>†</sup>	333.11	332.92	23016
400-500	453.18	447.14	121

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$



**Figure 76.** Comparison of the average PFRQ's from 100 to 200 kHz (every 5 kHz) generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.05 for 5024 within 105-110 kHz according to the least square mean.

\*Standard Error = 0.08 for 8237 within 105-110 kHz according to the least square mean.

\*Standard Error = 0.08 for 5024 within 140-145 kHz according to the least square mean.

\*Standard Error = 0.11 for 8237 within 140-145 kHz according to the least square mean.

\*Standard Error = 0.17 for 5024 within 155-160 kHz according to the least square mean.

\*Standard Error = 0.19 for 8237 within 155-160 kHz according to the least square mean.

\*Standard Error = 0.17 for 5024 within 160-165 kHz according to the least square mean.

\*Standard Error = 0.05 for 8237 within 160-165 kHz according to the least square mean.

\*Standard Error = 0.14 for 5024 within 165-170 kHz according to the least square mean.

\*Standard Error = 0.04 for 8237 within 165-170 kHz according to the least square mean.

**Table 11.** Average PFRQ for *E. coli* strains 5024 and 8237 from 100-200 kHz in 5 kHz increments.

Peak Frequency (kHz)	Average PFRQ for <i>E. coli</i> Strains without Alginate		Number of Observations
	5024	8237	
100-105	101.58	101.52	6275
105-110 <sup>†</sup>	106.34	106.67	1319
110-115	111.88	112.12	330
115-120	117.03	117.39	177
120-125	122.53	121.86	100
125-130	126.98	127.26	74
130-135	133.65	133.57	231
135-140	138.87	138.72	841
140-145 <sup>††</sup>	141.95	141.11	787
145-150	146.20	146.49	140
150-155	151.70	151.67	35
155-160 <sup>†</sup>	157.61	158.44	116
160-165 <sup>†</sup>	162.69	163.35	548
165-170 <sup>†</sup>	167.80	168.22	1022
170-175	172.01	171.55	220
175-180	177.40	177.22	48
180-185	182.00	182.54	27
185-190	187.43	187.69	20
190-195	192.33	192.80	24
195-200	198.62	198.71	20

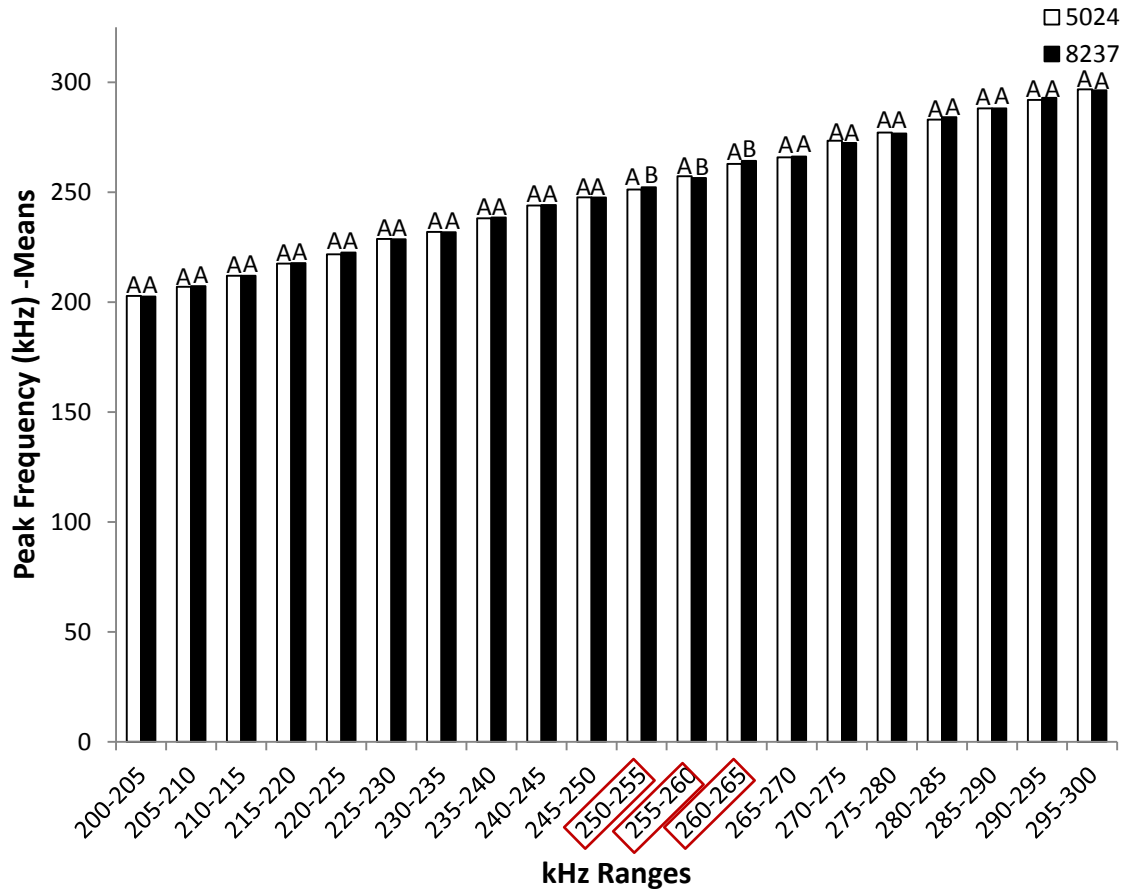
<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$

