CHAPTER – VII

OPTIMIZATION OF PROCESS PARAMETERS INFLUENCING GI PRODUCTIVITY BY STREPTOMYCES SP. SB - P1

7.1 INTRODUCTION

Production of any metabolite from microorganisms can be achieved by providing proper physiological and chemical conditions. All microbes require specific set of process parameters for optimum production of any metabolite. The parameters like temperature, pH, agitation rate and inoculum size must be optimized for developing any bioprocess technology [Stanbury et al. 2003].

The process parameters are essential because if the growth and production parameters are different than the inoculum preparation and fermentation process needs fine tuning for providing biomass accumulation time separately. Our investigation which is based on production of glucose isomerase from Streptomyces sp. SB - P1 is an enzyme required in primary metabolism of the organism. The time scale analysis for growth and production already reported in Chapter – V reveals the overlapping of both parameters. Other physiological parameters required for growth and production like temperature, pH and agitation rates should also be worked out.

The medium used for development of inoculum influences the morphology as well as metabolism of the microorganism. The presence of suitable nutrients and the ingredients directing the metabolism towards product formation can be used to achieve fast growth of the organism. The initialization of the production during inoculum development reduces the lag phase in production medium and the time required for the whole process [Manhas and Bala, 2004; Srih-Belghith et al., 1998].

The optimisation of temperature for fermentation and the growth of the organism is very important factor. The production of an enzyme by microorganism requires efficient functioning of the protein synthesis machinery
of the microorganism. The vital processes of the organism must also be provided optimum conditions in order to achieve maximum growth [Modi, 2009]. The pH of the medium exerts a profound effect on the metabolic machinery of the organism which is composed of enzymes. The optimisation of pH for growth and production enhances the productivity of the process greatly [Givry and Duchiron, 2008; Deshmukh et al., 1994].

An optimum rate of agitation shall provide thorough mixing of the microbial cells with the nutrients in the medium. This also helps in homogeneous availability of the oxygen to microbial cells in a submerged fermentation process.

The influence of process parameters on filamentous and non-filamentous organism varies greatly in submerged fermentations. Non filamentous organisms make the medium turbid on growth because of dispersed growth of bacterial cells. The filamentous organisms either grow in the form of loose filaments where hyphae form a homogeneous suspension dispersed through the medium or pellets consisting of compact discrete masses of mycelium. The filamentous form increases the viscosity whereas pellet forms allow better oxygen transfer and low viscosity. The environment provided to the cells in the filaments should be homogeneous for maximum productivity. It is observed that when same amount of oxygen is supplied to unicellular and filamentous microbial fermentations, the oxygen transfer rates in unicellular fermentations are better. Thus in the case of filamentous organisms efficient mixing becomes extremely important. The cells deep inside the pellet may become devoid of proper nutrients and oxygen. Therefore the pellet size should not be very large. At low agitation rates biomass within the pellets receive limited oxygen creating heterogeneity in the system. The higher agitation rates may provide smaller pellet size and better aeration but the shear forces may break up the mycelia and autolysis of some hyphal compartments [Stanbury et al. 2003].

This chapter deals with the optimisation of parameters influencing the GI production. The effect of inoculum size, temperature, pH and agitation rate for
extracellular production of glucose isomerase by *Streptomyces sp. SB-P1* and its growth were studied.

### 7.2 MATERIALS AND METHOD

The process parameters play an important role in enhancing the productivity of a fermentation process therefore they were optimized for GI production.

#### 7.2.1 Effect of Inoculum Size on production of Glucose Isomerase

The optimum inoculum size for production was optimized by using cup borerers of different diameters. The isolate *Streptomyces sp. SB - P1* was inoculated on wheat bran agar medium [Appendix -I] as this medium gives relatively fast growth. Five sets of triplicates of 100 mL conical flasks were prepared containing 20 mL of Bennett’s Broth and Media No. 9 separately [Appendix -I]. Inoculation was done by cutting discs of 5, 6, 7, 8 and 10 mm diameters in different sets. All the flasks were incubated at 30°C and 120 RPM in orbital shaker. The crude enzyme extract was prepared after the fermentation period of 96 h. The GI assay was performed according to section 4.2.2.1.

