

## GROWTH KINETICS OF *PASTEURELLA MULTOCIDA* ISOLATED FROM NILI RAVI BUFFALO (*BUBALUS BUBALIS*)

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### ABSTRACT

Hemorrhagic septicemia (HS), caused by *Pasteurella multocida*, is one of the highly fatal diseases of Buffalo and mostly killed vaccines are used to save the animals from this disease. Enhanced cell mass yield from *Pasteurella multocida* could help in better vaccine efficacy and process economics. In the present study, we characterized the growth pattern of *Pasteurella multocida* on various carbon sources by determining the specific growth rate ( $\mu$ ) and volumetric rate of cell mass formation ( $Q_x$ ). A field isolate of *Pasteurella multocida* Robert's type-1 was grown on standard casein yeast extract media using glucose, maltose, galactose or sucrose as carbon source (0.3%). Higher cell mass yields were noted at 48, 32, 26 and 22 h post inoculation, respectively for glucose, sucrose, maltose, and galactose. For a further insight, carbon source concentration was increased from 0.3 to 1.0% and dry cell mass yields on glucose, fructose, maltose, galactose and sucrose were noted in shake flask culture. Maximum dry cell mass ( $\text{g L}^{-1}$ ) was obtained when sucrose used as carbon source followed by fructose. Specific growth rates of 0.319, 0.091, 0.183, 0.156 and 0.147 ( $\text{h}^{-1}$ ) respectively were noted on sucrose, glucose, fructose, maltose and galactose, respectively. Similarly,  $Q_x$  was found to be significantly higher on sucrose compared with other sugars. Volumetric rate of cell mass formation were 0.205, 0.202, 0.093, 0.124, 0.166  $\text{g L}^{-1} \text{h}^{-1}$  on sucrose, glucose, fructose, maltose and galactose respectively. There was an increase of 1.7% or a decrease of 38.1, 53.5 and 17.8% in  $Q_x$  values on sucrose, fructose, maltose and galactose, respectively compared with that on glucose. The results indicated that carbon source type and concentration has significant effects on cell mass yield and optimized concentration and type of the carbon source could help in better vaccine production for HS disease control.

**Key words:** *Pasteurella multocida*, carbon sources, growth, kinetics, buffalo.

### INTRODUCTION

Hemorrhagic septicemia (HS) is an acute disease caused by particular serotypes (E: 2 and B: 2) of *Pasteurella multocida* and manifested by an acute and highly fatal septicemia in cattle and buffaloes (Arun and Krishnappa, 2004). The causative organisms during intervening periods survive in the tonsillar and nasopharyngeal mucosa of the animals (Blood, *et al.* 1989; Foged, 1992). Vaccination against this disease is widely practiced. Plain broth bacterins, or alum precipitated and aluminium hydroxide gel vaccines are administered twice a year since these vaccines offer an immunity of 4-6 months (Verma and Jaiswall 1998; Hodgson *et al.* 2005). Immunogenic property of the killed vaccines is directly proportional to the quantity of bacteria per ml of the vaccine which requires process yields to be improved. In this context, the density of culture can be increased by using improved culture medium and techniques (Bain, *et al.*, 1982). In broth, after 24 hours at 37°C, there is moderate growth with slight turbidity and a slight powdery to viscous deposit. Later the turbidity increases and a heavy viscous deposit from disintegrating partly on shaking but leaving irregularly sized wisp like masses of growth in suspension. An incomplete surface pellicle forms with an

inconspicuous ring growth (Topley and Wilson, 1998). Little information is available in terms of cell mass yields in time course from local field isolates of *Pasteurella multocida* in Pakistan. This study was conducted to determine growth pattern and some of the parameters of the growth kinetics of a field isolate of *P. multocida* from Buffalo (*Bubalus bubalis*).

### MATERIALS AND METHODS

**Test Strain:** *Pasteurella multocida* used in this study was isolated from a field outbreak of Hemorrhagic septicemia in the periphery of Faisalabad, Pakistan. The isolate was confirmed by standard microbiological and biochemical tests (on the basis of morphology, staining reaction and culture characteristics) before starting work related to growth kinetics.

