International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 3 Issue 8, July 2016

ISSN: 2394-4404

Preliminary Study To Enhance The Growth Rate Of Bacteria

Muthuraj.K

Research Scholar, PG Department of Microbiology, Madras Christian College

Mahalakshmi.V

Corresponding Author and Head, PG Department of Microbiology, Madras Christian College

Abstract: Bacteria reproduce by binary fission and the doubling time of different bacterial cultures differ based on the nutrient requirements, environmental conditions and growth in batch and continuous cultures etc. The ability to increase the doubling time and hence the growth rate was attempted by increasing the enzymatic activity, growing fastidious organisms with normal bacteria, growing pure culture with mixed culture to promote metabolic activity and providing pyruvate and citric acid as a substitute for glucose.

I. INTRODUCTION

To obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients. Nutrients are substances used in biosynthesis and energy production and therefore are required for microbial growth. Environmental factors such as temperature, oxygen levels, and the osmotic concentration of the medium are critical in the successful cultivation of microorganisms. [1]. Bacterial growth is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission. Providing, no mutational event occurs, the resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry)[2], direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements[3] .

BACTERIAL GROWTH CURVE\KINETIC CURVE

In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: lag phase (A), log phase or exponential phase (B), stationary phase (C), and death phase (D).[4]

- ✓ During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
- ✓ The log phase (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling. [5] The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.[5] The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day.[6] Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

- ✓ The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase.
- ✓ At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria[7] and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations. The doubling time of S. cerevisiae grown in YEPD at 30^{o} C may be between 90 and 100 minutes, while a 200-minute doubling time may be expected in minimal medium. At 37^{o}C, a typical *E.coli* strain doubles in 20-30 minutes in rich medium and the same *E.coli* strain doubles in 50-60 minutes in minimal medium. Mandelstam *et al*. (1982). This work aims at reducing the doubling time of bacteria by increasing the enzymatic activity of the cells and by substituting intermediate components of metabolism than the substrate itself.

II. AIM AND OBJECTIVES

AIM:

To reduce the doubling time of a bacteria by using various promoters.

OBJECTIVES:

- ✓ To increase enzyme activity of a cell.
- ✓ Ability of fastidious bacteria to grow in standard media.
- ✓ Utilization of pyruvate and citric acid as substitute for glucose.
- ✓ Comparison between the growth rate of pure and mixed cultures.
- ✓ Uptake of enzymes from culture supernatant to increase metabolic activity of a cell.

III. METHODOLOGY

A. ISOLATION OF BACTERIAL CULTURES

Bacterial cultures of *Escherichia coli, Proteus, Pseudomonas, Klebsiella, Vibrio cholera, Salmonella, Staphylococci* were isolated and identified by standard lab procedures.

B. CHECKING OF NUTRIENTS UPTAKE

Conical flasks were taken and in the first conical flask 25ml of Nutrient broth and 1 gram of Lactose was added, in the second conical flask 25ml of Nutrient broth and 1 gram of Glucose was added, in third conical flask 25ml of Nutrient broth was added, in the forth conical flask 25ml of Nutrient broth and 1 gram of beef extract was added and in fifth conical flask 25ml of Nutrient broth and 1 gram of fructose was added and autoclaved. *E.coli* culture (1ml) was added to each conical flask and incubated at 37^{o}c for 5 hours and OD value was measured at every half an hour interval. (Chart 4.1)

C. BREAKDOWN OF CELLS AND COLLECTION OF ENZYMES FROM THE SUPERNATANT

Nutrient broth(10 ml) was poured in five 15ml test tubes and autoclaved.. *E.coli* culture (1ml) was added to these tubes and incubated for 5 hours, cell lysate solution was added and then centrifuged at 10000 rpm for 10 minutes to breakdown the cells. Supernatant was collected and 1ml of *E.coli* culture was added in those supernatant and incubated at 37^{o}C for five hours and OD values were taken at ever half an hour interval .(Fig 4.1)

D. LAWN CULTURE OF E.COLI WITH SPOT CULTURE OF LACTOBACILLUS

Colonies of *Lactobacillus* were isolated in MRS plates from curd sample and identified by standard lab procedures. Five Nutrient agar plates were prepared with the lawn culture of *E.coli* and spot inoculated with Lactobacillus and incubated at 37^{0}C for 3days. (Fig 4.2)

