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DIAUXIC GROWTH OF *RAOULTELLA SP SBS2* FAVORING ENHANCED DEGRADATION OF PHENOL IN MINERAL SALT PHENOL MEDIUM

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ABSTRACT
Raoultella sp SBS2 was proved as a potent species capable of degrading phenol to less toxic or non toxic intermediates. The ability of the bacteria to grow at various concentrations of phenol from 7.5mM to 37.5 mM concentrations was evaluated in terms of both growth and percentage reduction of phenol. On analysis of the growth curve, it was observed that the organism exhibited diauxic growth in all the cases of effective biodegradation. The extent of log phase in each of the phases in the diauxic growth pattern decreased upon increasing the concentration of phenol from 7.5 mM to 37.5 mM. The results showed similar trend in the reduction of percentage of phenol biodegradation on raising the concentration from 7.5mM to 37.5 mM. At various concentrations of phenol, the growth pattern showed a better degradation rate in second phase of logarithmic growth rather than in first phase. Phenol concentration of 37.5 mM yielded only one significant lag phase from 4 to 8 hours giving a total phenol removal of only 33% in MSPM.

KEYWORDS: Phenol, *Raoultella* sp, Diauxic growth, biodegradation.

INTRODUCTION
Our environment is deeply polluted with several toxic pollutants. Phenol, which is listed as a priority pollutant by EPA is one among them. Biodegradation is more advantageous and inexpensive when compared to physical or chemical methods of degradation as it offers complete mineralization to less toxic or non toxic intermediates.

Microbial cell remediation of xenobiotics was found to be a challenging task for microbial ecologists and process engineers (Satya Sunder Mohanty, 2017). Use of competitive microorganisms in the biodegradation of phenol was a challenging strategy. *Raoultella sp SBS2*, a gram negative and aerobic, non-motile, facultative anaerobic rods exhibited immense potential in biodegrading phenol.

Bi-phasic or diauxic growth was observed when bacteria were allowed to grow on chemically defined medium. A phase of arrested growth separates two logarithmic phases. The lag phase represents a consequential loss of growth during this switch over (Chu & Barnes, 2016). The change in substrate concentrations and dilution rates may be the key factors that facilitate the diauxic growth (Nakamura et al, 1996). The provision of higher growth rate by the substrate is an intrinsic property of induction and dilution kinetics (Narang & Pilyugin, 2006). There exists a dynamic analogy between the microbial growth on substrate and the increase in population of competitive species. When a complex condition is arrived, the bacteria will adapt themselves to regulate its gene expression to select and survive under the best available carbon sourc (Zhan et al, 2009).

We have been concentrating our research on phenol biodegradation for the past few years and have already selected four bacterial species as efficient degraders. These organism includes *Enterobacter* sp SBS 1, *Raoultella* sp SBS 2, *Alcaligenes* sp d2 and *Bacillus megatherium* sbs 3.We have explored the mechanism, enzymology and growth kinetics of phenol biodegradation by all these isolates. Here in this paper we report the significance of diauxic growth pattern exhibited by *Raoultella* sp on degrading phenol.

MATERIALS AND METHODS
Selection of the bacterial strain
The bacteria, *Roultella sp SBS 2*, was collected from culture collection centre of Bioprocess and Biotechnology lab, School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala. The bacteria was maintained by periodic subculturing in nutrient agar media.

Biodegradation of phenol
Raoultella sp SBS 2 was allowed to utilize different concentrations of phenol in Mineral Salt Phenol Medium

(MSPM) at different incubation periods from 4 to 36 on an interval of 4. The percentage reduction in phenol concentrations were estimated with modified Mordoco *et al* method. (Mordocco, 1999). A graph showing the percentage reduction in phenol concentrations against different incubation periods was plotted.

Growth curve of *Raoultella sp SBS 2* at different concentrations of phenol

16-24 hour old culture of *Raoultella sp SBS 2* was allowed to grow in MSPM containing phenol as the only source of carbon and energy. Different concentrations of phenol from 7.5mM to 37.5mM were used for the study. The optical density was measured at 600nm for the sample withdrawn on every 4 hour interval of time. A standard graph with optical density on Y axis and incubation time in hours on X axis was plotted.