**Preparation of media:** Initially *Pasteurella multocida* was grown on Casein yeast based broth by adding sucrose, glucose, galactose, maltose and fructose as carbon sources. Chemical composition of Casein yeast media with 0.3% of the stated carbon sources was Casein hydrolyzate: 3.0; Yeast extract: 5.0; NaCl: 5.0;  $\text{KH}_2\text{PO}_4$ : 2.0 g/L. Media were prepared by dissolving the ingredients in distilled water and autoclaving the contents at 121°C and 15 bar pressure for fifteen minutes until

otherwise stated. Respective to carbon source type, the sterilized media in aliquots of 50ml in 250ml flasks were allowed to cool down and then inoculated with a loopful of *Pasteurella multocida* from preserved slants. The pH of liquid medium was determined by a pH meter and adjusted to  $7.4 \pm 0.1$  by the addition of 1M NaOH.

**Inoculum preparation:** Fifty ml of CSY medium (Sucrose 1%) in 250 ml cotton wool plugged Erlenmeyer flask, was sterilized. Small amount of bacteria from the slant was aseptically transferred with the help of inoculating needle. The flask was incubated at 37°C in a rotary incubator shaker (Gallenkamp, UK) at 200 rpm for 24 h. The growth in inoculum was monitored volumetrically and at a turbidity of about 1.2 at 540 nm was used, until otherwise stated, in case of growth parameter studies based on dry cell mass yield.

**Dry weight measurement:** Casein yeast extract media was prepared as stated above with the exception of an elevated level of sugars at 10g/l concentration. Media in aliquots of 50ml in triplicate was taken with the stated. The flasks were autoclaved for 15 minutes at 121°C. After cooling the media, inoculated and incubated in a time course study in the rotary shaker at 200rpm. The flasks were monitored for growth and removed the flasks according to their sampling time ranging from 2h to 48h. The contents were centrifuged for 15 minutes at 4,000 rpm to obtain the pellet by discarding the supernatant. The weight of dry cells was determined by drying the pellet at 120°C for 2 hours.

**Fermentation technique:** At laboratory scale stainless steel stirred shaker of 16 flasks capacity was used for studying growth kinetics in time course fermentation. The fermentation medium consisted of sucrose or otherwise stated sugars. The inoculum was transferred to the medium at a rate of 2% (v/v) based on total working volume of the fermentation medium. All the experiments were run parallel in triplicate.

**Determination of Growth Kinetic Parameters of *Pasteurella multocida*:** *Pasteurella multocida* was grown on stated carbon sources at 10g/l concentration in shake flask culture. In time course study dry cell mass was noted and Kinetic parameters viz. specific growth rate ( $\mu$ ) and volumetric rate of cell mass formation  $Q_x$  were determined after Pirt (1975).

## RESULTS

**Comparison of *Pasteurella multocida* growth on various carbon sources:** When the field isolate of *Pasteurella multocida* was grown on five carbon sources viz; glucose, fructose, maltose, galactose and sucrose in casein yeast extract based media, there was a variable cell mass formation (Fig.-I). Maximum cell mass was

obtained when sucrose was used as carbon source followed by fructose. Dry cell mass per liter of 48 h grown media at 200rpm and 37 °C were 1.5, 1.4, 0.83, 1.04 and 0.62 (g/L) on sucrose, fructose, maltose, glucose and galactose, respectively. The concentration of 3.0 g/l carbon source was increased to 10.0 g/l in further studies and the results are as follows;

**Growth of *Pasteurella multocida* on fructose:** In casein yeast extract based media growth started on fructose as function of time. Time course growth pattern is presented in fig. It is obvious from the Fig.II that 1.3g, 1.6g, 2.6g, 1.8g dry cell mass per liter of culture broth was obtained on 14h, 21h, 32h and 48h respectively. There was exponential growth till 32h while growth ceased after 48h.

**Growth of *Pasteurella multocida* on maltose:** In casein yeast extract based media when maltose used as carbon source, cell mass formation started as function of time. Time course growth pattern is presented in Fig.II. It is obvious from Fig that 1.19g, 1.54 g, 1.71g and 1.15g dry cell mass was obtained at 14h, 21h, 32h, and 48h respectively. Exponential growth was evident till 32h while growth was ceased at 48h.

**Growth of *Pasteurella multocida* on galactose and sucrose:** In casein sucrose based media growth started on galactose as function of time. Time course growth pattern is presented in Fig.-II. It is obvious from the Fig that on 14h 1.4g cell mass on 21h 1.7g on 32h 1.8g and on 48h 1.4g cell mass was noted. Similarly, in casein sucrose based media growth started on sucrose as function of time.