E. LACTOSE DEGRADATION TEST

Lactose agar plates (10% lactose, 100ml distilled water, 1.8 grams of agar) and MacConkey agar plates were prepared and autoclaved. These plates were inoculated with lawn cultures of *E.coli* and spot cultures of *Proteus, Klebsiella* and *Pseudomonas were made on the lawn culture* and incubated at 37^{o}C for overnight. (Fig 4.3)

F. REINOCULATION TECHNIQUE

Nutrient broth was prepared and autoclaved. Each tube was inoculated with 1ml of *Vibrio cholera* and incubated at 37^{o}C for 2 hours, cell lysate added and centrifuged at 10000 rpm for 10 minutes in order to destroy the bacterial cells. Supernatant was collected and reinoculated with 1ml of *Vibrio cholera* and further incubated for 5 hours and OD values were noted. (Fig 4.4)

G. COMPARISON BETWEEN PURE AND MIXED CULTURES

- ✓ Nutrient broth was prepared in four tubes and autoclaved. The first tube was kept as control, second tube was tube was inoculated with 1ml of *Proteus*, third tube was inoculated with 1ml of Klebsiella and

the forth tube was inoculated with both *Proteus* and *Klebsiella*. The tubes were kept for incubation at 37°C for 5 hours and OD value was measured at every half an hour interval.

- ✓ Nutrient broth was prepared in four tubes and autoclaved. The first tube was inoculated with 1ml of E.coli, second tube was inoculated with 1ml of Vibrio cholera, third tube was inoculated with E.coli and Vibrio cholera and the forth tube was inoculated with E.coli, Vibrio cholera and Klebsiella and incubated at 37° C for 5 hours. The OD values were measured at every half an hour interval. (Fig 4.5)

H. REINOCULATION TECHNIQUE 2

Double strength Nutrient broth was prepared in five tubes and autoclaved. Each tube was inoculated with *Klebsiella* and incubated at 37°C for 2 hours, OD values were measured at every half an hour interval and centrifuged. The supernatant was collected and reinoculated with *Proteus* and incubated for 37°C for five hours and OD values were measured at every half an hour interval. (Fig 4.6)

I. LACTOSE DEGRADATION TEST (BROTH)

Lactose broth (10% lactose and 10ml distilled water) was prepared in four test tubes and autoclaved. The first tube was kept as control, second tube was inoculated with 1ml of *Klebsiella* (Lactose degrading), the third tube was inoculated with 1ml of *Pseudomonas* (non-lactose degrading) and the forth tube was inoculated with both *Klebsiella* and *Pseudomonas* and incubated at 37° C for 5 hours and OD values were measured at every half an hour interval. (Fig 4.7)

J. USE OF CITRIC ACID AS THE CARBON SOURCE

Nutrient broth and lactose broth (10% lactose) were prepared, 1% of citric acid was added to few tubes and autoclaved. *E.coli culture(1ml)* was inoculated in nutrient broth and lactose broth tubes and 1ml of *Klebsiella* was inoculated in nutrient broth and lactose broth tubes with 1% citric acid. All the tubes were inoculated at 37°C for five hours and OD values were taken at every half an hour interval. (Tab. 4.1)

K. USE OF PYRUVATE AND CITRIC ACID AS CARBON SOURCE

Test tubes(10 number of 15ml capacity) were taken and to the first tube added 1% pyruvate and 1% citric acid, to the second tube added 1% pyruvate alone, to the third tube 1% citrate alone, to the forth tube nutrient broth, to the fifth tube1% lactose, to the sixth tube 1% pyruvate and 1% lactose, to the seventh tube1% lactose and 1% citric acid, to the eigth tube nutrient broth and 1% citric acid, to the ninth tube 1% pyruvate and nutrient broth and the tenth tube was kept as control with distilled water. *E.coli* culture (1ml) was added to all the test tubes. All tubes were incubated at 37°C for ten hours and OD values were taken in every half an hour interval. (Chart 4.2)