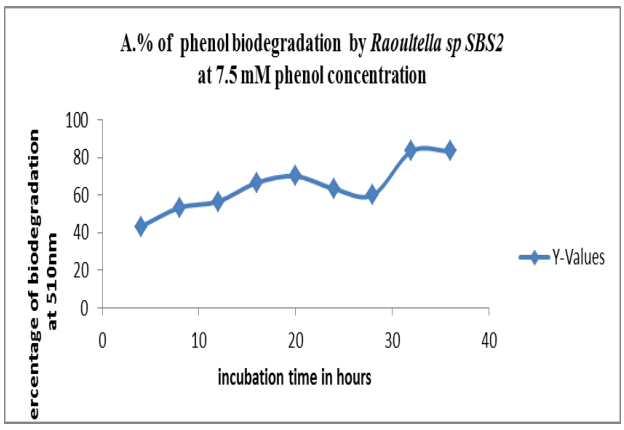
Diauxic growth analysis of *Raoultella sp SBS 2* on phenol

The growth curve of the *Raoultella* sp SBS2 was studied and the extent of the logarithmic phase of bacterial growth was noted. The time period, extent of growth and the percentage of biodegradation on the stipulated phases were recorded.

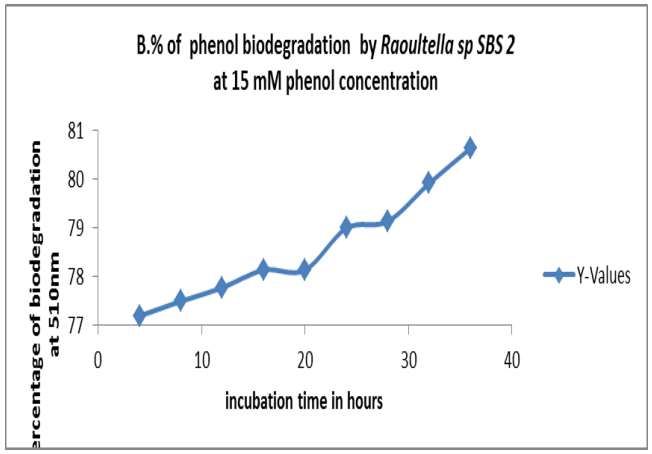
RESULTS AND DISCUSSION

The present work brings out the phenol metabolizing ability of *Raoultella sp SBS2* in mineral salt phenol medium. Phenol in concentrations 7.5mM, 15mM, 22.5 mM, 30 mM, 37.5 mM were used for the study. High concentrations of substrates exhibited inhibitions on cell growth as well as its own rate of degradation (Bai Jing et al, 2006).

Raoultella sp SBS2 was found to be degrading 86% of 7.5mM phenol whereas the rate of biodegradation was found to decrease on increasing the phenol concentration from 7.5mM to 37.5mM. It was noted that the percentage of degradation was maximum at 24 hours of incubation in all the cases of phenol concentrations (Fig.1 to Fig.5).



"Fig 1"



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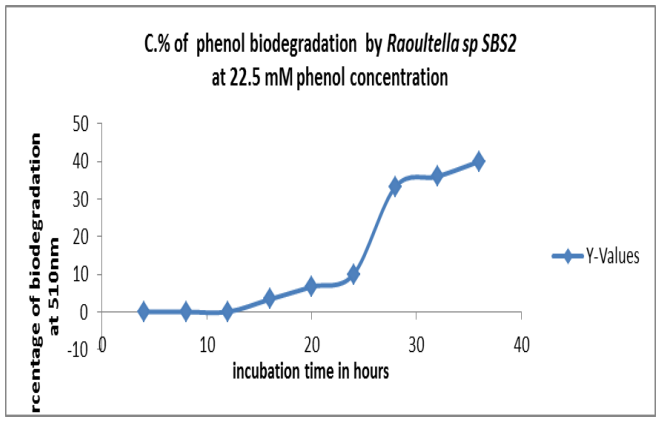

