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With 3 tables and 6 figures in the text

The Effect of pH, Working Volume and Rate of Aearation on Production of Live Anthrax Spore Vaccine

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ملخص البحث

تم إجراء هذه الدراسة باستخدام مخمر قو تنج بغرض تحديد افضل المؤثرات الآس الهيدروجيني ،حجم الزرع البكتيري ،معدل الأوكسجين الداخل لنمو وتكوين ابواغ عصوية الحمي الفحمية (عترات استيرن).وقد وجد ان افضل المؤثرات هي 6.98 للأس الهيدروجيني ، 2 لتر حجم الزرع البكتيري و1286 م م /دقيقه لمعدل الهواء الداخل.

Summary

This study was carried out using a IBT-Goettingen bioreactor to detemine the effect of certain physical parameters on the production of anthrax live spore vaccine. pH 6.98, 2L working volume, and rate of aeration at 1286 mm/min. gave the best results.

Introduction

Anthrax vaccine produced from the attenuated culture of Pasteur had been replaced by the more safe spore vaccine prepared from the avirulent uncapsulated strain of Sterne. The latter gave very good results in the field, and is now being used extensively in different parts of the world. In the Sudan anthrax live spore vaccine was prepared for the first time on solid media in Roux bottles (Babiker *et al.*, 1986). The amounts produced were limited and insufficient to meet the demand of the Sudan. A new system of mass production of bacterial vaccine has been introduced in the Sudan since 1984 using the Gottingen bioreactor (Mohamed *et al.*, 1989).

This work investigates the effect of varying some physical parameters for the production of anthrax live spore vaccine in the bioreactor.

Materials and Methods

Bacteria:

Bacillus anthracis, Sterne strain 34 F_2 was obtained from the International Laboratory for Biological Standards, CVL, Weybridge, England.

Bioreactor:

The IBT-Gottingen bioreactor was essentially used as described by Bohnel (1986).

Medium:

Meat infusion broth was used and prepared and follows (g/L): 500g minced lean meat (beef), 5g sodium chloride and 10g peptone (Sigma) were mixed in one liter of tap water and kept overnight at 4°C.Next day any floating fat particles were removed and the mixture was boiled sufficiently, then cooled and filtered through clean gauze.The pH was adjusted to 7.8, before autoclaving the medium at 121°C (20lb/in^2) for 20 minutes, then it was cooled and filtered again through clean gauze;this was followed by a final filtration through filter paper (0.2 M) by negative pressure. It was then dispensed in 5 litres amount in flasks and finally autoclaved at 121°C (20lb/in^2) for 15 minutes.To this sterile broth medium, 0.5g/L of agar (Merck) were added.The suspension was heated and finally autoclaved again.

Parameters and Counting:

The parameters used in the bioreactor were temperature, pH, aeration for growth and for sporulation, stirring and working volume. Temperature, aeration for growth and stirring were kept constant throughout the experiments. Variable values of pH, aeration for sporulation and working volume were employed to determine their effect on both growth and sporulation.

Percentage rate of sporulation was determined microscopically by counting spores (free and prespores) and the vegetative cells. Viable count was carried out according to Miles and Misra (1938).

Determination of most appropriate pH:

Temperature (37°C), working volume (2L), aeration for growth (857mm/min), stirring (300rpm), and aeration for sporulation (1286mm/min) were constantly used.The variable pH values used were 6.88, 6.89, 7.08, 7.18, 7.28, 7.38.

Determination of the best working volume:

The constant parameter used were maintained.The pH was adjusted to 6.98.The variable working volumes used were 2L,3L,4L,5Land 6L.

Determination of the most appropriate rate of aeration for sporulation:

The constant parameters, which mentioned previously, were preserved. The variable aeration rates,used for sporulation were no aeration, 1286 mm/min and full aeration (4500 mm/min).

Results

The effect of different pH values on the percentage of sporulation and the viable spores count are shown in table(1).Accordingly, pH 6.89 was taken as the best pH for viableSpores production.Figure (1) also shows the effect of pH on percentage of sporulation and that the optimal pH was 7.18.

