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ULTRASONIC INVESTIGATIONS ON NON-LINEAR GROWTH PHASES OF DIFFERENT BACTERIA CULTURES BY USING ULTRASONIC PARAMETERS

Physics

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ABSTRACT

Ultrasonic velocity and attenuation coefficient were measured in batch cultures to study non-linear growth properties of E.coli, S. epidermidis, P.multocida and B.subtilis. An unconventional ultrasonic Pulse Echo Overlap (PEO) technique was adopted for measurement of ultrasonic parameters. The results were compared with the conventional establishing measuring techniques involve direct microscopic counts and indirect viable cell counts. The batch cultures were prepared for growth periods of 6, 12, 18, 24, 30, 36, 42 and 48 hrs. The 2 and 10 MHz ultrasonic frequencies were selected based on Rayleigh-plesset's bubble activation theory. It was found that ultrasonic velocity and attenuation coefficient were linearly increased in exponential phase, almost constant in stationary phase and slightly decreased in death phase. The results of ultrasonic velocity and attenuation coefficients of different bacteria cultures were found in the right order of magnitude and aligned with the theory proposed by A Zips and U Fast. It was learnt that the ultrasonic velocity and attenuation coefficients depend on relative cell size, shape, wall structure and characteristic arrangement of cell groups. Finally, in this paper it was proposed that the measurement of ultrasonic parameters with pulse echo overlap technique could be emerged as alternate technique to estimate the non-linear growth properties of different bacteria cultures.

KEYWORDS

Ultrasonic Velocity, Attenuation, Pulse echo overlap technique and Bacteria cultures

1. INTRODUCTION

In the recent years, the understanding of biological effects caused by ultrasound in macromolecules, microorganisms, tissues, organs and bones are enormously required in industries like medical, pharmaceutical, food, packaged and processing technology. The sound speed in tissues was evaluated using the sound speed in fluid medium which the specimen was placed[1-7] and measured *in vitro* ultrasonic velocities in porcine and bovine bone samples by using dual transducer technique. Yuko Kanayama et. al.[8] proposed a method for real-time ultrasound attenuation imaging and quantification for diffuse fatty liver disease. These findings suggest that real-time attenuation parametric imaging may be able to replace CT for quantifying the degree of fatty infiltration of the liver[9-10]. Beside widespread well established studies of ultrasonic velocity and attenuation coefficient of biological materials in basic research, more recently the adiabatic compressibility and intermolecular free length has also been considered as an indicator with high potential in the advanced medical diagnoses and as a process control parameter in the food industry[11-14]. In contrast to the extensive knowledge of biological effects induced by ultrasound in tissues, organs, microorganisms and bones[15-21], the knowledge of biological effects of microbes induced by ultrasound were rather limited, especially when it comes to the measurement of ultrasonic parameters with growth phases of bacteria.

Growth characteristics of prokaryotic microbes are very complex that includes four phases of growth cycle via. lag phase, exponential phase, stationary phase and death phase. The conventional lab techniques of microbiologists involve direct counts, visually or instrumentally, and indirect viable cell counts. In direct microscopic counts, dead cells cannot be distinguished from living ones and only dense suspensions can be counted (>10^7 cells per ml) [22-23]. For electronic counting chambers the size of bacteria in the suspending medium must be very clean[24]. Disadvantages of indirect viable cell counts are only living cells develop colonies that are counted, clumps or chains of cells develop into a single colony and colonies develop only from those organisms for which the cultural conditions are suitable for growth. In view of demerits of the above techniques, the need for alternative Pulse Echo Overlap (PEO) technique[25-30] is arisen to measure the growth characteristics of microbes by evaluating ultrasonic parameters.

However, the scope of this paper is to investigate the variation of ultrasonic parameters with different phases of growth cycle of bacteria. Because, there exists a continuing need for reliable ultrasonic data of bacteria in medical and process technology industry. This is particularly true for systems involved in industrial process with their objectives in mind, an attempt has been made to measure ultrasonic velocity (v), attenuation Coefficient () and density () in bacteria cultures for different phases of growth cycle at constant temperature of 303K.

Data of ultrasonic velocity (v), attenuation Coefficient (), density () are utilized in evaluating some useful properties like acoustical impedance (z), adiabatic compressibility (), intermolecular free length (L_f) which can be utilized as a qualitative guide to predict the extent of ultrasound interaction with bacterial suspensions. This work is a first of its kind towards to estimate the growth kinetics of bacteria.

