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Enhancement of Biomass Production of Bacillus Thuringeinsis Serovar. Israelensis by Fed-Batch Fermentation

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Fed – batch fermentations of Bacillus thuringeinsis serovar. israelensis

Abstract: *Bacillus thuringeinsis serovar israelensis is the most widely and effectively used mosquito larvicide used in mosquito control programmes, although the industrial production of this biopesticide remains expensive .Here an attempt has been made to develop cost effective fed- batch fermentations of these Bacilli based on a variety of carbon sources like Sucrose, tapioca powder, jaggery and glucose. Fed batch methods have considerably improved both it's biomass and toxicity. Batch fermentations of Bacillus thuringeinsis serovar israelensis with glucose based medium cannot accommodate glucose concentration above 3% (w/v).Therefore this investigation suggests a fed – batch fermentation technique for more economical industrial production of Bacillus thuringeinsis serovar israelensis.*

Keywords*: Bacillus thuringeinsis serovar. israelensis, biomass productivity, catabolite repression, delta-endotoxin, down time, Fed-batch fermentations .*

I. Introduction

Although chemical based insecticides has been effectively used for the past several decades, its use has been restricted recently due to various factors including resistant development in the vectors, environmental pollution and harmful effects on target species.Bacillus thuringeinsis serovar israelensis is the most effective microbial control agent active against mosquitoes that is available to date.(De Bajrac 1978; Tyrell et al.1979; De Bajrac & Thiery 1984; Federici et al.1990; Mahmood 1998; Su & mulla 1999).

Bacillus thuringeinsis serovar israelensis (Bti) is highly toxic to lepidopteran larvae and is very much safe for non target organisms including humans and they do not pose any threat to the environment (Goldberg and margalit 1977; Kalfon. et al. 1983.).It is very critical for the Bt. biopesticide industry to be able to achieve a high yield in the Bt fermentation process in order to reduce its cost and compete with chemical pesticides in the market. Several studies have been reported on the standardization of media composition for increasing the endotoxin production in Bt (Salama et al.1983; Mummigatti & Raghunathan1990; Morris etal. 1997; Vora & Shetna 1999).Several studies on the raw materials used for the production of Bt based microbial pesticide was carried out (Fernando Hercos et al. 2010; Rozul marzban, 2012; Li na qui et al. 2013 and Wu sing quing et al. 2014).

Fed batch mode of reactor operation is a potential means of achieving high cell densities. They were used in the production of bakers yeast (Reed and Nagodavithana 1991). Experiments with Bacillus thuringensis serovar kurstaki involved batch fermentations followed by incremental feeding of concentrated nutrient solution and the flow rate of the nutrient medium is manipulated to grow the cells under different conditions of nutrient availability to the cells.(Vinod Bihari 1998). Fed –batch fermentation technology was used to obtain high cell densities for the production of Bacteriocin, through the continuous supply of fresh medium (Raf Callewart and Lue De Vuyst 2000). Bacillus thuringeinsis variety kurstaki biopesticide was produced in Fed-batch mode using stach industry waste water as sole subsrate(Vu Kd et al. 2010). As per reports Bacillus thuringeinsis sub species kurstaki was also grown in batch and fed-batch cultures using waste water sludge as a raw material (Yezza et al. 2005).

Bacillus thuringeinsis serovar israelensis is the most widely and effectively used mosquito larvicide used in mosquito control programmes. The industrial production of this biopesticide remains expensive and there are not many reports of fed–batch operation of fermentation involving spore formers especially regarding fed-batch fermentations involving Bacillus thuringeinsis serovar.israelensis. Here an attempt has been made to develop cost effective fed- batch fermentations of these Bacilli based on a variety of carbon sources like Sucrose, tapioca powder, jaggery and glucose.

II. Materials And Methods

Bacteria : Cultures of Bti strains H14 were obtained from ETH, Zurich,Switzerland.

Bacteria culture and maintenance

Bacteria used in this study was Bti serotype H-14.The growth temperature and the shaker speed were 30 degree Celsius and 180 rpm respectively.