The effect of working volume on the percentage of sporulation and viable spores count is shown in table(2) and illutraed in figure (3) and figure(4).According to the results in table (2),two liters was considered to be the most appropriate working volume for obtaining higher value of both sporulation and viable spores.

The effect of the rate of aeration on sporulation and viable spores count is shown in table (3) and accordingly, 1286 mm/min. was found to be best. Figure (5) and figure (6) show the effect of aeration on the rate of sporulation and the spores count respectively.

Table 1: Effect of pH on the percentage of sporulation and viable spores count.

pH	*Percentage of sporulation(%)*	*Viable spores count(CFU/ml)*
6.88	83.3	$1.50x10^9$
6.89	89.3	$5.00x10^9$
7.08	94.3	$1.25x10^9$
7.18	100	$2.50x10^9$
7.28	92.1	$1.50x10^9$
7.38	N.D.	$2.50x10^9$

N.D. not determined.

Table 2: Effect of working volume (w.v) on percentage of sporulation and viable spores count.

W.V.(L)	*Percentage of sporulation(%)*	*Viable spores count CFU/ml*
2	89.3	$5x10^9$
3	83.7	$1.0x10^8$
4	34	$1.0x10^7$
5	30	$1.0x10^7$
6	22	$1.0x10^6$

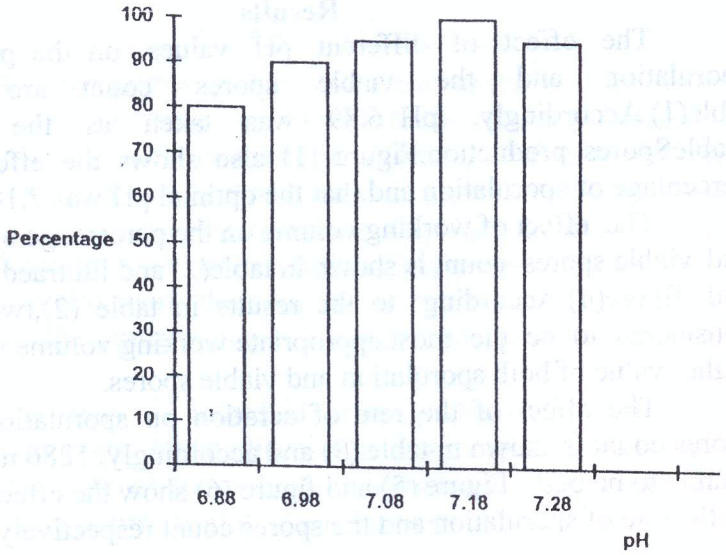

Fig.1: Effect of pH on the percentage of sporulation of *Bacillus anthracis*,Sterne strain in meat infusion broth using IBT-Goettingen bioreacter

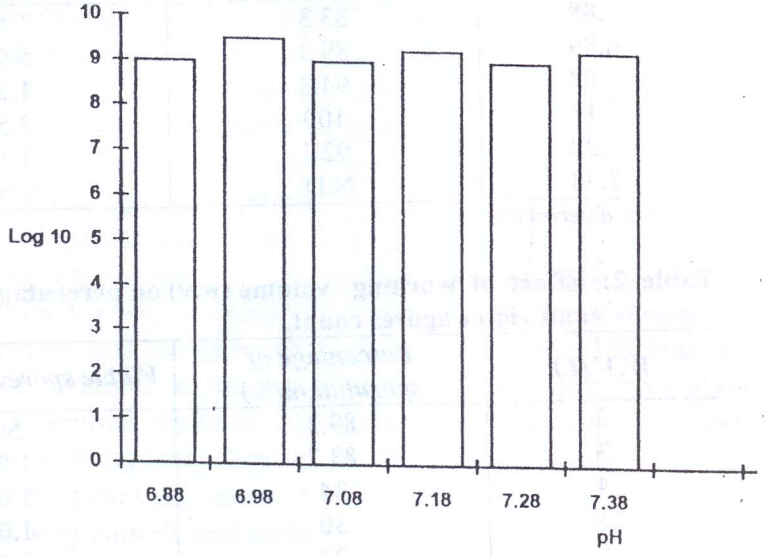

Fig.2: Effect of pH on the viable spores count of *Bacillus anthracis*,Sterne strain in meat infusion broth using IBT-Goettingen bioreacter