#### 7.2.2 Effect of Temperature on production of Glucose Isomerase

The effect of temperature was studied on the production of glucose isomerase by varying the temperatures. Seven sets of triplicates of 100 mL conical flasks were prepared containing 20 mL of Bennett’s Broth and Media No. 9 separately [Appendix -I]. The pH of the media was set to 7 and was sterilized by autoclaving. Each set was incubated in orbital shaker set at different temperatures, 20, 25, 30, 35, 40, 45, 50 and 55°C. All the flasks were inoculated by 8mm disc cut from 72 h growth of *Streptomyces sp. SB - P1* grown on wheat bran agar as inoculum. The fermentation process was terminated after 96 h. The flasks were harvested and crude enzyme extract prepared. The effect was noted by performing GI assay for all the flasks according to the details given in section 4.2.2.1.
7.2.3 Effect of pH on production of Glucose Isomerase

The influence of varying pH on glucose isomerase production was studied. A range of pH from 4 to 11 was selected for the study. Eight sets of triplicates of 100 mL conical flasks were prepared containing 20 mL of Medium No. 1 and Medium No. 9 separately [Appendix -I]. The media were prepared in different buffers. Acetate buffer of pH 4 and 5 was prepared separately and the medium ingredients were dissolved in it. The pH was again checked after dissolving all the ingredients. Phosphate buffer was used for preparing media having pH 6 and 7, Tris for pH 8 and 9 and Glycine buffer for pH 10 and 11 [Appendix -II]. Inoculation was done by cutting 8mm disc of *Streptomyces* sp. SB - P1 spot inoculated on wheat bran agar plate. All the flasks were incubated at 30°C at 120 RPM. The fermentation process was terminated after 96 h and the broth was harvested in sterile centrifuge tubes and enzyme extract prepared. The response of glucose isomerase production on varying pH was measured by performing GI assay as described in section 4.2.2.1.

7.2.4 Effect of Agitation Rate on production of Glucose Isomerase

The effect of rate of agitation was checked on the production of glucose isomerase. Five sets of triplicates of 100 mL conical flasks were prepared containing 20 mL of Medium No. 1 and Media No. 9 separately [Appendix -I]. The pH of the media was set to 7 and incubation temperature was 30°C. Inoculation was done by cutting 8 mm disc of *Streptomyces* sp. SB - P1 spot inoculated on wheat bran agar plate. Each set was incubated at different agitation rates of 60, 80, 100, 120 and 140 Revolutions Per Minute (RPM). The fermentation process was terminated after 96 h and crude enzyme extract was prepared. GI activity was checked according to the details given in section 4.2.2.1.
7.3 RESULTS AND DISCUSSION

The factors influencing the production of GI like inoculum size, temperature, pH and agitation speed were studied.

7.3.1 Effect of Inoculum Size on production of Glucose Isomerase

The optimisation of fermentation process for industrial application also requires suitable medium for inoculum development. The time required for growth of inoculum should be as less as possible. This can be achieved by selection of a medium which supports fast growth of the organism and also initializes the machinery for the formation of desired product (glucose isomerase) in the organism. Wheat bran medium is reported to induce GI production as well as growth of Streptomycetes [Manhas and Bala, 2004; Srih-Belghith et al., 1998]. During our studies on diversity dealt in chapter II, we studied the growth of our isolates on different types of media. We observed the fastest growth of all the Streptomycetal isolates on wheat bran agar medium. Therefore this medium was used throughout the investigation for development of inoculum. The inoculation of *Streptomyces sp. SB - P1* was done by cutting discs of varying sizes, 5, 6, 7, 8 and 10 mm diameter with the help of a cup borer. The enzyme production, growth response and total protein content were measured for all the flasks. The enzyme yield, biomass as well as total protein increased in the flasks on increasing the inoculum size. The increase in all the parameters was progressive from 5 mm disc containing flask to 8 mm disc containing flask, but there was no remarkable increase beyond this. The optimisation of industrial processes is based on utilizing minimum resources and getting maximum outputs therefore the marginal increase beyond 8 mm diameter is not considered significant and 8 mm disc is picked up as the optimum size of inoculum. The graphical representation of effect of varying inoculum size is shown in Fig. 7.1 for GI production, Fig. 7.2 for growth response in terms of biomass accumulated, and Fig. 7.3 for total protein content in the fermented broth.
Fig. 7.1: Effect of inoculum size on GI production

