

# GROWTH CURVE ANALYSIS OF BACTERIAL ISOLATES FROM FRESH COAL MINE SPOIL

**KHULANA SETHY AND NIRANJAN BEHERA\***

School of Life Sciences, Sambalpur University,  
Jyotivihar, Burla - 768 019  
Sambalpur, Orissa, INDIA  
E-mail: nbehera2001@yahoo.com

## KEY WORDS

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**\*Corresponding  
author**

## ABSTRACT

The study deals with growth curve analysis of a Gram negative bacilli and a Gram negative cocci, isolated from fresh coal mine spoil, over a temperature range of 35-50°C. The analysis indicated normal optimal growth curve for both the isolates at 35°C. However with increase in temperature, both the isolates showed deviated growth curve with reduction in the exponential phase and specific growth rate. The study concludes both the isolates to be mesophilic with capacity to tolerate higher temperature regime.

## INTRODUCTION

Coal mine spoil overburden represents a physically disturbed habitat for the existence of soil organism (Juwarkar *et al.*, 2004; Ghose, 2005; Machula *et al.*, 2005), due to internal high temperature profile (Bos *et al.*, 1994; Johnson 2002; Satyanarayana *et al.*, 2005 ) and low pH (Blowes *et al.*, 1995; Kristjanson and Hreggvidsson, 1995; Ledin and Pedersen, 1996; Schippers *et al.*, 2000). In spite of such extremities, the coal mine spoil is not a microbiologically sterile habitat and often harbours specific group of thermoacid tolerant, chemolithotrophic and heterotrophic bacteria (Darland *et al.*, 1970; Belly and Brock, 1974). Our earlier microbiological studies (Sethy and Behera, 2009) on coal mine spoil overburdens of Basundhara coal field area of Mahanadi coal field limited, Orissa revealed the isolation of thermo and pH resistant Gram negative bacilli and cocci. In the present study, the growth performances of these two isolated group of bacteria with respect to a temperature range of 35 to 50°C was analysed.

## MATERIALS AND METHODS

Pure culture of Gram negative bacilli and cocci (isolated through our earlier sampling: Sethy and Behera, 2009) were used for the present study. A loop of bacteria from the pure culture slant of each bacterial isolate was aseptically transferred to a sterilized 250mL Erlenmeyer flask containing 100mL of nutrient broth (peptone-5g/L, Beef extract-3g/L and NaCl-5g/L) and was activated by incubating for 24hrs at 35°C.

Fifteen number of Erlenmeyer flasks (capacity: 150mL) and each containing 50mL of sterilised nutrient broth were taken and to the 12 number flasks, one ml of activated Gram (-ve) bacilli culture was aseptically transferred. These flasks were then incubated in a shaker incubator over a temperature range of 35 to 50°C for a period of 285-300 minutes. Simultaneously, three number of Erlenmeyer flask (capacity: 150mL) having only nutrient broth (un inoculated) were taken as control. The culture content of the flasks were spectrophotometrically analysed for growth of the bacteria at 640nm with respect to different time (15 minutes) interval. Same procedure was adopted for the growth assessment of Gram negative cocci bacterial isolate.

Specific growth rate ( $\mu$ ) of the bacterial isolates at different temperature was calculated as follows (Heritage *et al.*, 2002):

$$\frac{\log N_t - \log N_0}{t_t - t_0} = \frac{\mu}{2.303}$$

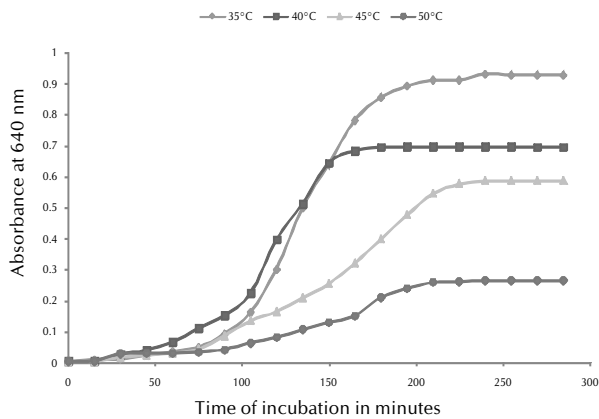
Where,  $N_0$  = Absorbance at initial of the exponential phase of growth