range, strain 8237 also generated higher ( $P < 0.0001$ ) frequencies in the ranges of 250-255 kHz and 260-265 kHz, while strain 5024 was significantly higher ( $P < 0.05$ ) in the 255-260 kHz range, shown in **Figure 77**. The frequencies were significantly higher ( $P < 0.05$ ) in strain 5024 in the ranges of 300-305 kHz and 350-355 kHz, while strain 8237 was significantly higher ( $P < 0.05$ ) in the 365-370 kHz range, shown in **Figure 78**. In the 400-500 kHz range (**Figure 79**), strain 8237 was significantly higher ( $P < 0.05$ ) in the range of 405-410, while strain 5024 was significantly higher ( $P < 0.05$  and  $P < 0.0001$ ) in the ranges 425-430 kHz and 475-480 kHz, respectively. **Tables 11-14** precede Figures 74-77, showing the means and significant differences for both strains of *E. coli*, for each kHz range and the number of observations.

**4.8.5. Summary of PFRQ Ranges.** Significant differences were observed in the generated PFRQ's, at different kHz levels, between strains 5024 and 8237. Initial observations revealed the frequencies generated from 5-50 kHz were 2% higher in strain 5024, while frequencies generated beyond 50 kHz were 44% higher in strain 8237. Upon further investigation, observations from 100-500 kHz, revealed strain 8237 overall generated frequencies 23% higher than strain 5024. In the 100-200 kHz and 200-300 kHz, the frequencies generated from strain 8237 were 15% and 5% higher than strain 5024, respectively. However, in the 300-400 kHz range, strain 5024 generated frequencies 0.06% higher than strain 8237. Analysis from the observations of 5 kHz increments, between 100-500 kHz, revealed significant differences between both strains within 14 different kHz ranges. In 8 of the kHz ranges, strain 8237 generated higher frequencies than strain 5024, while in the other 6 kHz ranges strain 5024 generated





**Figure 77.** Comparison of the average PFRQ's from 200 to 300 kHz (every 5 kHz) generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