Fig. 7.2: Effect of inoculum size on production of biomass of *Streptomyces sp. SB - P1* for GI production
7.3.2 Effect of Temperature on production of Glucose Isomerase

The production of glucose isomerase was found to be maximum at 30°C as also reported by Gong et al., (1980) for GI production by Actinoplanes missouriensis and Dhungel et al., (2007) for psychrotolerant Streptomyces sp. and Kim and Pyong-Su, (1992) for mesophilic Streptomyces sp. Givry and Duchiron, (2008) observed maximum GI production from a non-filamentous organism Lactobacillus bifermantans at 37°C. The results are presented in Fig. 7.4 for Medium No. 1 and Fig. 7.5 for Medium No. 9. The growth was also observed highest at 30°C which is a beneficial result as there will be no need to provide separate biomass accumulation time at industrial level. The growth was also scanty at above and below the optimum temperature. The scanty and loose fragmented growth can be seen in Fig. 7.6a in Medium No. 1 and Fig. 7.6b in Medium No. 9. The flasks incubated at temperature lower and higher than 30°C had very low concentrations of enzyme as also observed by Givry and Duchiron, (2008) for L.bifermantans. The temperature optima were determined on Bennett’s broth as well as Medium No. 9. Highest enzyme production in
Medium No. 9 was observed at 40°C. The organism exhibited a wider range of temperature optima in this medium for production. This may be due to the presence of mineral salts (magnesium sulphate and cobalt chloride) in the medium. As already stated in earlier chapters that these two divalent cations support the production, activity and thermostability of the enzyme molecule, therefore increase in the incubation temperature for fermentation in this medium did not lower the amount of GI production to great extents. Many of the earlier researchers also have reported the production of enzyme between 28°C to 30°C [Bhosale et al., 1996]. Wang et al., (1998) reported maximum GI production at 25°C by a recombinant strain of Streptomyces lividans. Thermophilic organisms are also reported to produce glucose isomerase in considerable concentrations having high optimum temperature of 50°C as optimum for GI production from Streptomycetes isolated from hot springs [Manhas and Bala, 2004]. Debnath and Majumdar, (1985) produced GI from Streptomyces kanamiyceticus at 28°C.

![Graph](image.png)

**Fig. 7.4: Effect of temperature on production of Glucose Isomerase in Medium No. 1**
Optimization of Process Parameters influencing GI Productivity by Streptomyces sp. SB