L. NUTRIENT DIFFUSION TEST

Lactose agar plates (10% lactose, 2grams of agar and 100ml of distilled water) were prepared and autoclaved. Lines were drawn at 0.5cm distance and lactose degrading organisms were inoculated (*Klebsiella*) on the line and non-lactose degrading organisms *(E.coli, Pseudomonas, Proteus, Vibrio cholera, Salmonella)* were inoculated next to the lines, and incubated at 37°C overnight. (Fig 4.8)

IV. RESULTS AND DISCUSSION

A. CHECKING OF NUTRIENTS UPTAKE

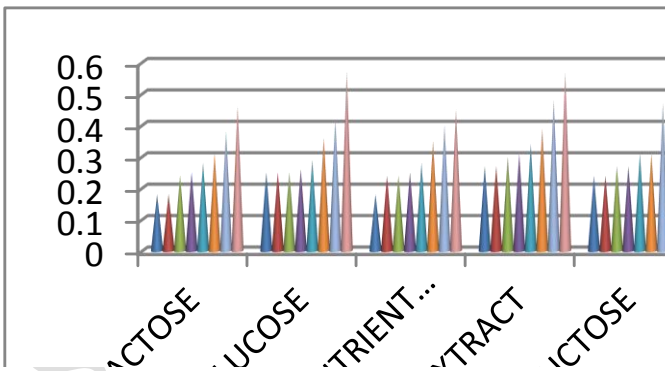

Chart: 4.1: Showing notable difference in nutrient uptake

From this it was inferred that the time taken for a monosaccharide (glucose, fructose), nutrient broth nutrients and beef extract degradation greatly differed from disaccharide (lactose) degradation and also that the metabolic activity of E.coli cells decreased when it utilized disaccharides like lactose. Thus it was confirmed that Bacterial cells preferred simple compounds than complex compounds whereby the enzymatic activity of a cell can be increased in a short span of time.

B. BREAKDOWN OF CELLS AND COLLECTION OF ENZYMES FROM THE SUPERNATANT

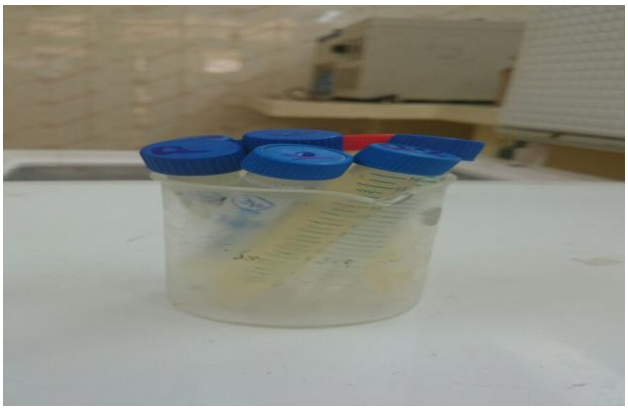

Figure 4.1: Showing centrifugation tubes containing Nutrient broth medium inoculated with E.coli.

Since neither growth nor turbidity was observed in the inoculated nutrient broth tubes after centrifugation, it was inferred that the first inoculated organism had already exhausted all the nutrients in the medium or the accumulation

ISSN: 2394-4404

of secondary metabolites (toxins and other secretary products) would have inhibited the growth of newly inoculated organisms.

C. LAWN CULTURE OF E.COLI WITH SPOT CULTURE OF LACTOBACILLUS

Figure 4.2: Showing lawn culture of E.coli

Lactobacilli colonies were not seen in the lawn culture of *E.coli* in nutrient agar plates. This showed that the lawn culture of *E.coli* did not support the growth of *Lactobacilli*. Another probability could be that since Lactobacilli is a slow growing bacteria, *E.coli* would have gained advantage over *Lactobacilli* and did not allow it to grow and this experiment also indicated that the enzymes secreted by *E.coli* cells could not be utilized by *Lactobacilli*.