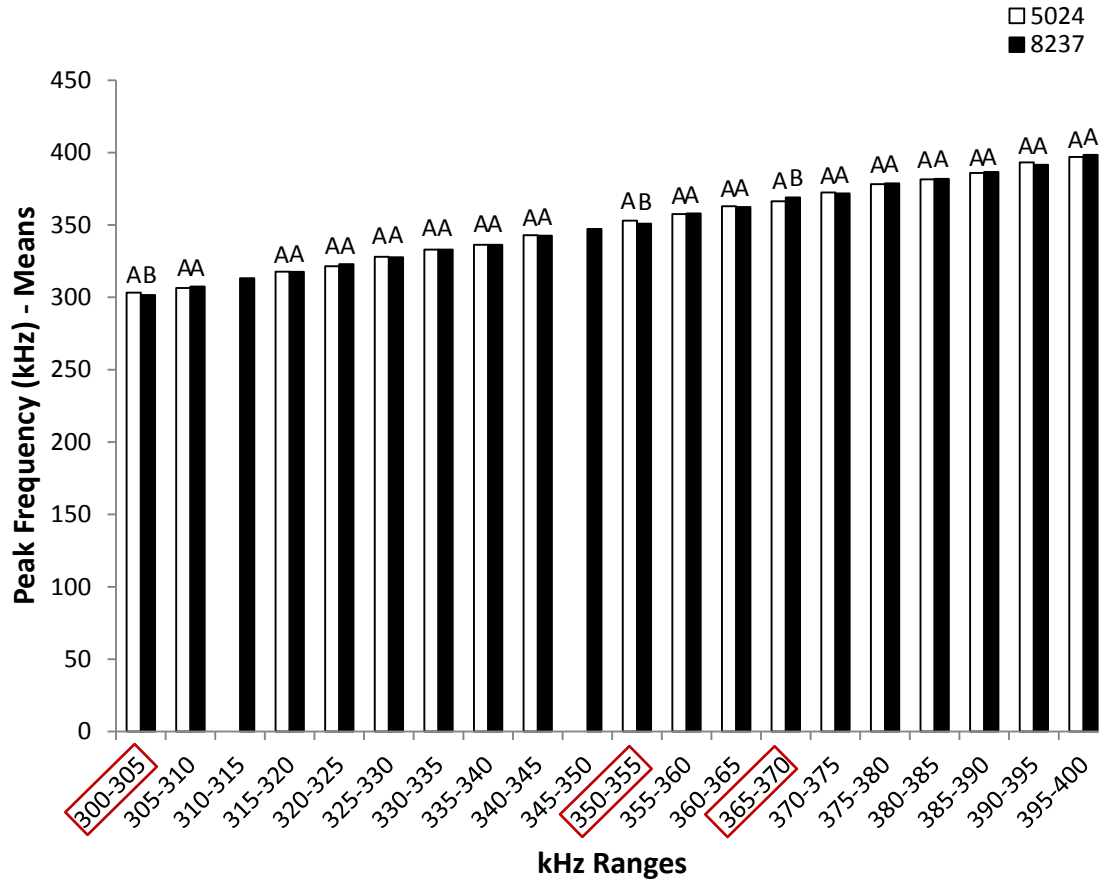
- \*Standard Error = 0.12 for 5024 within 250-255 kHz according to the least square mean.
- \*Standard Error = 0.05 for 8237 within 250-255 kHz according to the least square mean.
- \*Standard Error = 0.27 for 5024 within 255-260 kHz according to the least square mean.
- \*Standard Error = 0.15 for 8237 within 255-260 kHz according to the least square mean.
- \*Standard Error = 0.14 for 5024 within 260-265 kHz according to the least square mean.
- \*Standard Error = 0.02 for 8237 within 260-265 kHz according to the least square mean.

**Table 12.** Average PFRQ for *E. coli* strains 5024 and 8237 from 200-300 kHz in 5 kHz increments.

Peak Frequency (kHz)	Average PFRQ for <i>E-Coli</i> Strains without Alginate		Number of Observations
	5024	8237	
200-205	202.81	202.55	58
205-210	207.00	207.29	16
210-215	212.00	212.00	14
215-220	217.50	217.71	15
220-225	221.75	222.56	17
225-230	228.74	228.59	223
230-235	231.96	231.80	534
235-240	238.14	238.46	33
240-245	243.92	244.15	468
245-250	247.69	247.57	1309
250-255 <sup>††</sup>	251.21	252.23	964
255-260 <sup>†</sup>	257.27	256.46	144
260-265 <sup>††</sup>	262.85	264.27	1379
265-270	265.89	266.20	1252
270-275	273.43	272.41	46
275-280	277.11	276.73	39
280-285	283.00	284.09	74
285-290	288.14	288.10	222
290-295	292.00	292.89	637
295-300	296.80	296.34	425

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$



**Figure 78.** Comparison of the average PFRQ's from 300 to 400 kHz (every 5 kHz) generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.75 for 5024 within 300-305 kHz according to the least square mean.

\*Standard Error = 0.17 for 8237 within 300-305 kHz according to the least square mean.

\*Standard Error = 0.91 for 5024 within 350-355 kHz according to the least square mean.

\*Standard Error = 0.27 for 8237 within 350-355 kHz according to the least square mean.

\*Standard Error = 0.68 for 5024 within 365-370 kHz according to the least square mean.