$N_t$  = Absorbance at end of the exponential phase of growth

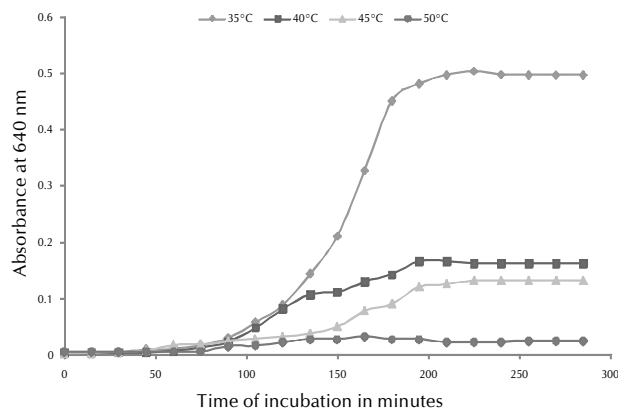
$t_t - t_0$  = Time difference to achieve absorbance from  $N_0$  to  $N_t$

## RESULTS

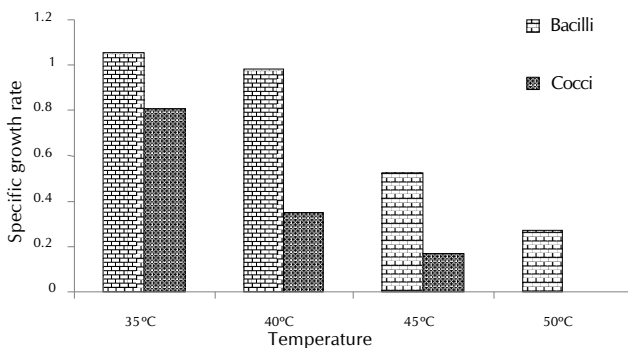
Fig.1 illustrates the growth curve of Gram negative bacilli at a temperature regime of 35 to 50°C. The bacteria showed a lag phase up to 60 to 90 minutes. The exponential phase for the



**Figure 1: Growth curve of Gram negative bacilli at different temperature**



**Figure 2: Growth curve of Gram negative cocci at different temperature**



**Figure 3: Specific growth rate of Gram negative bacilli and cocci at different temperature regime**

bacteria growth at 35°C lasted up to 195 minutes, after which the bacteria entered in to stationary phase. However, the growth patterns of the bacteria at 40, 45 and 50°C were marked to be different. Growth at 40°C showed relatively earlier culmination of exponential phase. At 45°C, although exponential phase did extend up to 225 minutes, the slope of the phase was much less compared to 35°C or 40°C. At 50°C, the bacteria did exhibit little growth showing still less slope during the exponential phase. Fig. 2 illustrates the growth curve of Gram negative cocci over a temperature regime of 35 to 50°C. The

bacteria showed a lag period up to 90 to 135 minutes. The exponential phase for the bacteria growth at 35°C lasted up to 195 minutes of incubation time, there after the stationary phase started. Where as at 40°C, the bacteria exhibited relatively earlier culmination of the exponential phase. However at 45°C the bacteria did exhibit little growth and the slope of the phase was much less than 35 and 40°C. In response to 50°C the growth of bacteria was found to be insignificant.

Specific growth rate ( $\mu$ ) of the bacteria at different temperature (Fig. 3) indicated highest value of " $\mu$ " at 35°C and the value decreased with gradual increase in the temperature.

## DISCUSSION

Our earlier study (Sethy and Behera, 2009) revealed Gram negative bacteria (both bacilli and cocci) to be in a major proportion of total colony forming units of bacterial population in the fresh coal mine spoil. There have been also reports about the prevalence of Gram negative bacteria from coal mine spoils of different geographical regions (Marsh and Norris, 1983; Ghauri and Johnson, 1991; Zhou *et al.*, 2007). Relatively greater tolerance of Gram negative bacteria to different extremities of habitat is usually explained on the basis of their external lipopolysaccharide layer over and above the cell wall (Moat *et al.*, 2006). The growth pattern of these isolates over a temperature range starting from 35°C to 50°C, as observed in the study depicts their adoptibility for the higher temperature tolerance, confirming the observation of Hallberg and Lindstrom (1994) and Zhou *et al.*, (2007). Analysis of different growth phases with respect to different temperature regime indicated the optimal growth of the isolates at 35°C and increase in the temperature above 35°C affected mostly these exponential growth phase resulting relatively early initiation of stationary phase. Such observation clearly reveals about their basic mesophilic character with adoptibility for the higher temperature tolerance. Growth pattern of the Gram negative cocci at higher temperature of 50°C showed total absence of the exponential phase resulting the specific growth rate to be zero. This reflects the poor thermal adoptibility of the cocci in comparison to the bacilli. However, both the isolates showed maximum value of specific growth rate at 35°C and the rate declined with increase in the temperature which very conclusively points out their mesophilic character. Further studies on the physiological viability and cellular enzymatic characterization of these two isolates with respect to the higher temperature regime may throw more light on their bioprospecting ability in the context of their industrial application.

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