2. MATERIALS AND METHODS

2.1 Selection of bacteria

The selection of bacteria species covers all *shapes* and *sizes* i.e. *rods, spheres* and *spirals* and also overlay *facultative anaerobic*, *aerobic, motile* and *non-motile* belongs to *gram +ve* and *gram –ve* bacteria. Therefore, in view of this, within the limitations the four species of bacteria were selected and they were; *Escherichia coli, Bacillus subtilis, Pasteurella multocida* and *Staphylococcus epidermidis*. The four organisms were model organisms in their category that covers the study of most of the bacteria[22-24].

2.2 Selection of ultrasound frequencies

According to Rayleigh-plesset's bubble activation theory the resonance frequency of microbes may fall in the range of 0.1 MHz to 10 MHz for the vacuole radius of microbes 0.1 7μm[31]. It was discovered that ultrasound biological interaction mechanism was highly effective with irradiation of resonance frequency ultrasound. It was obviously that the biological interaction did not appear during the irradiation of non-resonance frequency ultrasound. Due to monetary constraints, it was fixed us, therefore, to carry out the research with irradiation of ultrasound frequencies of 2 MHz and 10 MHz for selected microbes.

2.3 Media preparation

The most common growth media for microorganisms were *nutrient broths* (liquid nutrient medium) or *LB medium* (*Lysogeny Broth*). Liquid media were often mixed with agar and poured via sterile media

dispenser into Petri dishes to solidify. These agar plates provide a solid medium on which microbes would be cultured. Nutrient agar and nutrient broth used for bacterial growth were prepared according to the methods recommended by Harrigan and Mc Cance (1976) and Harrigan (1998)[32-33].

2.4 Sample preparation

The samples were prepared at *"center for biotechnology laboratory", University College of technology, Osmania University, Hyderabad.* From each sample, serial dilutions (10 fold) were made by following the method of Harrigan (1998)[33]. The samples were stored under refrigeration conditions for subsequent experiments.

2.5 Ultrasonic studies

The first of its kind "Pulse Echo Overlap (PEO)" technique introduces to measure ultrasonic velocity and attenuation coefficient for different growth periods of bacteria. Firstly, the 10 mL nutrient broth (without inoculum) was poured into the sonication vessel (15 ml glass bottles, internal diameter 21mm, flat base, 2.5 mm wall thickness). Next, the low power (2 mW) ultrasonic transducer of 10mm gap between transmitting and reflecting surfaces (this gap was filled with medium) was immersed into the vessel. The other end of the transducer is connected to the ULTRASONIX 4400M (fabricated and supplied by Roop Telsonic Ultrasonix Limited, Mumbai.) by BNC cable and Pulse Echo pattern was observed on the CRO screen and automatically displays the ultrasonic velocity (control).

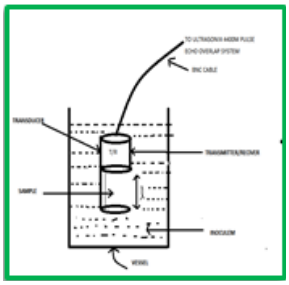

Figure 1: Schematic view of immersion of transducer immersed in cultures.

Further, the selected single colony of bacteria inoculated into the nutrient broth and incubated at 37°C for 6 hours. Then, the 10 mL inoculum poured into the sonication vessel, the above procedure was repeated to measure the parameters. Similarly, the above procedure was repeated for growth periods of 12 hrs, 18 hrs, 24 hrs, 30 hrs, 36 hrs, 42 hrs and 48 hrs.

The sonication vessel is kept inside the water bath up to its neck. The temperature of the water bath was maintained at constant temperature of 303K with aid of automatic temperature control unit. The each reading is repeated per six times and average values were reported.

2.6 Measurement of Ultrasonic Velocity

The ultrasonic transducer generates a pulse of ultrasound which travels across the sample, was reflected from the back wall of the measurement cell, travels back through the sample, and was then detected by the same transducer[34-35].

The ultrasound velocity in the medium was found from the measured delay time difference (t) and earlier found length of the measurement chamber *(d)*.

$$v = \frac{2d}{\Delta t}\ cm/sec$$

The uncertainty in velocity measurements by this technique were 0.02%.