D. LACTOSE DEGRADATION TEST

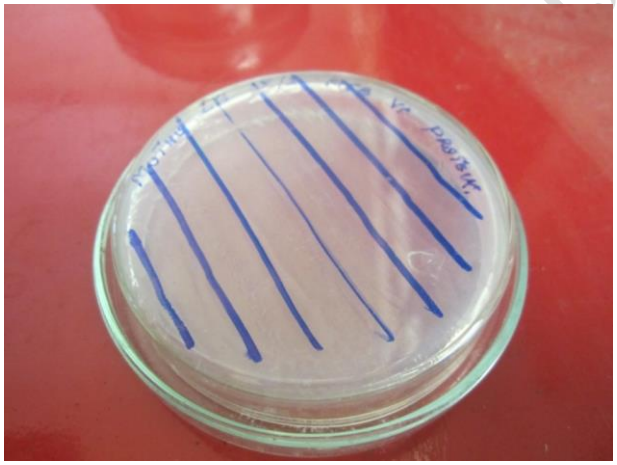

Figure 4.3: Showing lactose agar plates

Colonies weren't observed in Lactose agar plates but colonies were seen in MacConkey agar plates. This shows even lactose fermenting organisms like (*E.coli*) could not grow in Lactose agar plates (10% lactose, 2% agar and distilled water) with lactose alone but could grow in MacConkey agar plates showing lactose fermenting colonies.

E. RE-INOCULATION TECHNIQUE

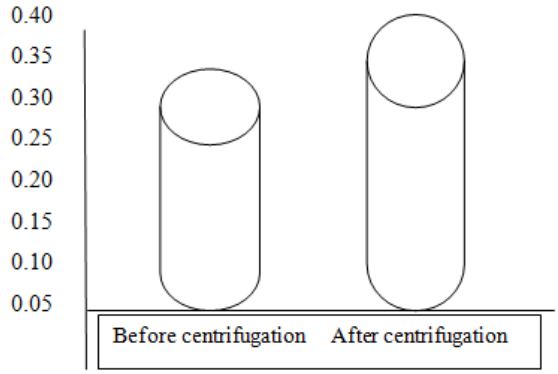

Figure 4.4: Showing enhanced Vibrio cholera growth in a gap of 2 hours of readings

In this experiment considerable difference was observed in the time taken by *Vibrio cholera* to reach the log phase for after centrifugation, there was a drastic difference in the growth rate but the growth stopped suddenly which showed that the culture supernatant promoted growth but when it got exhausted, growth stopped.

F. COMPARISON BETWEEN PURE AND MIXED CULTURES

Figure 4.5: Showing nutrient broth tubes with both pure and mixed cultures

Notable difference weren't seen between pure cultures and mixed cultures in nutrient broth. This shows that the time taken for the organism to reach log phase for both pure culture (*Proteus*) (*Klebsiella*) and mixed culture (*Proteus, Klebsiella*) (*Proteus, Klebsiella* and *Vibrio cholera*) was more or less the same. This may be due to the fact that the organisms present in the mixed cultures did not support each others growth.

ISSN: 2394-4404

G. RE-INOCULATED TECHNIQUE 2

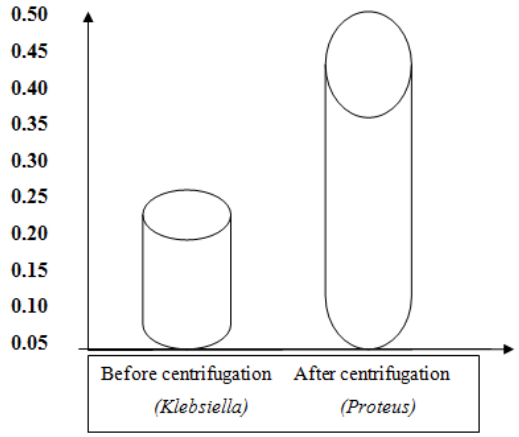

Figure 4.6: Shows considerable growth difference of Klebsiella and Proteus in 4 hour of incubation

From the result it was inferred that *Klebsiella* culture took 2 hours to enter log phase, whereas, Proteus culture took just one hour. This may be due utilization of one or more primary metabolites produced by Klebsiella by Proteus.