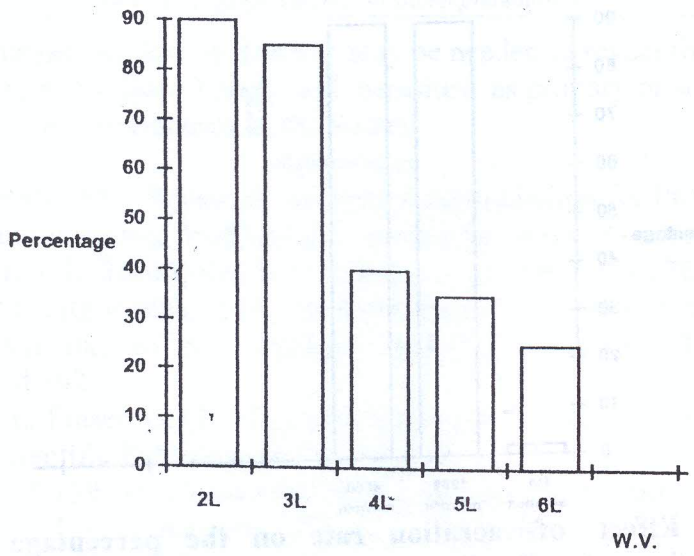

Fig.3: Effect of working volume (W.V) on the percentage of sporulation of *Bacillus anthracis*,Sterne strain in meat infusion broth using IBT-Goettinge bioreacter

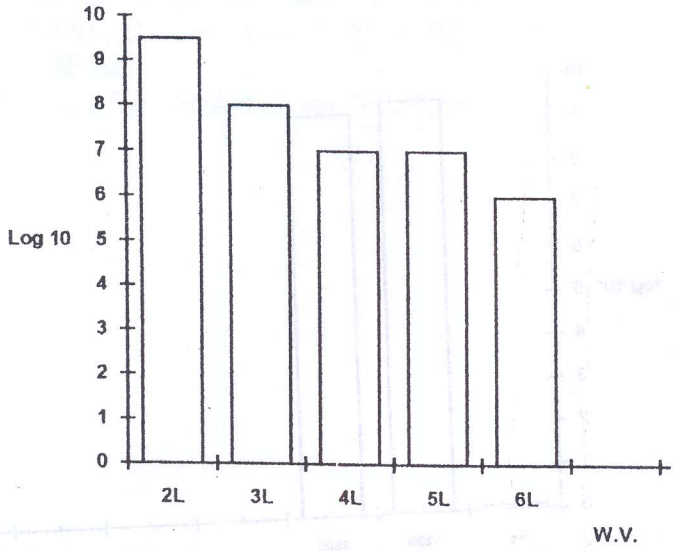

Fig.4:Effect of working volume (W.V) on spores count of *Bacillus anthracis*,Sterne strain in meat infusion broth using IBT-Goettingen bioreacter bioreacter

