Ultrasonic Interactions of Aerobic and Anaerobicspp.Cultures at Various Growth Stages

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Abstract **- The Ultrasonic investigations of Velocity, Attenuation and Density have been made in both aerobic bacterial cultures of Streptococci species,Staphylococci species and anaerobic cultures of Klebsiella, Salmonella species at various growth stages in nutrient broth at constant temperature of 30^0 C. These bacterial cultures were grown at Microbiology Laboratory of VeterinaryCollege, Bidar, Karnataka. The pulse echo selection technique has been adopted for measuring velocity and echo amplitudes of ultrasonic waves of 2 MHz frequency under log phase of Bacteria grown in nutrient broth.**

Keywords - Bacterial cultures, Nutrient broth, Logphase, Ultrasonic velocity, Attenuation.

I. INTRODUCTION

The relationships between aerobic petroleum-degrading and anaerobic sulfate-reducing bacteria in oil degradation are presented [15, 16]. Neither pure nor mixed cultures of sulfate-reducing bacteria were able to grow on oils under anaerobic conditions. Such cultures, however, were able to grow on the residues from the aerobic bacterial degradation of some oils. Growth of sulfate-reducers was determined in part by the nature of the aerobic population used to degrade oil, the chemical composition of the oil and whether the sulfate-reducing bacteria were in pure or mixed culture.[13,14] to be used because of the production of nitrate bymixed cultures. The results presented to support the hypothesis that sulfate-reducing bacteria cannot initiate degradation of oil but If mixed cultures were grown, then a nitratefree medium had rather grow on the residues resulting from the aerobic degradation of oil [10-12]

In continuation of the work on bacteria [1-6], the authors have tried to measure the ultrasonic parameters [7-9] in Salmonella species, Streptococci species and Klebsiella species at various stages of its growth (Log Phase) such as: Acoustic Impedance (Z),IsotropicCompressibility (K_s), Intermolecular Free length (L_f) and attenuation coefficient (α).

II. EXPERIMENTAL DETAILS

The measurement of Ultrasonic velocity and Attenuation were carried out by Electronic Pulse Echo Selection (PES) method supplied by Vivace-sonics, INDIA at a frequency of 2 and 5 MHz and at 30 ^{0}C temperature the block diagram of which is shown in Figure A. The PES technique is new and more accurate for measuring ultrasonic velocity and echo-amplitudes in low absorptive solids as well as liquids. The accuracy of the technique is 2parts in 10^4.

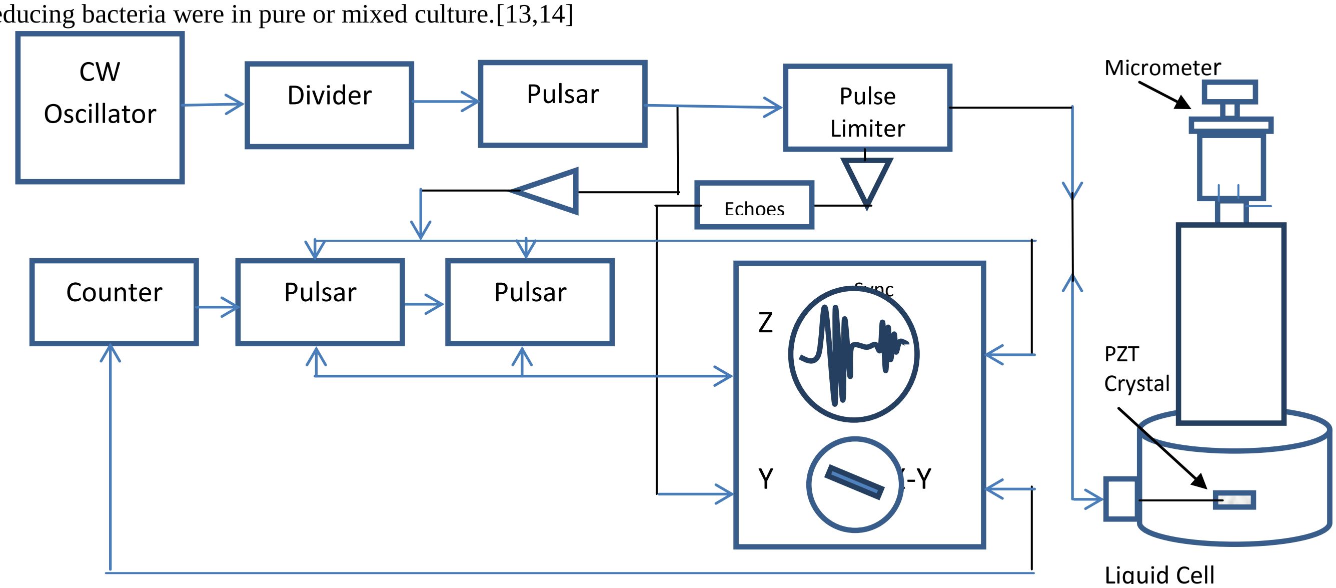

Figure A: Block diagram of Pulse Echo Selection (PES) Technique.