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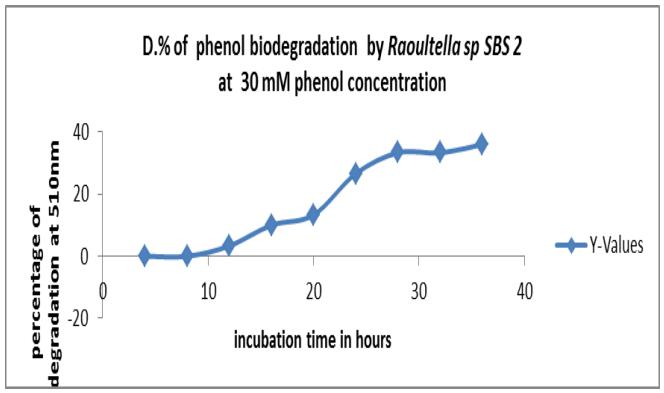

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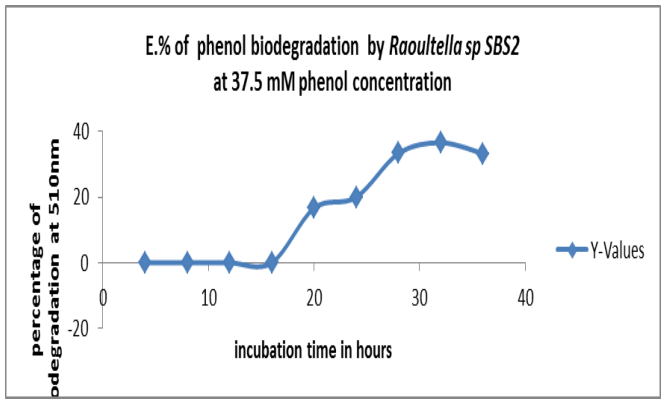
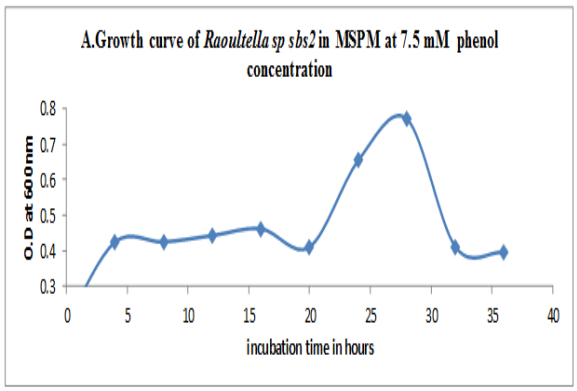


"Fig 5"

In most of these cases phenol degradation was effected during two phases of bacterial growth. The bacterium metabolized 50% of 7.5mM phenol in 4 hours of incubation where as it showed nearly 70 percent of reduction in 15mM phenol under same condition. At phenol concentrations of 22.5mM, 30mM and 37.5 mM, the initial log phase growth of the culture could not contribute much in the percentage reduction of phenol. The higher percentage of phenol removal was observed in the second phase of growth after an incubation of nearly 24 hours.

The bacterial species showed diauxic growth nature on metabolizing phenol in mineral salt medium. Generally at all cases of phenol concentrations the initial growth phase was shown on 0-8 hours of incubation period and the second logarithmic phase was shown after 24 hours of incubation(Fig. 6 to Fig.10).



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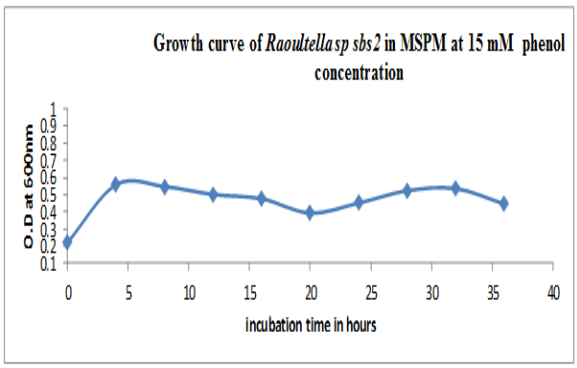

