

“Media Optimization Studies for Cellulase Production from *Lactococcus lactis* and *Cellulomonas fimi*”

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Abstract

Cellulases are a group of hydrolytic enzymes and are capable of degrading lignocellulosic materials. Cellulases have wide range of applications. This work focuses on media optimization for production of cellulase by using *Lactococcus lactis* and *Cellulomonas fimi*. Different cultural conditions were examined to assess their effect in optimizing enzyme production. Several other parameters like pH, temperature, time duration, and its concentration were also optimized for the cellulase production. Media of pH 4 gave 2.13 IU/mL cellulase activity. Optimum pH for cellulase production was between 4.0 and 4.5. Submerged fermentation at 120 rpm at 28°C gave higher yields of cellulase compared to static condition.

Key words: Cellulase, *Cellulomonas fimi*, *Lactococcus lactis*

1. Introduction

Cellulose is an organic compound, formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand $\beta(1\rightarrow4)$ linked D-glucose units. The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria (Baig et al., 2004). Large quantities of lignocellulosic wastes are generated through forestry, agricultural practices and industrial processes, particularly from agro-allied industries such as breweries, paper pulp, textile and timber industries. These wastes generally accumulate in the environment thereby causing pollution problem (Abu et al., 2000). Cellulose is the most common organic compound on Earth. About 33% of all plant matter is cellulose (the cellulose content of cotton fiber is 90%, that of wood is 40–50% and that of dried hemp is approximately 45%).

2. Materials and Methodology

Micro-organism used :- *Lactococcus lactis* , *Cellulomonas fimi*.

Media :- NAM (Nutrient Agar media)

Extraction media :- Medium composition described by Mandles and Weber for 1L.

The pH of media was adjusted to 5.0 ± 0.2 . Then, 100 ml of the liquid medium was placed in 250 ml flask and sterilized by autoclaving 121°C for 15 min.

2.1. Optimizing parameters

2.1.1. Optimization of temperature

Optimization of temperature was carried out by incubating the medium at 20°C , 30°C and 34°C , in orbital shaker incubator at 120 rpm , Room temperature and soil incubator respectively. After regular intervals, enzyme assay was performed.



Figure.1 Media inoculated and kept in different temperature conditions



Figure 2. Soil Incubator

2.1.2. Optimization of substrate concentration

The substrate used was taken in different concentration , in 100 ml fermentation media, in 250 ml flasks. The flask was inoculated *Lactococcus lactis* and *Cellulomonas fimi* and incubated at $28 \pm 2^{\circ}\text{C}$ at 120 rpm in orbital shaker-incubator.

2.1.3. Optimization of fermentation time

Fermentation time was optimized by putting various flasks, containing fermentation medium, at from 24 to 192 h, at $28 \pm 2^\circ\text{C}$ in to orbital shaker-incubator at 120 rpm. Enzyme assay is carried out at regular intervals.

2.1.4. Effect of static and agitated condition

There were two sets prepared, to check the effect of static and agitated condition on enzyme activity. In both sets, all the conditions (pH, temperature, substrate concentration) applied were kept similar. One set was put in orbital-shaker incubator at 120 rpm, while other set was kept in static condition (soil incubator).

After regular intervals, enzyme assay is carried out.

2.2. Protein estimation is done by Folin – Lowry Method and O.D is taken at 660 nm.

Test tube samples :-

1. Blank
2. 5 Standard (S1, S2, S3, S4, S5) with different concentrations of working BSA (wBSA)

Test Samples :- 1ml of Enzyme was added to each of the sample. T1 *Lactococcus lactis*, T2 *Cellulomonas fimi*.

3. Formula Used**3.1. Enzyme Activity,**

$$\text{Unit/ml of enzyme extract} = \frac{\text{mg of cellulose released}}{\text{volume of enzyme used}} \times 30$$

4. Result and Discussion**4.1. Optimization of temperature**

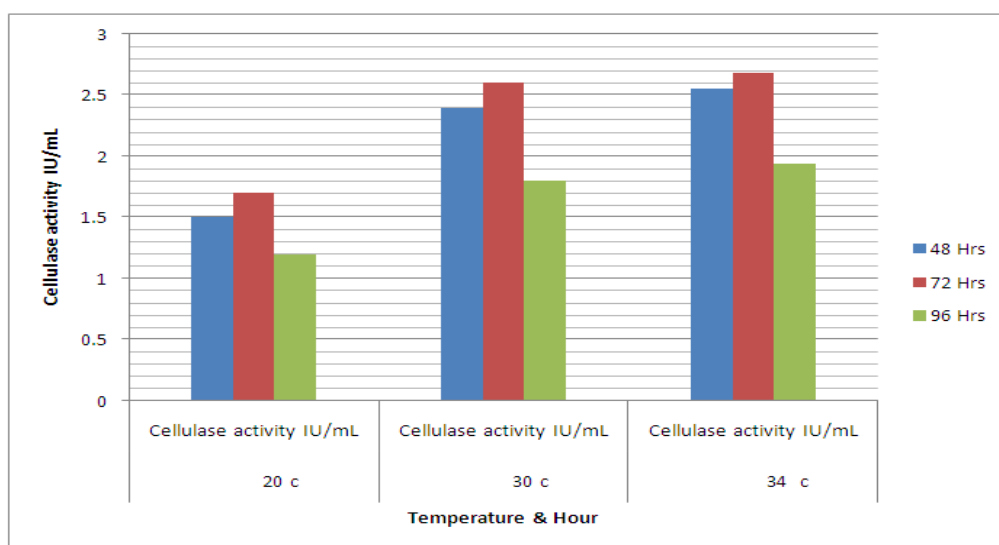
Optimization was carried out by incubating the fermentation flask for 72 hours at 20°C , 30°C and 34°C and the cellulase activities were 1.7, 2.6, 2.68 IU/mL, respectively.