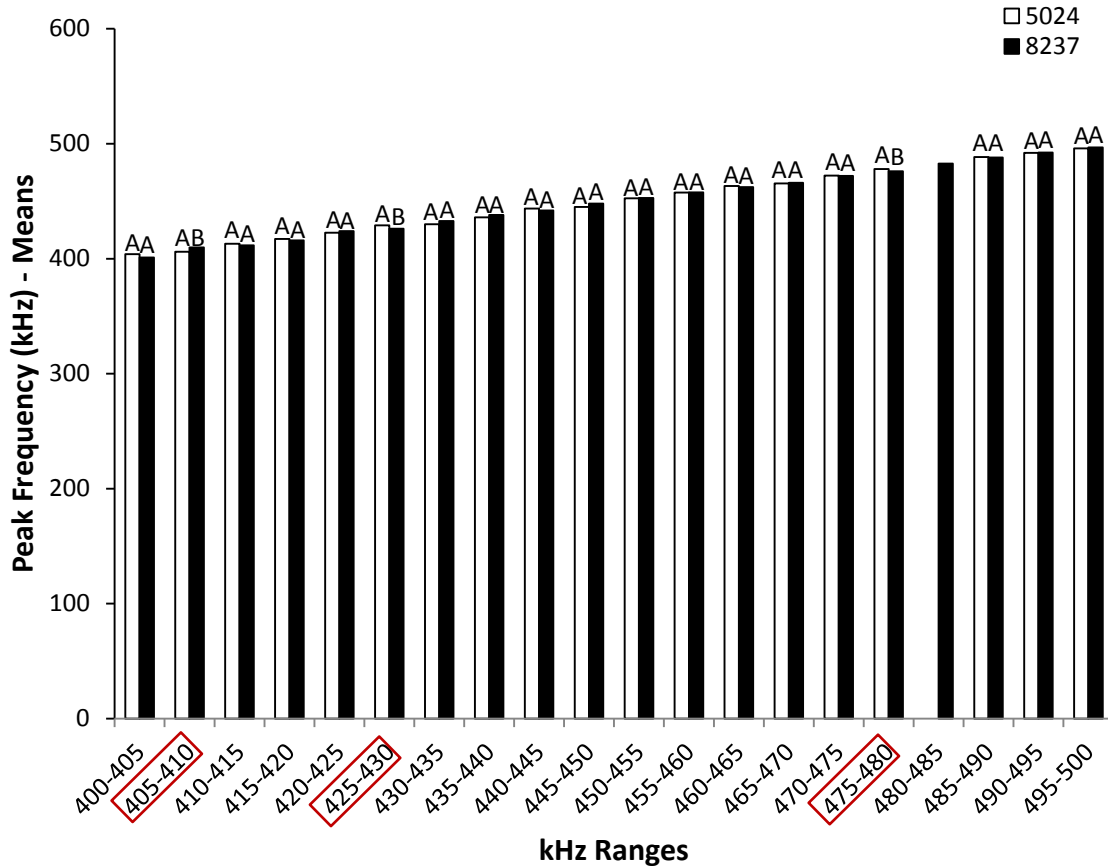
\*Standard Error = 0.58 for 8237 within 365-370 kHz according to the least square mean.

**Table 13.** Average PFRQ for *E. coli* strains 5024 and 8237 from 300-400 kHz in 5 kHz increments.

Peak Frequency (kHz)	Average PFRQ for <i>E-Coli</i> Strains without Alginate		Number of Observations
	5024	8237	
300-305 <sup>†</sup>	303.25	301.65	84
305-310	306.50	307.42	33
310-315	N/A	313.25	24
315-320	317.75	317.64	54
320-325	321.50	322.91	35
325-330	328.00	327.68	40
330-335	332.99	332.99	22582
335-340	336.28	336.30	63
340-345	343.00	342.66	56
345-350	N/A	347.30	37
350-355 <sup>†</sup>	353.00	350.91	25
355-360	357.50	358.00	5
360-365	363.00	362.73	11
365-370 <sup>†</sup>	366.40	369.00	12
370-375	372.50	371.77	15
375-380	378.25	378.78	13
380-385	381.50	381.90	14
385-390	386.00	386.67	7
390-395	393.25	391.60	9
395-400	397.00	398.50	6

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$



**Figure 79.** Comparison of the average PFRQ's from 400 to 500 kHz (every 5 kHz) generated by *E. coli* strains 5024 and 8237, when grown in medium without alginate.

\*ABC=Means with different letters are significantly different ( $P < 0.05$ ) between each strain.

\*Standard Error = 0.59 for 5024 within 400-405 kHz according to the least square mean.

\*Standard Error = 0.42 for 8237 within 400-405 kHz according to the least square mean.

\*Standard Error = 0.74 for 5024 within 425-430 kHz according to the least square mean.

\*Standard Error = 0.37 for 8237 within 425-430 kHz according to the least square mean.

\*Standard Error = 0.88 for 5024 within 475-480 kHz according to the least square mean.

\*Standard Error = 1.53 for 8237 within 475-480 kHz according to the least square mean.