The parent strain was maintained as sporulated cultures on sterile modified glucose yeast extract salt(mGYS) agar slants containing 0.3% glucose, 0.2% ammonium sulphate,0.5% Di-pottassium hydrogen phosphate,0.2% yeast extract,0.02% magnesium sulphate, 0.008% calcium chloride and 0.005%manganese sulphate (w/v),all dissolved in 100.ml distilled water and pH is adjusted to 7.3 before the addition of agar.

In all the cases the cultivation of bacteria began with a preculture stage. A loopful of the refrigerated preserved culture was transferred to 20 ml. of mGYS broth in 100.ml Erlenmeyers flask and incubated stagnant for 12-15 hours. For further cultivation 1.ml of the preculture was used as an inoculum for 100.ml of the medium.

Catabolite repression studies

In order to asses the maximum level of initial glucose concentration which this bacteria can tolerate in liquid culture a number of media containing varying concentrations of glucose starting from 1%(w/v) to 9%(w/v) glucose were prepared. The glucose medium contained in addition to glucose, peptone 0.5% gm and yeast extract 0.1% (w/v). The pH was adjusted to 7.3 before autoclaving. 1.ml.of Bti preculture was inoculated asceptically and all the 9 Erlenmeyer flasks of 250.ml.volume were simultaneously placed on the rotary shaker for growth. After 36 hours of growth, the cultures were checked for cell density by the optical density measurements taken at 600 nm. Similar experiments were conducted using Jaggery, Tapioca powder and Sucrose.

Fed- batch experiments

To improve cell density and in turn toxin production Fed-batch mode of operation was attempted .Usually 4%(w/v) carbon (glucose)is inhibitory to most of the bacteria. In order to address this problem and to accommodate high glucose concentration in the medium , the operation was done in fed-batch mode starting with an initial concentration of 3%(w/v) glucose (3.gm. glucose in 100.ml distilled water) and with an additional increment of 1. gm. glucose, every 12^{th} hour after inoculation.

A media containing 3.gm. Glucose, 0.5 gm. Peptone and 0.1 gm. yeast extract (w/v) dissolved in 100.ml. distilled water was prepared in a 250.ml.Erlenmeyers flask. The pH was adjusted to 7.3.The media was sterilized, cooled and the inoculated with one loopful of Bti preculture and placed on a rotary shaker for incubation. 1.gm. of glucose was added every 12th hour after inoculation, in order to achieve a final concentration of upto 8% (w/v). Similarly fed-batch fermentations were done using Sucrose and Jaggery, as the carbon sources in the medium.

Estimation of cell density: The cell density measurements were done using UV-visible spectrophotometer at 600 nm. The zero correction in each case was made using the respective fermentation media without the inoculum. 1:10 dilutions of the harvested culture was taken for each of the cases to note the absorbance value. The absorbance values thus observed were multiplied by the dilution factor to arrive at the appropriate cell density values.

III. Results And Discussion

Usually the quantity of product produced is heavily dependant on rich sources of nutrients which contains abundant quantities of carbon, nitrogen and other nutrients. It is found from previous studies that most of the fungi can tolerate increased level of carbon substrates compared to bacteria. It is always advisable to incorporate more carbon into the media preparations in order to boost productivity in terms of both biomass and product. There is a limit to which the level of carbon can be increased in the medium, without affecting the productivity .Simple carbon sources like glucose has got a repressive effect on both cellular growth as well as product accumulation above a particular concentration, where as complex carbohydrates are found to have less inhibitory effect on growth as well as product accumulation. Biosynthesis of different secondary metabolites is repressed by rapidly metabolisable sugar, particularly glucose. In the manufacture of antibiotics, carbon source other than glucose or glucose is fed at low rates (Fed-batch mode of reactor operation) to minimize catabolite repression,(Wulf cruger and Anneliese cruger 1989).

In the present study the repressive effect of Glucose, Sucrose, Jaggery and Tapioca powder were determined, when Bti is grown in batch mode using these carbon sources. The overall growth of Bti in all four media with varying concentration of each of the substrate were shown in figure 1and 2 and in Table .1 A-D.

Fig-1: Effect of glucose concentration on the cell density of Bacillus thuringiensis serovar. Israelensis(Batch fermentations).