2.7 Measurement of Attenuation Coefficient

The attenuation coefficients of the bacteria cultures were carried out by measuring the amplitudes of transmitted pulses of selected two successive echoes on CRO screen[34-35]. The ultrasonic attenuation coefficient was calculated by using the following formula.

$$\alpha = \frac{1}{2l}\ln\left(\frac{A_n}{A_{n+1}}\right) \quad \text{nep/cm}$$

Where $2l$ is distance travelled and A_n / A_{n+1} is the ratio between two successive echoes of A_n and A_{n+1}. The uncertainty in attenuation coefficient () measurements are ± 0.0015%.

2.8 Measurement of Density of Bacteria cultures

The density of bacteria cultures are carried out by using bicapillary pyknometer of 10 ml volume[12]. The transfer of bacteria cultures was made by using micro pipette in the laminar flow chamber to avoid contamination with air and body.

The density of bacteria cultures was measured by using the following procedure

Mass of the empty bicapillary pyknometer = w_1 gm
Mass of the bacteria culture + pyknometer = w_2 gm
Mass of the culture (m) = $w_2 - w_1$ gm
Volume of the culture = V cm^3

Density of the bacteria culture

$$(\rho) = \frac{Mass\ \ of\ \ the\ culture\ (m)}{Volume\ \ of\ the\ \ culture\ (V)} \quad gm/cm^3$$

The accuracy in measuring density of bacteria cultures was 2 parts in 10^5.

A single pan electrical balance was used to find the masses of the samples to find out density. The accuracy of measuring the masses is ±0.01mg.

2.9 Calculation of Adiabatic Compressibility

The Adiabatic compressibility can be calculated as

$$\beta_s = 1/\rho v^2 \quad cm^2/dynes$$

Where is the density and is the ultrasonic velocity

2.10 Calculation of Inter Molecular Free Length

The equation to calculate Intermolecular free length is

$$L_f = K\beta_s^{\frac{1}{2}} \quad A.U.$$

Where K is Jacobson's temperature constant K= 631 x 10^{-6} at 303.

2.11 Calculation of Acoustical Impedance

The equation to calculate acoustical impedance is

$$Z = \rho v$$

Where is the density and is the ultrasonic velocity

2.12 Measurement of optical density

The OD of a sample was measured in a 10 mm cuvette at a visible wavelength using a Spectrophotometer[36]. The amount of light of a specific wavelength that absorbed by a culture was related to the number of cells. Set the transmittance to 0 with no sample in the chamber. Set the absorbance to 0 using un-inoculated NB medium as a blank. The turbidity of the inoculated NB medium of four microbes was recorded for growth periods of 6hrs, 12hrs, 18hrs, 24hrs, 30hrs, 36hrs and 42hrs. This method counts all cells whether they were alive or not.

2.13 Estimation of colony forming units per mL

The plate-count method was used for counting cells in which serially dilute the original sample and spread-plate the resulting dilutions[37]. The colonies that arise after incubation were counted to determine the original cell density. The cell density was determined in the form of colony forming units (cfu) for four microbes for growth periods of 6hrs, 12hrs, 18hrs, 24hrs, 30hrs, 36hrs and 42hrs.

The one important thing to remember when doing plate-counts was that it only counts the viable cells (those cells that are able to grow under the specific incubation conditions used).

3 RESULTS

3.1 Density

The results of effect of growth period on density of microbes are detailed in figure (1) and tabulated in tables (1-8). It is observed that density of microbes increases in exponential growth and reached to maximum in stationary phase and finally slight decreased in the death phase. The stationary phase for microbes can be considered as 1500 cfu/ml. It is measured that the maximum density of *E.coli* is 1.135

gm/cm^3, *B.subtilis* is 1.165 gm/cm^3, *S.epidermidis* is 1.197 gm/cm^3 and *P.multocida* is 1.172 gm/cm^3 per 1500 cfu/ml.