**Table 14.** Average PFRQ for *E. coli* strains 5024 and 8237 from 300-400 kHz in 5 kHz increments.

Peak Frequency (kHz)	Average PFRQ for <i>E-Coli</i> Strains without Alginate		Number of Observations
	5024	8237	
400-405	404.00	401.00	4
405-410 <sup>†</sup>	406.00	409.75	6
410-415	413.00	411.60	7
415-420	417.25	416.00	8
420-425	422.60	424.00	8
425-430 <sup>†</sup>	429.00	426.13	10
430-435	430.00	432.75	9
435-440	436.00	438.13	9
440-445	443.67	442.00	9
445-450	445.00	448.00	7
450-455	452.50	452.75	8
455-460	457.50	457.67	13
460-465	463.25	462.25	8
465-470	465.50	466.00	3
470-475	472.33	472.00	4
475-480 <sup>††</sup>	478.00	476.00	4
480-485	N/A	482.75	4
485-490	488.50	488.00	7
490-495	492.00	492.40	9
495-500	496.00	496.75	7

<sup>†</sup>  $P < 0.05$

<sup>††</sup>  $P < 0.0001$

higher frequencies than strain 8237. These findings suggest that AE differences exist and can be distinguished between strains that are genetically similar.

## CHAPTER 5

### CONCLUSION

All three strains of *E. coli* (5024-parent strain, 8237-mutant strain and 8279-random/unrelated strain) when grown in media with or without alginate, generated acoustic emissions (AE's) within 5 min post inoculation and continued throughout the entire growth phase. The generated AE's from *E. coli* were more pronounced for peak frequency (PFRQ) (74%) than absolute energy (ABSE) (18%) when the strains were grown in medium without alginate. This may be attributable to the settling of cells atop the sensor, thus increasing the overall AE intensity. Distinct differences in the generated AE by each strain were easily determined, as strains 5024 and 8237 generated stronger ABSE and higher PFRQ's than did strain 8279. This may be attributable to the overall difference in each strains genotype, as strain 8279 was genetically quite different from that of the parent strain 5024 and mutant strain 8237 and grew slowly. Through extensive analysis between the parent strain 5024 and mutant strain 8237 distinct differences in the generated AE's were identified. The ABSE generated by strain 8237 was overall 12% stronger during the 3<sup>rd</sup> to 8<sup>th</sup> h of growth and the generated PFRQ's were 51% higher than strain 5024 up to the 8<sup>th</sup> h. However, strain 5024 generated 15% stronger ABSE and 31% higher PFRQ's than strain 8237 during the final hour of growth. The generated PFRQ's differed significantly between both strains. Strain 5024 generated higher frequencies from 5-50 kHz, while strain 8237 generated higher frequencies beyond 50 kHz and from 100-500 kHz. The generated PFRQ's observed within 5 kHz



increments, from 100-500 kHz, revealed fourteen distinct and significant differences ( $P < 0.05$ ) between both strains. Strain 8237 generated higher frequencies within eight of these kHz ranges, while strain 5024 generated higher frequencies within the other six kHz ranges. The AE differences may be attributable to the difference in the strains genotypes, as strain 5024 possesses the rpoS396 (the sigma factor=  $\sigma^s$ ), which is the master regulator in the general stress response and/or differences in cellular functions.

These data suggest that the AE generated from microbial sub-species of *E. coli*, as well as, genetically related strains such as *E. coli* 5024 and 8237, can be distinguished. These distinguishable differences could provide a novel method to identify bacteria by its unique “fingerprint” of sound. While no cellular functions have been determined for generated ABSE and PFRQ, these data identified AE differences that seemed to be based upon the rpoS396 gene. Further research is needed to monitor the AE generated from other genera and sub-species of bacteria, as well as, investigate the cellular functions associated with AE.

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## VITA

Traci Jane Cox was born in Evansville, Indiana. She graduated from Eldorado High School, Eldorado, Illinois in May 1988. In 1992, she graduated from Murray State University with a Bachelors of Science in Food and Nutrition. In 1995 she completed the AP-4 program at Southern Illinois University (SIU) at Carbondale. During this time she began her career as a Registered Dietitian, providing nutrition education and counseling with the Vanderburgh County WIC program. In 1998, she became a consultant dietitian, providing nutrition services to numerous long term care facilities and Rescare group homes throughout southern Indiana and Kentucky. In 1999, she graduated from Southern Illinois University (SIU) at Carbondale with a Master of Science in Food and Nutrition. She continued consulting for long term care facilities, as well as, providing nutrition services for the Head Start and First Steps programs. Throughout her career, she was involved with the Southwestern Indiana Dietetic Association, Louisville District Dietetic Association, Kentucky Dietetic Association and American Dietetic Association.

In 2011, she began to pursue a Master of Science in Food Science at the University of Kentucky. While at the University of Kentucky, she was the recipient of the Dan C. Hutson Fellowship, inducted into the Gamma Sigma Delta Honor Society and a member of the Food Science club.