In this experiment, we have measured the ultrasonic properties both aerobic bacterial cultures of Streptococci species, Staphylococci species and anaerobic cultures of Klebsiella, Salmonella species which were brought from the Microbiology laboratory, Veterinary college, Bidar, Karnataka and were inoculated in broth at room temperature in our laboratory for 24 hrs for their study.

Data Collection:

The experiment was repeated three times for every sample by using Electronic Pulse Echo Selection (PES) technique at 2 and 5 MHz frequencies at room temperature to measure the ultrasonic velocity (v_l) and attenuation (α).

The longitudinal ultrasonic velocity, $v_l = \frac{2l}{T}$

where l – length of liquid column

T – travel time of ultrasonic wave

The Ultrasonic attenuation, $\alpha = \frac{\ln(\frac{a0}{an})}{2ln}$

where a_o= the amplitude of transmitted pulse

a_n= amplitude of n^{th} echo

n = the number of echo

III. RESULTS AND DISCUSSION

It is observed that the values of velocity and density were increasing gradually up to 9 hrsand decrease up to 12 hrs and then become constant up to 24 hrsas shown in figures 1 to 4 whereas the attenuation coefficient values are decreasingup to 12 hrsand then increase up to 15 hrs and become constant up to 24 hrs as shown in figures 9 to 12 at various growth stages from 6 hrs to 24 hrs at regular intervals of 3 hrs in the bacterial cultures of Staphylococci spp., Streptococci spp., Klebsiella spp. and Salmonella spp.grown at room temperature.

From figures 5 to 8 it is clear that the acoustic impedance and adiabatic compressibility values are found to be more changing (active growth) at around 9 hrs of growth time and after that both values become constant (log phase) up to 24 hrs of growth time.

The results of the study indicated that physical properties were depending on the shape, size, motility and also density of the bacteria.In view of the extensive applications of ultrasound in the medical diagnostics, there is a need for thorough study of these parameters in different growth phases like lag, log and decline phases of bacteria.

IV. CONCLUSION

From all the plots it is clear that all physical properties are more effective at around 9 hrs of bacterial growth time and at turbidity 0.2. This is mainly due to the fact that the bacteria are found to be more motile at around 9 hrs of growth. Therefore, it is clear that the motility and density values of bacteria influence the physical properties.

ACKNOWLEDGEMENT

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Table: 1: Growth dependency of Measured and computed parameters of in Different Bacteria at room Temperature and turbidity 0.2

Staphylococci*spp*:

Growth Time	Density	Velocity	Attenuation	Echo amplitudes			Acoustic Impedance	Adiabatic Compress-ibility	Inter molecular Free length
T	**ρ**	**v x10^2**	**α**	**a_0**	**a_1**	**a_2**	**Z**	**K_ax10^{-11}**	**L_fx10^{-8}**
6	1	1538.96	0.4268	6.1	2.6	1.3	153896.00	4.22	0.0041
9	1.232	1544.99	0.4268	6.1	2.6	1.3	190342.77	3.41	0.0037
12	0.998	1528.58	0.3893	6.1	2.8	1.4	152552.28	4.29	0.0041
15	0.994	1540.17	0.4459	6.1	2.5	1.4	153092.90	4.24	0.0041
18	1.004	1533.65	0.4459	6.1	2.5	1.4	153978.46	4.23	0.0041
21	0.987	1538.49	0.4459	6.1	2.5	1.4	151848.96	4.28	0.0041
24	0.986	1527.88	0.4459	6.1	2.5	1.4	150648.97	4.34	0.0041

Streptococci*spp*:

Growth Time	Density	Velocity	Attenuation	Echo amplitudes			Acoustic Impedence	Adiabatic Compressibility	Inter molecular Free length
T	ρ	v x 10^2	α	a_0	a_1	a_2	Z	K_ax 10^{-11}	L_f x 10^{-8}
6	0.9896	1523.58	0.4504	6.4	2.6	1.5	150773.48	4.35	0.0041
9	1.0021	1544.99	0.4133	6.4	2.8	1.5	154823.45	4.18	0.0041
12	0.9904	1510.8	0.4504	6.4	2.6	1.5	149629.63	4.42	0.0041
15	0.9972	1518.6	0.4504	6.4	2.6	1.5	151434.79	4.34	0.0041
18	1.0023	1525.32	0.4504	6.4	2.6	1.5	152882.82	4.28	0.0041
21	1.0089	1517.45	0.4504	6.4	2.6	1.5	153095.53	4.3	0.0041
24	0.9889	1512.01	0.4504	6.4	2.6	1.5	149522.67	4.42	0.0041

***Salmonella*:**

Growth Time	Density	Velocity	Attenuation	Echo amplitudes			Acoustic Impedance	Adiabatic Compressibility	Inter molecular Free length
T	ρ	v x 10^2	α	a_0	a_1	a_2	Z	K_ax 10^{-11}	L_f x 10^{-8}
6	0.9992	1490	0.4504	6.4	2.6	1.5	150773.48	4.35	0.0042
9	1.012	1530.22	0.4315	6.4	2.7	1.5	154858.26	4.22	0.0041
12	1.002	1506.82	0.4504	6.4	2.6	1.5	150983.36	4.39	0.0042
15	0.999	1510.57	0.4504	6.4	2.6	1.5	150996.57	4.38	0.0042
18	1.008	1514.62	0.4504	6.4	2.6	1.5	152673.69	4.32	0.0042
21	0.999	1512.86	0.4504	6.4	2.6	1.5	151164.97	4.37	0.0042
24	1.005	1511.15	0.4504	6.4	2.6	1.5	151870.57	4.35	0.0042

Klebsiella:

Growth Time	Density	Velocity	Attenuation	Echo amplitudes			Acoustic Impedance	Adiabatic Compressibility	Inter molecular Free length
T	ρ	v x 10^2	α	a_0	a_1	a_2	Z	K_ax 10^{-11}	L_f x 10^{-8}
6	0.999	1524.18	0.4504	6.4	2.6	1.5	152357.03	4.3	0.0041
9	1.096	1554	0.4315	6.4	2.7	1.6	170442.72	3.77	0.0039
12	0.999	1509.66	0.4504	6.4	2.6	1.5	150935.80	4.39	0.0042
15	0.998	1515.15	0.4504	6.4	2.6	1.5	151348.33	4.36	0.0042
18	0.998	1522.1	0.4504	6.4	2.6	1.5	151966.46	4.32	0.0042
21	1.0096	1516.3	0.4504	6.4	2.6	1.5	153085.65	4.3	0.0041
24	0.988	1511.32	0.47	6.4	2.5	1.4	149409.09	4.42	0.0042

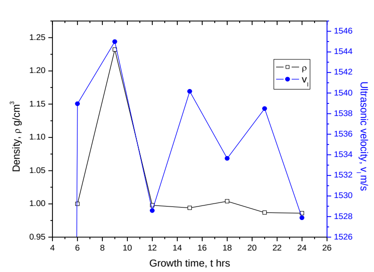

Figure 1: Variation of Density and Ultrasonic velocity versus Growth time of Staphylococci spp.

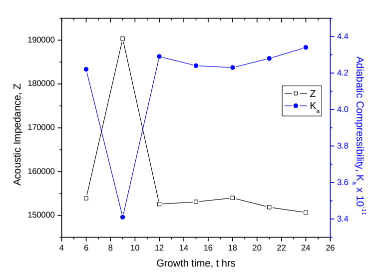

Figure 5: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Staphylococci spp.

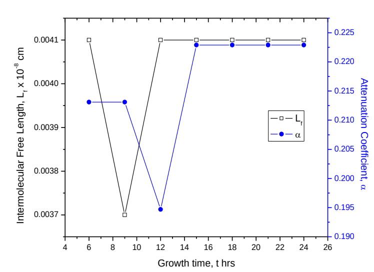

Figure 9: Variation of Intermolecular Free Length and Attenuation coefficient versus growth time of Staphylococci spp.

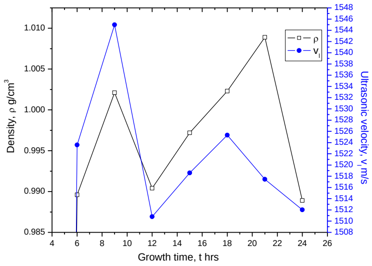

Figure 2: Variation of Density and Ultrasonic velocity versus growth time of Streptococci spp.