3.2 Ultrasonic Velocity

Figure (2) shows that the results of ultrasonic velocity as a function of growth period of microbes and measured data are tabulated in tables (1-8). It observed that ultrasonic velocity linearly increased in the exponential phase of the microbes. The maximum ultrasonic velocities for microbes are recorded in the stationary phase; *E.coli*: 1640 m/sec, *B.subtilis*: 1690, *S.epidermidis*: 1720 m/sec and *P.multocida*: 1705 m/sec per 1500 cfu/ml. During the death phase ultrasonic velocity slightly decreased for four microbes.

3.3 Attenuation coefficient

Graphic presentation of effect of growth period on attenuation coefficient at 2 MHz and 10 MHz frequencies are expressed in figure (3) and recorded in table (1-8). Attenuation coefficient linearly increased in exponential growth phase of microbes at 2 MHz to 10 MHz frequencies. The maximum attenuation coefficient is obtained in the stationary phase of microbes. The measured values of attenuation coefficient at 2 MHz; E.coli: 0.2346 nep/cm, *B.subtilis*: 0.2423 nep/cm, *S.epidermidis*: 0.2931 nep/cm and *P.multocida*: 0.2801 nep/cm per 1500 cfu/ml and at 10 MHz; *E.coli*: 0.4146 nep/cm, *B.subtilis*: 0.4568 nep/cm, *S.epidermidis*: 0.6013 nep/cm and P.multocida: 0.5901 nep/cm per 1500 cfu/ml. Attenuation coefficient slightly decreased in death phase of microbes.

Table 1. Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and free length of Bacteria species for 6 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.106	1552	0.1146	0.2956	0.32	700	1.716	3.754	3.919
S.epidermidis	1.117	1536	0.1158	0.2351	0.18	240	1.715	3.794	3.942
P.multocida	1.112	1541	0.1194	0.2398	0.25	400	1.713	3.786	3.937
B.subtilis	1.104	1516	0.1084	0.2215	0.15	120	1.673	3.941	4.017

Table 2. Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 12 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7(CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.135	1640	0.2346	0.4146	0.72	1500	1.861	3.276	3.663
S.epidermidis	1.137	1620	0.1951	0.4087	0.36	600	1.841	3.351	3.704
P.multocida	1.132	1612	0.2234	0.4568	0.45	900	1.824	3.399	3.731
B.subtilis	1.124	1540	0.1192	0.3126	0.29	400	1.731	3.751	3.919

Table 3. Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 18 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.135	1640	0.2346	0.4146	0.72	1500	1.861	3.275	3.662
S.epidermidis	1.167	1670	0.2621	0.5642	0.65	1100	1.948	3.072	3.547
P.multocida	1.172	1705	0.2801	0.5901	0.74	1500	1.998	2.935	3.467
B.subtilis	1.138	1560	0.2132	0.3864	0.51	910	1.775	3.611	3.846

Table 4. Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 24 hrs growth period at 303K

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f)X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.130	1636	0.2336	0.4042	0.72	1450	1.848	3.306	3.679
S.epidermidis	1.197	1720	0.2931	0.6013	0.8	1500	2.058	2.825	3.401
P.multocida	1.172	1705	0.2801	0.5901	0.74	1500	1.998	2.935	3.467
B.subtilis	1.151	1620	0.2313	0.4123	0.62	1240	1.864	3.310	3.682

Table 5. Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 30 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.125	1630	0.2246	0.3894	0.72	1398	1.833	3.345	3.701
S.epidermidis	1.197	1720	0.2931	0.6013	0.8	1500	2.058	2.824	3.401
P.multocida	1.170	1698	0.2799	0.5898	0.74	1490	1.986	2.964	3.484
B.subtilis	1.165	1690	0.2423	0.4568	0.77	1500	1.968	3.005	3.508

Table 6 Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 36 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.120	1621	0.2136	0.3678	0.71	1340	1.815	3.397	3.731
S.epidermidis	1.190	1715	0.2899	0.5982	0.8	1480	2.040	2.857	3.421
P.multocida	1.162	1680	0.2608	0.5616	0.73	1410	1.952	3.049	3.533
B.subtilis	1.165	1690	0.2423	0.4568	0.77	1500	1.968	3.005	3.508

Table 7 Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance, Adiabatic compressibility and Free length of Bacteria species for 42 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.119	1614	0.1836	0.3456	0.7	1296	1.806	3.431	3.748
S.epidermidis	1.178	1701	0.2798	0.5808	0.79	1410	2.003	2.933	3.466
P.multocida	1.158	1667	0.2587	0.5314	0.73	1360	1.930	3.107	3.567
B.subtilis	1.160	1681	0.2356	0.4425	0.77	1479	1.949	3.050	3.534

Table 8 Density, Ultrasonic velocity, Attenuation coefficient, Optical density, Colony forming units, Acoustical impedance,Adiabatic compressibility and Free length of Bacteria species for 48 hrs growth period at 303K.