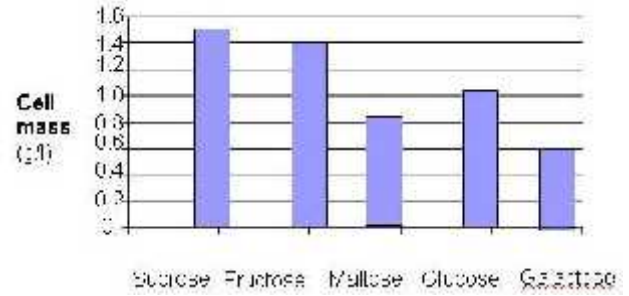
**Specific growth rate ( $\mu$ ) of *Pasteurella multocida*:** From the time course study, growth of *Pasteurella multocida* was undertaken on various carbon sources. Specific growth rate ( $\mu$ ) in *Pasteurella multocida* on various carbon sources values are presented in Table-I. Maximum ( $\mu$ ) value was noted when sucrose used as carbon source. On average basis, when sucrose, glucose, fructose, maltose and galactose were used as carbon source they resulted in specific growth rate values of  $0.319 \pm 0.018$ ,  $0.091 \pm 0.013$ ,  $0.183 \pm 0.021$ ,  $0.156 \pm 0.016$  and  $0.147 \pm 0.014$  ( $h^{-1}$ ), respectively.

**Volumetric rate of cell mass formation:** Volumetric rate of cell mass formation ( $Q_x$ ) values are presented in Table-I. Maximum  $Q_x$  value was noted when sucrose used as carbon source. On average basis, when sucrose, glucose, fructose, maltose and galactose used as carbon source they resulted in 0.2056, 0.2021, 0.0939, 0.1249, 0.1667 g/L/h, volumetric rate of cell mass formation, respectively. There was an increase in  $Q_x$  values 1.7% or a decrease of 38.1, 53.5 and 17.8% over value on glucose, were used as carbon sources, respectively at 10g/l concentration.

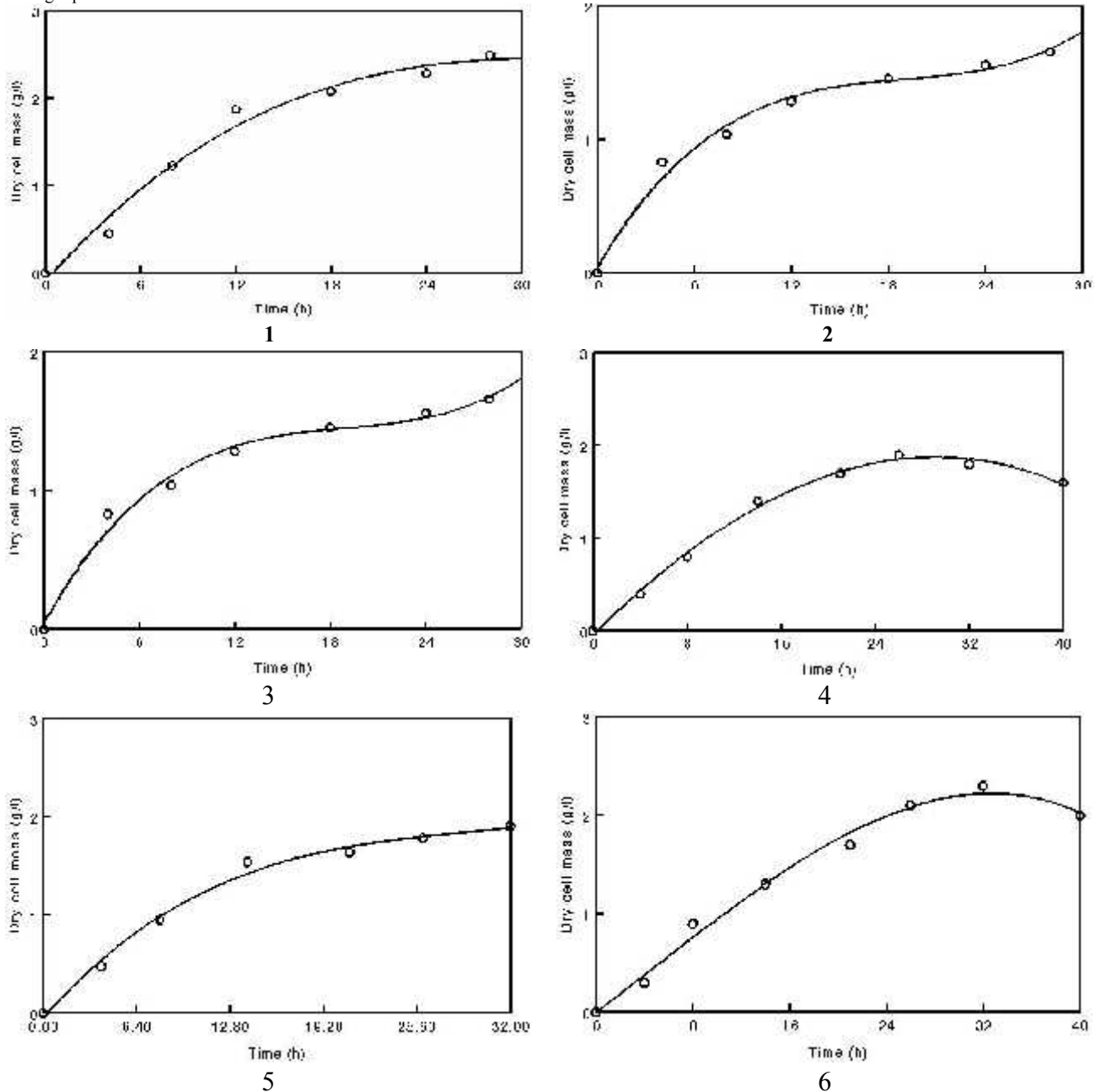
**Table-I Specific growth rate ( $\mu$ ) and  $Q_x$  of *Pasteurella multocida* on various carbon sources**