4.2. Optimization of fermentation time

As seen in Table 1, the flasks were incubated at different time duration; 48, 72, 96 hr and cellulase activities of 2.55, 2.68, 1.94 IU/mL were obtained, respectively. Thus, at 72 h maximum degradation was observed. The highest cellulase level of was achieved on the 4th day of the fermentation period.

Table1. Optimization of temperature and fermentation time.

HOURS	20 ⁰ c	30 ⁰ c	34 ⁰ c
	Cellulase activity IU/mL	Cellulase activity IU/mL	Cellulase activity IU/mL
48	1.5	2.4	2.55
72	1.7	2.6	2.68
96	1.2	1.8	1.94

**Fig 3 .** Comparion of Cellulase activity at different temperature and time.

4.3. Optimization of pH

As shown in Figure 4, effect of pH on cellulase production was determined at pH values of 4.0, 5.0, and 6.0; and cellulase activity obtained were 2.13, 1.39 and 1.13 IU/mL, respectively. Optimum pH for cellulase activity was between 4.0 and 4.5. Maximum cellulase activity was 2.13 IU/mL found at pH 4.0.

Table2. Optimization of pH

pH	Cellulase Activity
4	2.13
5	1.39
6	1.13

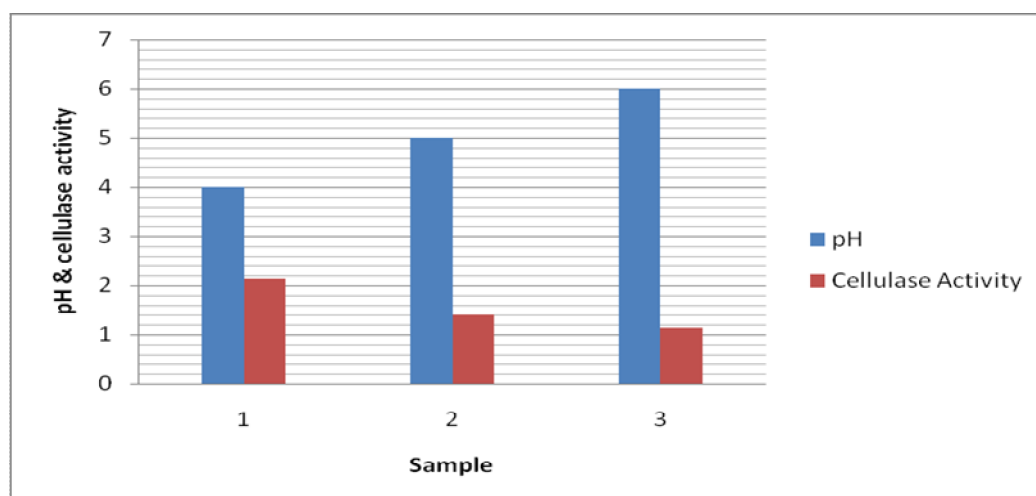


Fig 4. Comparison of Cellase activity at different pH

4.4. Effect of static and agitated condition

Maximum activity of cellulase enzyme was observed at agitated condition. The Cellulase activity was around 2.45 IU/mL at 120 rpm after 96 h incubation period. But in static condition, maximum cellulose activity was observed at 72 h which was 1.23 IU/mL. This was around half of the maximum activity than agitation condition.

Table3. Effect of conditions

Conditions	Cellulase Activity (IU/ml)
Agitated Condition	2.45
Static Condition	1.23

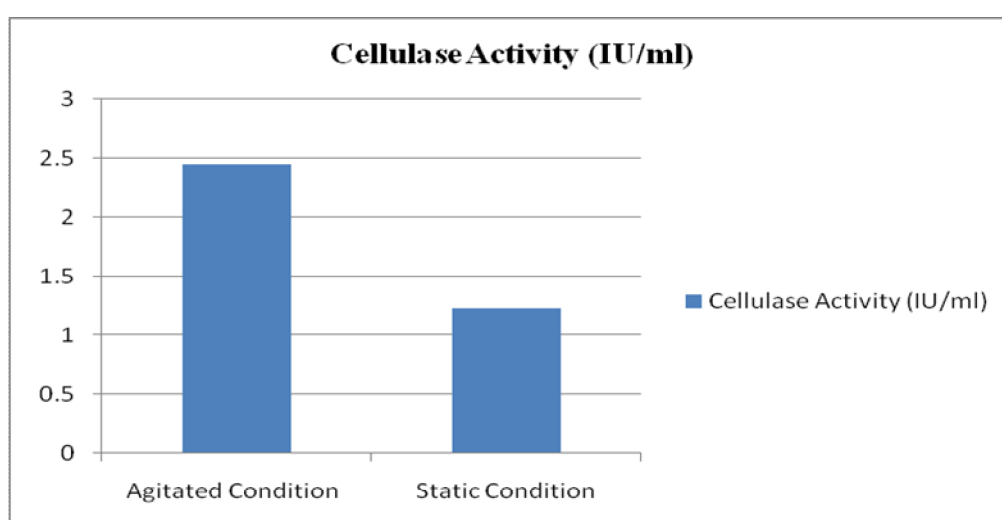


Figure 5. Cellulase activity at different conditions

5. Conclusion

The Media was optimized under various parameters like temperature, pH , fermentation time and static and agitated condition. We found that maximum cellulase activity was found at 34 degree Celsius , at agitated condition , at pH 4 for 72 hours fermentation time. Thus we concluded that for production of cellulose from *Cellulomonas fimi* and *Lactococcus lactis* the following conditions are required to be followed for maximum cellulase activity.

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