Bacteria	Density (gm/cm^3)	Ultrasonic Velocity (v) 10^2cm/sec	Attenuation Coefficient nep/cm		Optical Density	Colony Forming Units X 10^7 (CFU/ml)	Acoustical Impedance(z) 10^5 gm/cm^2-sec	Adia.Comp (β_s) X 10^{-11} cm^2/dyn	I.M.Free length (L_f) X 10^{-9} cm
			2 MHz	10 MHz					
E.coli	1.110	1607	0.1636	0.3115	0.7	1250	1.783	3.488	3.779
S.epidermidis	1.165	1692	0.2653	0.5686	0.79	1360	1.971	3.335	3.695
P.multocida	1.146	1651	0.2499	0.5110	0.72	1301	1.892	3.201	3.621
B.subtilis	1.154	1665	0.2241	0.4389	0.76	1423	1.921	3.199	3.619

4 DISCUSSIONS

The data presented for cfu/ml in tables (1-8) shows that generation time for *E.coli P.multocida S.epidermidis B.subtilis.* In the present investigations 1500 cfu/ml was considered as a stationary phase. The stationary phase reached as follows; *E.coli> P.multocida> S. epidermidis> B.subtilis.*

The varied density with phases of growth cycle of microbes is compared and depicted in figure (2). It observed that the density of microbes linearly increased with growth period in exponential phase due to constant growth rate of microbes. It is investigated that in the stationary phase the density of *S.epidermidis> P.multocida >B.subtilis> E.coli* because naturally sphere (*coccus*) forms of microbes (*S.epidermidis, P.multocida*) will take less space to occupy than rod (*bacillus*) forms of microbes (*B.subtilis, E.coli*). Due early occurrence of death phase of B.subtilis the density of B.subtilis is reduced relatively higher than other microbes.

It is reported that the gram–positive microbes are more resistant (high stiffness) than gram-negative[38] and the coccal forms of microbes possessed less adiabatic compressibility than rod forms of microbes[39]. The comparison of ultrasonic velocity with phases of growth cycle of microbes is depicted in figure (3). It observed that the ultrasonic velocity for bacteria linearly increased in exponential phase because free length and adiabatic compressibility of cultures linearly decreased with increased cfu/ml. In stationary phase the remarkable observation is that the ultrasonic velocity for *S.epidermidis> P.multocida >B.subtilis> E.coli*. Using this condition, we examined S.epidermidis is gram-positive and coccal form therefore; it has high stiffness and less adiabatic compressibility than other three microbes and hence possessed highest ultrasonic velocity. Even though, *P.multocida* is gram-negative but, its coccal form provided next highest ultrasonic velocity due its observed low free length and adiabatic compressibility [figures (4-5)]. On the other hand, *B.subtilis* is gram-positive but, due its rod forms their free length and adiabatic compressibility is more than *P.multocida* and hence possessed less ultrasonic velocity than *P.multocida*. Finally, *E.coli* is gram-negative and rod forms possessed lowest ultrasonic velocity among of four species of bacteria.

Finally, it is admitted that the ultrasonic velocity of *S. epidermidis >P. multocida >B.subtilis> E.coli.* i.e. *coccal forms > rod shaped & Gram +ve > Gram –ve.* It is because of high stiffness of coccal forms provided less compressibility and free length to cultures. The gram +ve bacteria offered more resistance than gram –ve bacteria, due to multilayer cell wall structure of gram +ve bacteria (20-80nm) as compared to 10 nm thin of gram –ve and also due to reported high acoustical impedance of gram +ve than gram –ve as shown in figure (6). And also it is reported that the density of *S.epidermidis> P.multocida >B.subtilis>E.coli* may be one of the reason for less compressibility of cultures.