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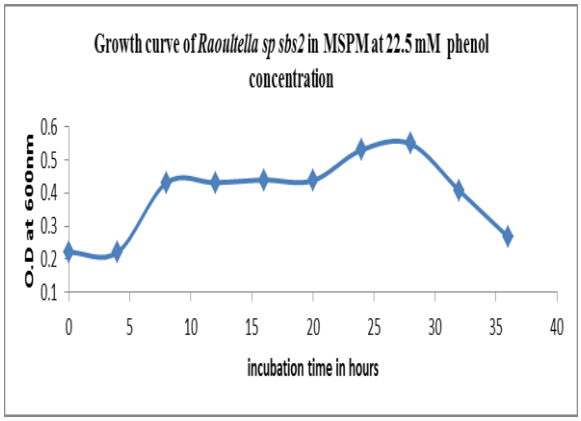

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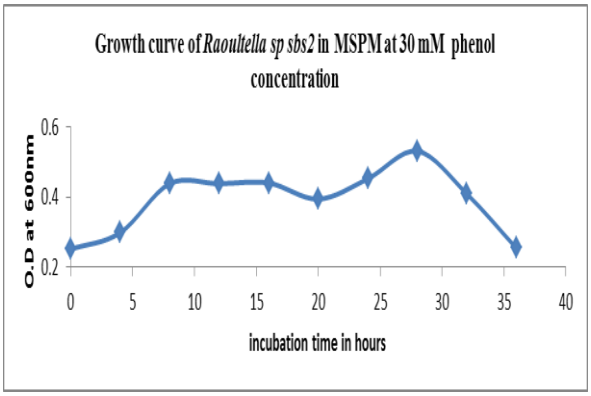

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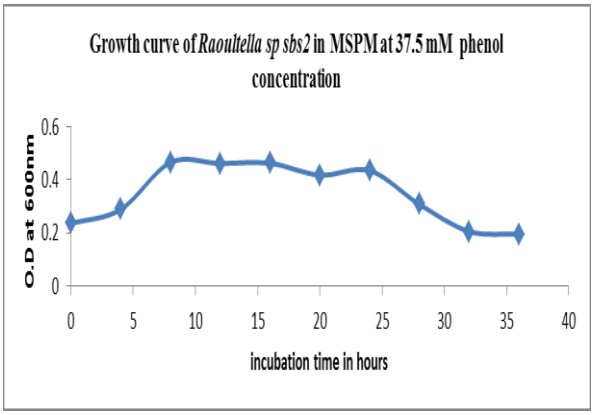

"Fig 10"

In all the cases of phenol concentrations from 7.5 mM to 30mm the bacteria exhibited diauxic growth. At the phenol concentrations of 7.5 mM and 15 mM the first phase of diauxic growth was within 4 hours of incubation and the second phase of diauxic growth could be observed from 20-28 hours of incubation. At 22.5 mm and 30 mM phenol concentration, 4-8 hours marked the first phase and 22-24 represented the second phase (Table 1). It is to be noted that the extent of both first and second phase of diauxuc growth got diminished upon increasing the phenol concentration. It followed that the length of the log phases in a diauxuc growth behavior was instrumental in bringing better biodegradation rate particularly in the case of stress situations. At the highest concentration of 37.5 mM the growth curve included

only one log phase from 20 hours onwards. The resultant percentage of phenol degradation was also considerably less.

Table 1:- Diauxic growth Vs % of phenol degradation of *Raoultella* sp SBS2 at different concentrations of phenol

concentration	1st Log phase of growth in hours	Rate of biodegradation	2nd Log phase of growth in hours	Rate of phenol biodegradation
7.5mM	0-4	50%	20-28	86%
15mM	0-4	71%	20-28	79%
22.5mM	4-8	Nil	20-24	10%
30mM	4-8	Nil	20-28	35%
37.5mM	4-8	Nil	nil	33%

CONCLUSION

Raoultella sp SBS2 has proved to be a potent strain in the biodegradation of phenol. The bacteria can easily metabolize lower concentrations of phenol effectively through a biphasic growth behavior. But at higher concentrations, the length of the diauxic phases decreased and the resultant degradation rate progressively got diminished. The present observation regarding the linkage between diauxic growth nature and biodegradation of a stress pollutant throw light into the possibility of developing new strategies for extension of diauxic phases under stress using methods like cometabolism.

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