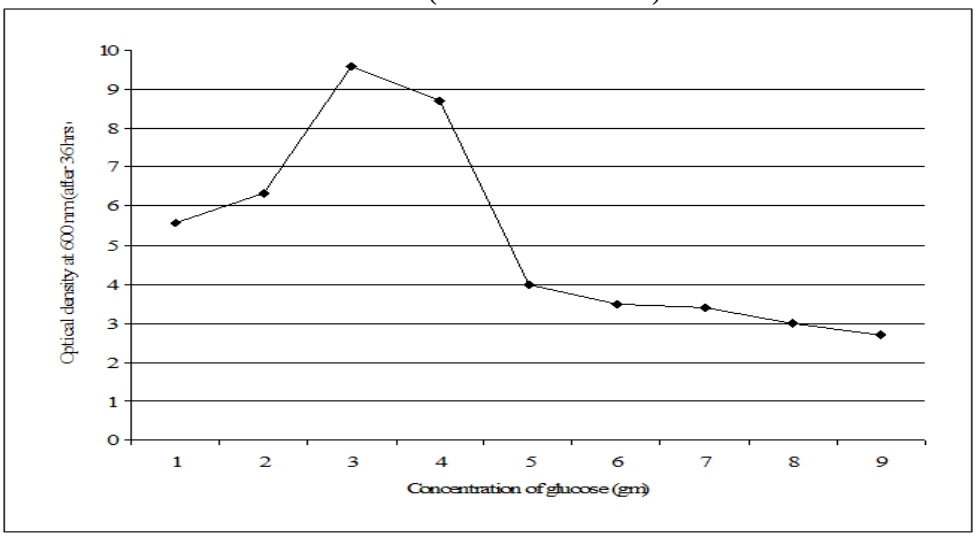

Fig-2: Effect of Substrate Concentration on the cell density of Bacillus thuringiensis serovar. Israelensis (Batch fermentations).