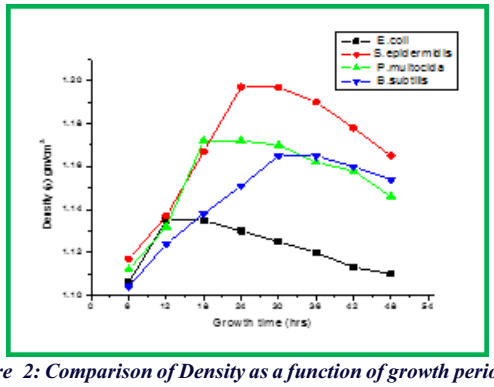

Figure 2: Comparison of Density as a function of growth period for Bacteria cultures.

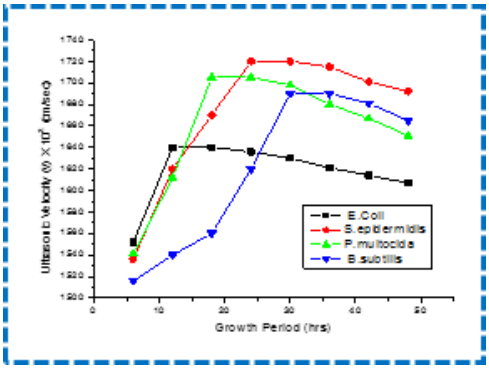

Figure 3: Comparison of Ultrasonic velocity as a function of growth period for Bacteria cultures.

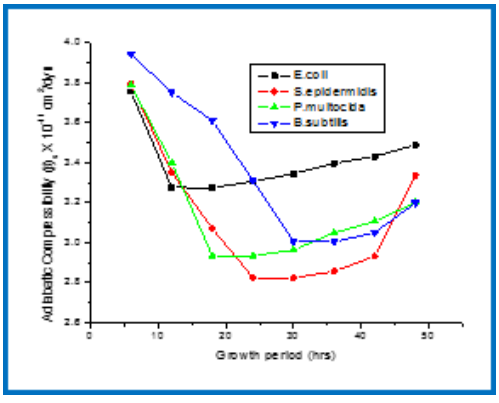

Figure 4: Graphic presentation of effect of growth period on adiabatic compressibility for Bacteria cultures microbes.

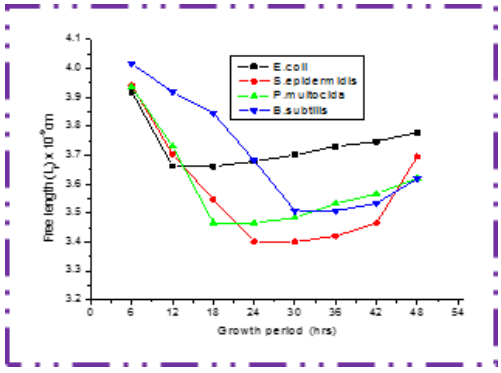

Figure 5 Graphic presentation of effect of growth period onfree length for Bacteria cultures

The four most important types of interaction between ultrasonic waves and microbes are intrinsic absorption, visco-internal dissipation losses, thermal dissipation losses and scattering losses[40-41]. In most suspensions, one or two of the above mechanisms usually dominate the overall attenuation in a particular frequency range. At relatively low frequencies, the visco-internal and thermal loss mechanisms usually dominate, but at higher frequencies the intrinsic absorption and scattering losses usually dominate. In this study 2 MHz and 10 MHz ultrasonic frequencies are relatively considered as high frequencies, therefore most of the attenuation is due to absorption and scattering losses. Even though 2 MHz and 10 MHz ultrasonic frequencies are longer than the size of the microbes, however, interaction of ultrasonic waves with microbes may be due to their cell size, cell forms and cell wall structural properties.

The species with normally faster growth rates were more affected by ultrasonic exposure[42]. It is generally admitted that cells of a bigger size are more sensitive to ultrasound [38] and rod shaped bacteria more than coccal forms[39]. The gram-positive are more resistant (high stiffness) than gram-negative[38].