Carbon source	Specific Growth Rate ( $\mu$ ) $h^{-1}$	Volumetric rate of cell mass formation $Q_x$ (g/L/h)
Sucrose	$0.319 \pm 0.018$	$0.205 \pm 0.006$
Glucose	$0.091 \pm 0.013$	$0.202 \pm 0.001$
Fructose	$0.183 \pm 0.021$	$0.093 \pm 0.009$
Maltose	$0.156 \pm 0.016$	$0.124 \pm 0.009$
Galactose	$0.147 \pm 0.014$	$0.166 \pm 0.007$

Each value is a mean value of triplicate samples.  $\pm$  Shows standard error among replicates.



**Fig-I Comparative dry cell mass formation on various carbon sources**



**Fig. II. Time course cell mass formation on sucrose (1), glucose (2), maltose (3), galactose (4) and fructose (5)**

## DISCUSSION

*Pasteurella multocida* was grown on casein yeast extract based media. Four carbon sources viz; glucose, maltose, galactose, fructose and sucrose were tested. The growth on sucrose based media was more consistent and there was a decreasing reduction in O.D value with the passage of time. Present finding is in accordance to that by Bain *et al.* (1982) and Henry (2002) who studied in detail role of sucrose on growth pattern of *Pasteurella multocida*. Similarly, our results are similar to those by Andrew (1961 and 1962) and Costin (1978) who studied different media components for optimization of growth. Similar to our work Jablonski, *et al.* (1996) developed a minimal medium supporting the growth of both toxigenic and non toxigenic strains of *Pasteurella multocida* to higher optical densities.

In comparative carbon source type study, *Pasteurella multocida* was grown on casein yeast extract based media. Five carbon sources viz; glucose, fructose, maltose, galactose and sucrose were tested for optimization of cell mass formation. Maximum dry cell mass was obtained when sucrose used as carbon source followed by fructose. Our results are similar to those by De-Elwis *et al.*, (1981) and Arawwewla *et al.* (1981) who studied role of casein based media for optimization of *Pasteurella multocida* growth. The improvement in dry cell mass yield in present study could be attributed to various optimized growth factors like work reported earlier (Popova, 1997).

The decrease in growth might be a result of depletion of essential nutrients in the medium. The situation represents genetics versus environment relationship as the genetic potential of *Pasteurella multocida* growth is affected by media components. Maximum Specific growth rate ( $\mu$ ) was noted when sucrose used as carbon source. Maximum  $Q_x$  value was noted again when sucrose used as carbon source. There was an increase in  $Q_x$  values of 1.7% or a decrease of 38.1, 53.5 and 17.8% over  $Q_x$  value on glucose at 10g/l concentration. It could be concluded that growth of *P. multocida* is better on sucrose compared with glucose which might be due to catabolite repression.

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