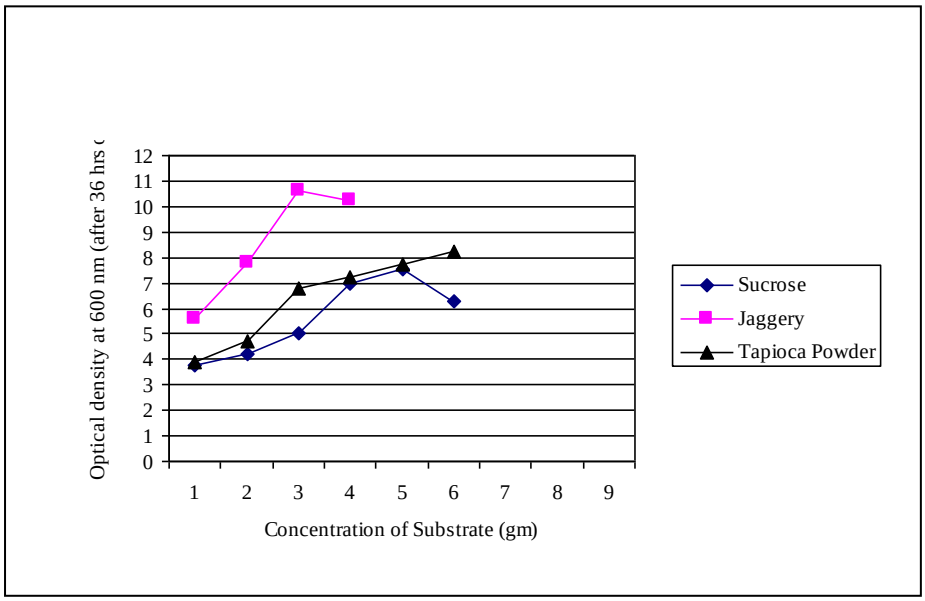

Fig-3: Fed-batch fermentations of Bti using Glucose as the carbon source

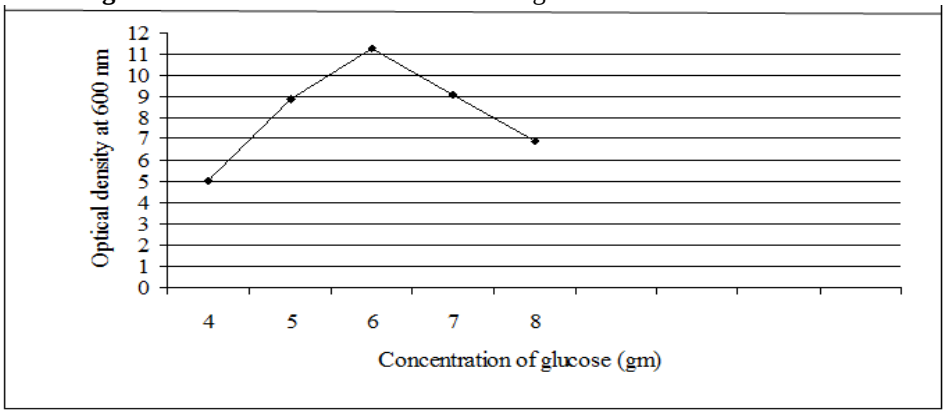

Fig-4: Fed-batch fermentations of Bti using different carbon sources

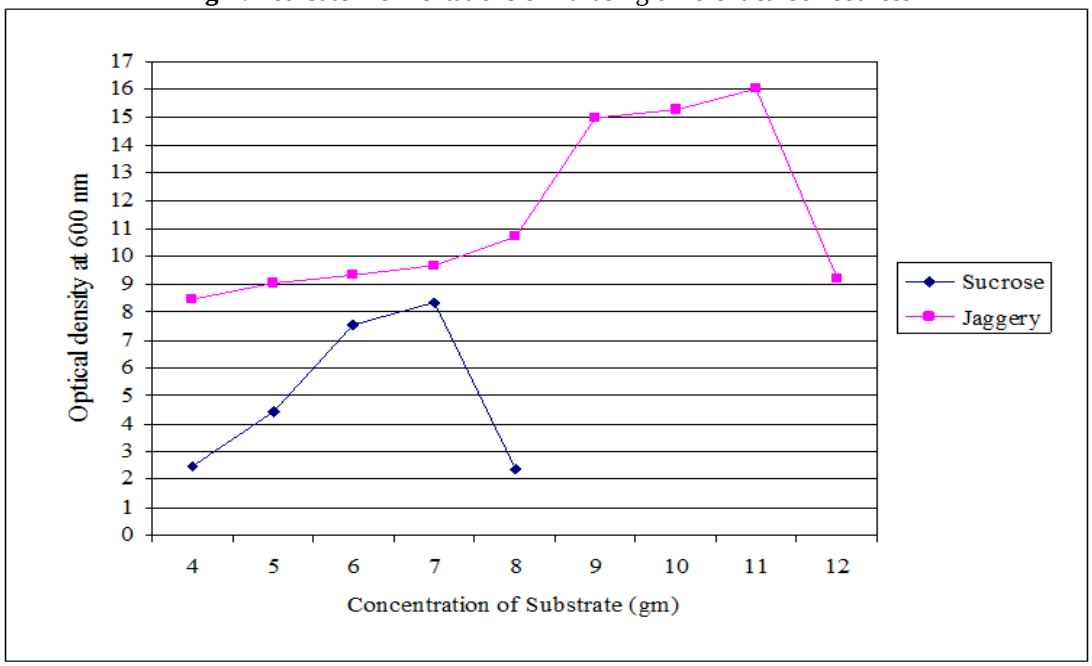

Table – 1A: Optical Density Values of Bti grown using Glucose in batch fermentations.

Percentage of Carbon used (Glucose) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
1	36	5.56
2	36	6.32
3	36	9.60
4	36	8.71
5	36	4.0
6	36	3.5
7	36	3.4
8	36	3.0
9	36	2.7

Table - 1B: Optical Density Values of Bti grown using Sucrose in batch fermentations.

Percentage of Carbon used (Sucrose) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
1	36	3.8
2	36	4.2
3	36	5.0
4	36	7.0
5	36	7.56
6	36	6.3

Table – 1C: Optical Density Values of Bti grown using Jaggery in batch fermentations.

Percentage of Carbon used (Jaggrey) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
1	36	5.6
2	36	7.8
3	36	10.6
4	36	10.25

Table – 1D: Optical Density Values of Bti grown using Tapioca Powder in batch fermentations.