Usually absorption losses are directly proportional to square of frequency in liquids. Therefore, more attenuation is observed at 10 MHz than 2 MHz. In the exponential phase, attenuation coefficient linearly increased due to high number of scatters in cultures with growth period caused to high occurrence of scattering losses. It is found that the time taken to reach maximum value of attenuation as followed E.*coli P.multocidaS.epidermidisB.subtilis* because of generation time for *E.coli P.multocida S.epidermidis B.subtilis.*

In the stationary phase, it is reported that attenuation of *S.epidermidis>P.multocida >B.subtilis>E.coli* as shown in the figure (7). It is investigated that the scattering losses are dominated in coccal forms, gram +ve bacteria than road forms, gram –ve bacteria. The reason could be that the scattering losses are inversely proportional to size ad also high resistance offered by coccal forms of bacteria. It is reported that the size of coccal forms are smaller than rod shaped forms and hence the scattering losses followed as *S. epidermidis> P.multocida >B. subtilis>E. coli.* During the death phase the scattering losses are reduced because the number of scatters present in the cultures is decreased.

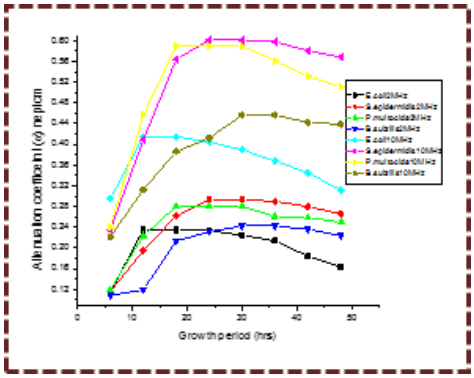

Figure 6: Comparison of Attenuation coefficient as a function of Growth period for Bacteria cultures.

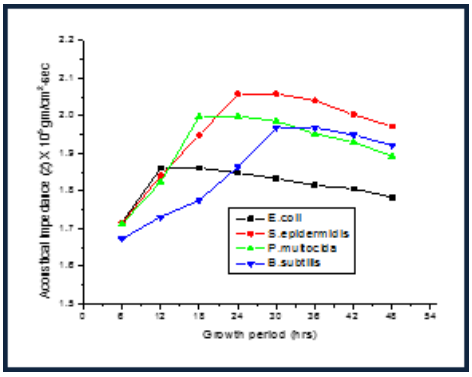

Figure 7: Graphic presentation of Acoustic impedance as a function of Growth period for Bacteria cultures.

It is observed that good agreement between ultrasonic measurements and conventional measurements on phases of growth cycle of bacteria [figures (8-9)]. The evaluated ultrasonic velocity and attenuation coefficient are compared with literature values of A Zips and U Fast11 and found in the right order of magnitude.

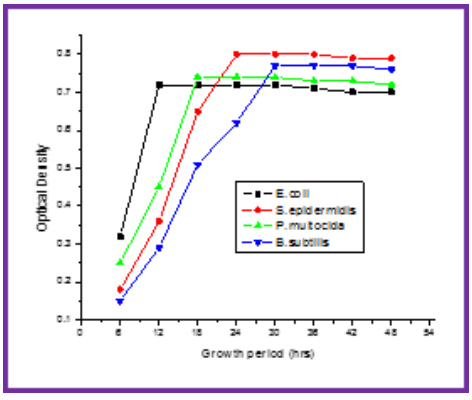

Figure 7: Graphic presentation of Optical density as a function of Growth period for Bacteria cultures

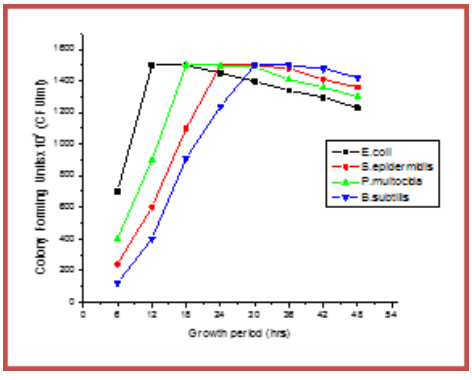

Figure 8: Comparison of CFU/ml as a function Growth period for Bacteria cultures

5. CONCLUSIONS

It was concluded that the variation of ultrasonic parameters strongly depending on relative size, shape and cell wall structural properties of bacteria. The high values of ultrasonic velocity and attenuation coefficient were reported for coccal forms than rod shaped forms and gram-positive than gram-negative. The data of ultrasonic velocity and attenuation coefficient could be used to estimate the phases of growth cycle of bacteria. The Pulse echo technique (PEO) would have been emerged as alternate technique to estimate growth period of bacteria.

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