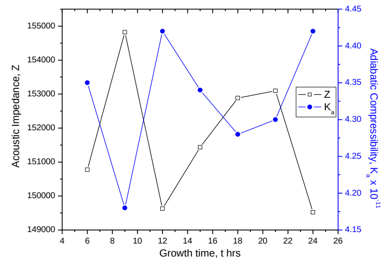

Figure 6: Variation of Acoustic Impedance and Adiabatic compressibility versus growth time of Streptococci spp.

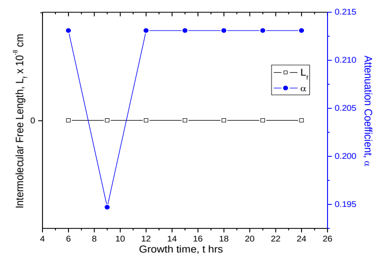

Figure 10: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Streptococci spp.

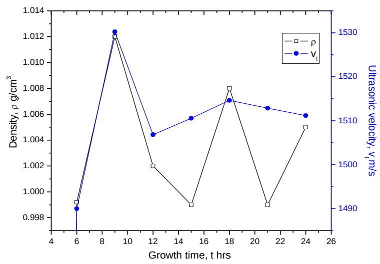

Figure 3: Variation of Density and Ultrasonic velocity versus growth time of Salmonella spp.

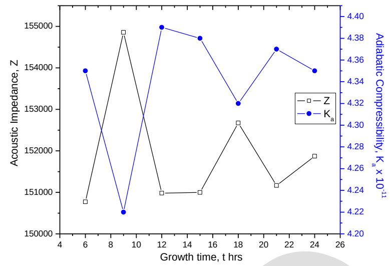

Figure 7: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Salmonella spp.

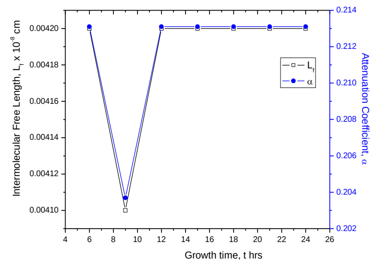

Figure 11: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Salmonella spp.

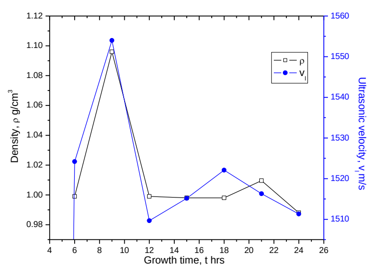

Figure 4: Variation of Density and Ultrasonic velocity versus growth time of Klebsiella spp.

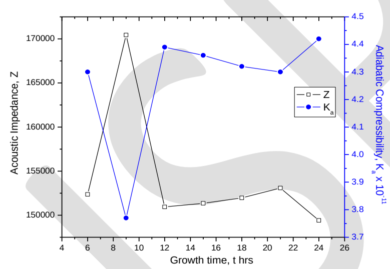

Figure 8: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Klebsiella spp.

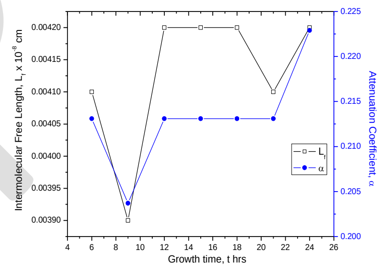

Figure 12: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Klebsiellaspp

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FIGURE CAPTIONS

Figure 1: Variation of Density and Ultrasonic velocity versus Growth time of Staphylococci spp.
Figure 2: Variation of Density and Ultrasonic velocity versus growth time of Streptococci spp.
Figure 3: Variation of Density and Ultrasonic velocity versus growth time of Salmonella spp.
Figure 4: Variation of Density and Ultrasonic velocity versus growth time of Klebsiella spp.

Figure 5: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Staphylococci

Figure 6: Variation of Acoustic Impedance and Adiabatic compressibility versus growth time of Streptococci spp.

Figure 7: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Salmonella spp.

Figure 8: Variation of Acoustic Impedance and Adiabatic Compressibility versus growth time of Klebsiella spp.

Figure 9: Variation of Intermolecular Free Length and Attenuation coefficient versus growth time of Staphylococci spp.

Figure 10: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Streptococci spp.

Figure 11: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Salmonella spp.

Figure 12: Variation of Intermolecular Free Length and Attenuation coefficient versus Growth time of Klebsiella spp