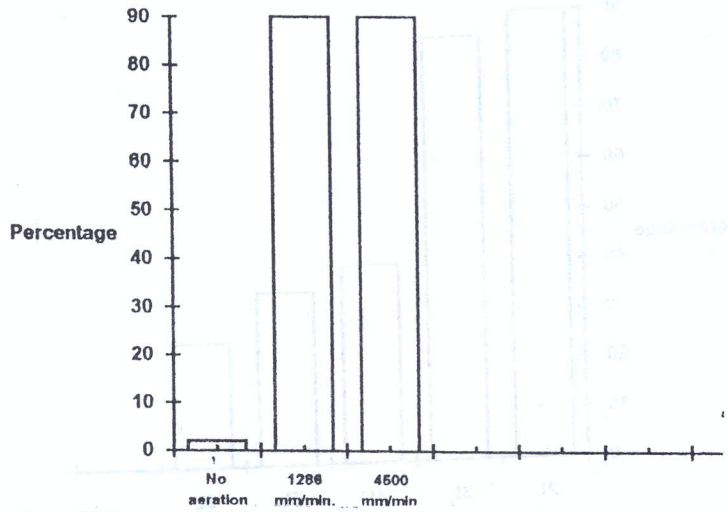

Fig.5: Effect of aeration rate on the percentage of sporulation of *Bacillus anthracis*, Sterne strain in meat infusion broth using IBT-Goettingen bioreactor.

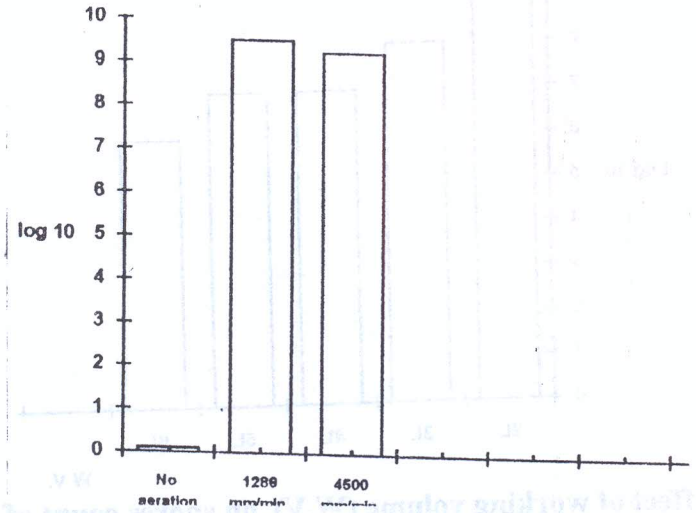

Fig.6: Effect of aeration rate on spore count of *Bacillus anthracis*, Sterne strain in meat infusion broth using IBT-Goettingen bioreactor.

Discussion

Anthrax in animals is a soil-borne disease. The pathogen is generally acquired orally as spores (Jorg and Bohnel, 1995). In soil, survival is favored by neutral or alkaline reactio, moisture content of at least 60% and atmospheric temperature between 21°C and 37°C which promotes sporulation (Davis, 1960; Kanke, 1985).

Table 3: Effect of rate of aeration on percentage of sporulation and viable spores count.

Rate of aeration (mm.min.)	*Percentage of sporulation(%)*	*Viable spores count CFU/ml*
No aeration	2	Negligible
1286	89.7	$5x10^9$
Full aeration (4500)	90	$2.5x10^9$

This study was carried out using IBT-Gottingen bioreactor. Several values of pH, aeration for sporulation and working volume, were investigated. The values which gave the best results in the preceding experiment were maintained as constant in the succeeding experiment.

In an unpublished study, we found that growth of *Bacillus anthracis* (Sterne strain) in meat infusion broth is not highly comparable to other chemically defined media, when used under the same parameters. However, sporulation was found to be higher in meat infusion broth than the other media used.

When testing for different pH values; the highest viable spore count ($5x10^9$) was encountered at pH 6.89. Charlene and others (1989) used the initial pH value of 6.80 when growing *Bacillus brevis* using BIO FIO fermenter. Joseph and Bruce (1983) used the initial pH 8.0 when growing some strains of *Bacilus anthracis* in flasks, and Mohamed *et al.* (1989) used the pH 7.2. We could not determine the rate of sporulation at pH 7.38 due to uncountable vegetative cells in some microscopic fields. We found that sporulation and hence viable spores count was high in 2 liters working volume than in other volumes.

According to our results, atmospheric pressure is an important factor in inducing high or low sporulation. Very few spores were developed when oxygen flow was turned off after 24 hours of growth.

Consequently, at full aeration, significant increase in viable spores count was encountered. According to Foster (1994) the exact environmental stimuli that cause sporulation are unknown and may requires the combination of several factors.

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