**Fig. 7.5**: Effect of temperature on production of Glucose Isomerase on Medium No. 9

**Fig. 7.6**: Scanty and loose fragmented growth at temperature, 50°C, a: Medium No. 1 b: Medium No. 9

### 7.3.3 Effect of pH on production of Glucose Isomerase

A broad range of pH was tested for optimisation of glucose isomerase production by *Streptomyces sp. SB - P1*. We observed the maximum production in Bennett’s broth flasks having pH 7 and maximum growth at pH 8 whereas optimum pH for growth and production both occurred at pH 8 in Medium No. 9. There was no production as well as growth observed at pH 4 and 5 but the flasks having pH 8 and 9 exhibited substantial production of GI. The
production was further reduced on pH 10 and 11 but the overall picture exhibited by the organism indicates the preference of alkaline pH range with maximum at pH 7. There are earlier reports on production of GI at alkaline pH but very few reports on acidophilic organism producing GI [Bok et al., 1984]. Givry and Duchiron, (2008) observed maximum production by *L. bifermentans* at pH 7.5 although the growth occurred on a broad range from pH 5 to 9. Streptomycetal isolates of Manhas and Bala, (2004) exhibited maximum GI production at pH 5 and Lobanok et al., (1998) observed the same at pH 6.8. Deshmukh et al., (1994) also observed *Streptomyces thermonitrificans*, a thermophile producing GI between pH 7 and 8. The results are presented in Fig. 7.7 for Medium No. 1 and Fig. 7.8 for Medium No. 9.

![Graph](image)

**Fig. 7.7:** Effect of pH on production of Glucose Isomerase in Medium No. 1
7.3.4 Effect of Agitation Rate on production of Glucose Isomerase

The production of GI from *Streptomyces sp. SB - P1* was studied on a range of agitation rates. The highest yield of GI was observed at 100 RPM on Medium No. 1. The results are presented in Fig. 7.9 for Medium No. 1 and Fig. 7.10 for Medium No. 9. Glucose isomerase production in the flasks incubated at 60 and 80 RPM was ample enough but those incubated at 120 and 140 RPM exhibited very poor yield. The varying agitation rates on Medium No. 9 also exhibited high yield at 100 RPM. Lobanok et al., (1998) have reported optimum GI production at 180 RPM. The lower agitation rates here also yielded better results than higher rates. This indicates the preference of lower agitation rate of the organism for GI production. Kim and Pyong-Su, (1992) produced GI in fermentor using *Streptomyces* sp. at 350 RPM.
Fig. 7.9: Effect of agitation rate on production of Glucose Isomerase in Medium No. 1

Fig. 7.10: Effect of agitation rate on production of Glucose Isomerase in Medium No. 9
The rate of agitation influences the growth and morphology of the organisms also as stated in the introduction. The highest biomass was accumulated at 80 RPM in Medium No.1 and 100 RPM in Medium No. 9. The pattern of growth was different at different agitation rates as also stated by Stanbury et al., (2003). At lower agitation rates the growth was not as compact pellets but dispersed and filamentous outgrowths could be observed clearly as shown in Fig. 7.11a and 7.11b. There are very few reports on optimisation of agitation speed for GI production. The size of pellets was reduced at high agitation rates as compared to medium rates as shown in Fig. 7.11c and 7.11d.

![Fig. 7.11: Fibrous outgrowths observed at very low agitation rates a: Medium No. 1; b: Medium No. 9, c: larger pellets at medium agitation rates; d: Very small pellets of mycelial growth at high agitation rate](image)
7.4 CONCLUSIONS

The parameters for the production of glucose isomerase in submerged fermentation and growth of *Streptomyces sp. SB - P1* were optimized. The development of inoculum is a crucial step which must not be time consuming. Streptomycetes being slow growers need to be grown on enriched medium which favors sporulation and also switches on the machinery for product formation. Wheat bran medium proved to be successful in both the aspects. The biomass accumulation and product formation conditions coincide which is a beneficial result from the industrial point of view. The organism produced maximum amount of the enzyme between a range of temperature from 30 to 40°C. The maximum growth was also observed in the same range in both the tested media. This result is useful for industrial application where maintenance of extreme temperatures require spending huge amount of energy. Working with any process at ambient temperature definitely cuts down the cost on energy. The pH range required by the organism for maximum GI production is 7 which falls in neutral range, so the use of extremely acidic or alkaline reagents can be avoided which leads to the extra care to be taken for fermentor vessel construction. The optimum pH required for growth was also in the same range 7 to 8 with highest growth on pH 8. The rate of agitation required by the organism is 100 RPM for maximum production. Maximum growth was observed at 80 and 100 RPM in Medium No. 1 and Medium No. 9 respectively. All the parameters required by *Streptomyces sp. SB - P1* are well in the range of economic feasibility therefore such a process has good enough chances for industrialization.