H. LACTOSE DEGRADATION TEST (BROTH)

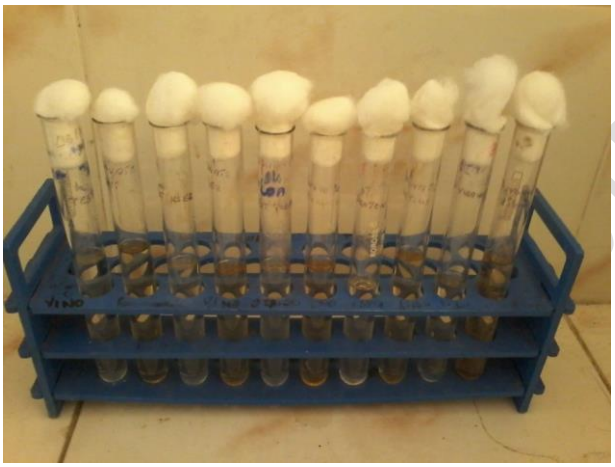

Fig 4.7 Showing lactose broth tubes

The lactose broth tubes (10% lactose and distilled water) with both pure culture and mixed cultures neither showed growth nor turbidity 5 hours of incubation but turbidity was seen in overnight incubated tubes. This may be because of the enzymatic activity of the cell based on their source of nutrients and organisms took longer time to utilize complex compounds than simpler compounds.

I. USE OF CITRIC ACID AS THE CARBON SOURCE

TIME/MIN	CONTROL		CITRIC ACID (E.coli)		CITRIC ACID (Klebsiella)		Nutrient broth (E.coli)	
	NB	LB	NB	LB	NB	LB	NB	LB
30	.22	.18	.21	.18	.21	.18	.20	.19
60	.22	.18	.22	.19	.24	.21	.24	.19
90	.22	.19	.23	.20	.26	.22	.22	.20
120	.23	.19	.24	.21	.26	.21	.24	.19
OVER NIGHT INCUBATION	.22	.18	.81	.44	1.10	.36	.84	.35
30	.24	.18	.81	.46	1.16	.38	.71	.35
60	.22	.21	.84	.41	1.21	.29	.64	.22
90	.23	.19	.77	.39	1.19	.28	.65	.25
120	.25	.20	.71	.35	1.19	.24	.57	.19
150	.24	.21	.69	.33	1.18	.23	.55	.18

Table 4.1: Showing OD values of Klebsiella and E.coli

Notable differences were observed between nutrient broth and lactose broth with and without 1% citric acid in the medium. But citric acid could not be readily utilized by bacteria. Therefore it may be inferred that the organism could not switch to Kreb's cycle directly by ignoring Glycolysis.

J. USE OF PYRUVATE AND CITRIC ACID AS CARBON SOURCE

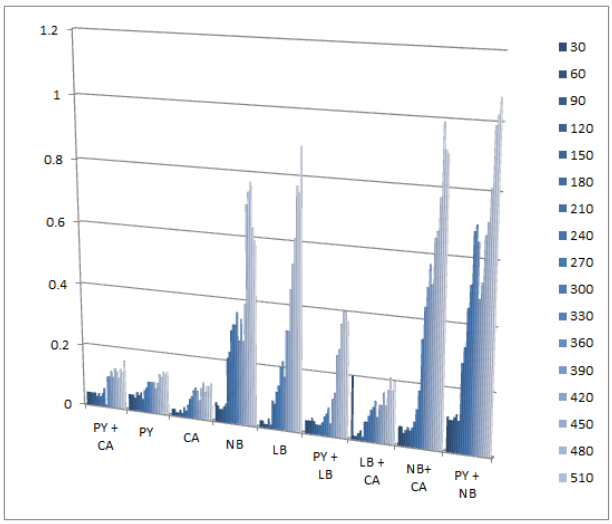

Chart 4.2: showing the growth differences between growth promoters

After ten hours of incubation, since considerable differences were noted between growth rate of cultures inoculated in Nutrient broth medium, Nutrient broth along with pyruvate, Nutrient broth along with citric acid, lactose broth and lactose broth with pyruvate. This may be due to the use of pyruvate and citric acid as good growth promoters.