Percentage of Carbon used (Tapioca Powder) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm (after 36 hrs of incubation)
1	36	3.9
2	36	4.7
3	36	6.78
4	36	7.2
5	36	7.71
6	36	8.23

Table – 2 A: Optical Density Values of Bti grown using Glucose in fed-batch fermentations.

Percentage of Carbon used (Glucose) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
4	12	5.010
5	24	8.913
6	36	11.29
7	48	9.050
8	60	6.900

Table – 2 B: Optical Density Values of Bti grown using Sucrose in fed-batch fermentations.

Percentage of Carbon used (Sucrose) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
4	12	2.500
5	24	4.450
6	36	7.540
7	48	8.341
8	60	2.341

Table – 2 C: Optical Density Values of Bti grown using Jaggery in fed-batch fermentations.

Percentage of Carbon used (Jaggery) + 0.1% yeast extract + 0.5% Peptone)	Culture time in hours	Absorbance at 600 nm
4	12	8.500
5	24	9.030
6	36	9.350
7	48	9.700
8	60	10.700
9	72	15.000
10	84	15.323
11	96	16.000
12	108	9.200

It is very evident from Fig.1.that the growth is maximum in glucose based media at 3%(w/v) glucose concentration, proving that a concentration of 4%(w/v) and above will have a repressive effect on cellular growth and thus product formation.

The repressive effect of Sucrose and Jaggery were shown in Fig.2. Sucrose based medium gives a maximum cell density at 5%(w/v) concentration ,while Jaggery based medium at 3% (w/v) has produced much more cell growth and maximum cell density compared to glucose based medium.Tapioca based medium did not show any repression upto 6%(w/v) concentration of tapioca powder. Increased concentrations of Tapioca powder based medium did not support any growth, due to the inherent viscocity problem with starch additive in the media. This would have considerably reduced oxygen solubility which in turn will affect the growth of bacteria.

Table.1A - D. shows the comparative absorbance values of Bti when grown on glucose, Sucrose, Jaggery and Tapioca powder based media, in batch mode of growth. Glucose based medium and Jaggery based medium gives a maximum absorbance of 9.60 and 10.60 respectively, at 3%(w/v) concentration ,while sucrose based medium gives a maximum absorbance of 7.56 at 5%(w/v) concentration. Improved tolerance of Sucrose by Bti can be attributed to the slow degradation of disaccharide by the enzymes ,produced by the bacteria. It is also very much evident from the data that the cell density at 3%(w/v) concentration of Jaggery is more compared to 3%(w/v) concentration of glucose. The increased biomass production is mainly due to the presence of growth factors like B- complex vitamins in Jaggerry. In the case of Tapioca powder based medium the absorbance values increased proportionally with increased concentration of tapioca powder, and it did not shows any repression upto 6%(w/v) concentration of Tapioca powder. Further additions more than 6% (w/v)) resulted in drastic decrease of growth mainly due to increased viscocity of the medium. The absence of repression is mainly due to the peculiar structure of starch. Starch being a polymer of glucose is cleaved by the extracellular enzymes produced by the bacteria as per the nutritional requirement. This will not give glucose concentration above catabolite repressive level. Thus addition of polymers is beneficial for improved biomass concentration and delta endotoxin production in batch mode operation.

Fig.3 and Fig.4 shows the growth profile of Bti grown using Fed –batch fermentation method, with different carbon sources. The optical density values of Bti grown in fed-batch fermentation using different carbon sources were given 2A-2C. In Fig.3.the repression was evident in the case of glucose only at 6% (w/v) concentration compared to repression at 3%(w/v) concentration in batch mode. The decrease of growth after 6%w/v) may be due to toxic accumulation of by products of metabolism.

Fig.4. shows the comparative growth profile of Bti grown using fed batch fermentation with Jagerry and Sucrose as the carbon sources. Sucrose based medium gave repression at 7%(w/v) concentration compared to 5%(w/v) concentration in batch mode, while in the case of Jaggery based medium repression was evident only at 11%(w/v), compared to 3% (w/v)concentration in batch mode.

IV. Conclusion

From the experiments conducted it can be concluded that Glucose, Sucrose and Jagerry based media with 0.1%(w/v) yeast extract and 0.5%(w/v) peptone can be successfully used for the production of Bti using Fed-batch fermentations, which will definitely improve both the biomass concentration and delta endotoxin production.

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