K. NUTRIENT DIFFUSION TEST

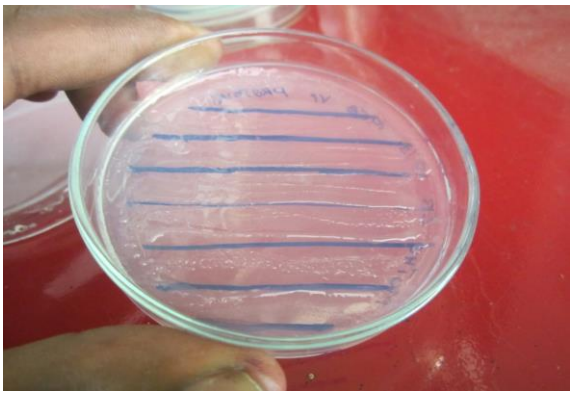

Figure 4.8: Showing lactose agar plate with the colonies of both Klebsiella and Proteus

ISSN: 2394-4404

This experiment was done to check whether the non-lactose degrading bacteria can utilize the primary metabolites from the lactose utilizing bacteria (Ex. *Klebsiella*). But colonies weren't observed in any of the plates. This may be due to none utilization of the the primary metabolites of the lactose degrading colonies by the non-lactose degrading bacterial colonies.

V. SUMMARY & CONCLUSION

Different bacterial cultures namely *Escherichia coli*, *Klebsiella*, *Proteus*, *Salmonella*, *Staphylococcus*, *Pseudomonas* were isolated and identified by using standard laboratory techniques. The approximate doubling time of the isolated bacteria was studied. Different experiments were employed to test the reduction in the doubling time of these bacteria. First, the time taken for a cell to utilize both simple compounds and complex compounds were analyzed. In another technique, nutrient broth tubes were inoculated with the bacteria and kept for overnight incubation. Those cultures were centrifuged, supernatant was collected and the organisms were re-inoculated in the supernatant medium but growth wasn't observed, this may be due to exhaustion of nutrients or inhibition by secondary metabolites. In a yet other technique, the lawn culture of *E.coli* with spot culture of *Lactobacilli was attempted*, no colonies were observed in spot inoculated part, which indicated that the enzymes secreted by *E.coli* cells could not be utilized by *Lactobacilli*.

Similar to above technique a lawn culture of lactose degrading organism was inoculated over that spot culture of non-lactose degrading organisms but no growth was observed. Again a re-inoculation technique was performed on *Vibrio cholera*, where notable results were observed in the OD values which showed that the culture supernatant promoted growth but when it got exhausted, growth stopped. In another technique, pure cultures and mixed cultures were both inoculated in nutrient broth to study whether organisms can promote each others growth rate, expected results were not obtained. In the diffusion technique, both non-lactose and lactose degrading organisms were inoculated next to each other on Lactose agar plates. No growth was observed which may be due to none utilization of the the primary metabolites of the lactose degrading colonies by the non-lactose degrading bacterial colonies.

The use of pyruvate and citric acid as good growth promoters was justified and future work is needed to obtain expected results.

REFERENCES

[1] Gottschall, J. C.; Harder,W.; and Prins, R. A. 1992. Principles of enrichment, isolation, cultivation, and preservation of bacteria. In *The prokaryotes*, 2d ed., A. Balows et al., editors, 149–96. New York: Springer-Verlag**.**

[2] *Skarstad K, Steen HB, Boye E (1983). "Cell cycle parameters of slowly growing Escherichia coli B/r studied by flow cytometry". J. Bacteriol. 154 (2): 656–62. PMC 217513. PMID 6341358.*

[3] *Zwietering M H, Jongenburger I, Rombouts F M, van 'T Riet K (1990). "Modeling of the Bacterial Growth Curve". Applied and Environmental Microbiology* ***56*** *(6): 1875–1881. PMC 184525. PMID 16348228.*

[4] *Fankhauser, David B. (17 July 2004). "Bacterial Growth Curve". University of Cincinnati Clermont College. Retrieved 29 sDecember 2015.*

[5] "http://www.ifr.ac.uk/bacanova/project_backg.html". Retrieved on May 7, 2008

[6] "Marshall T. Savage - An Exponentialist View"

[7] *Novick A (1955). "Growth of Bacteria". Annual Review of Microbiology 9: 97–110. doi:10.1146/annurev.mi.09.100155.000525. PMID 13259461.*