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Management of Glucose Production Process from Rice Husk by Solid State Fermentation Method

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Abstract. Introduction: Lignocellulosic substrates, such as rice husk is the agricultural wastes and by-products of rice hulling plants available in large amount of quantity and cheap especially in northern of Iran. Management branch in Chemical Engineering helps us change wastes to useful byproducts. The aim of this study is the feasibility of using rice husk for glucose production by Trichoderma Reesei under solid state fermentation.
Materials and Methods: In this experimental research, first lyophilized Trichoderma reesei (PTCC5142) under sterile hode, was transfer to serum physiologic and then to solid medium culture as slant. It was preserved in Malt extract Agar (M.E.A) at 4 ° C. Preparation of substrate, pre culture and main culture mediums were done. After the preparation of crude enzyme solution, concentration of glucose, protein, activity of filter paper and endogluconase enzyme using carboxymethylcellolus as substrate were measured. Finally, the optimum condition of main culture medium and optimization methods for production of glucose by solid state fermentation were investigated. Comparison of glucose production in culture groups under different condition of time and temperature incubation was analyzed using ANOVA test. The criterion for significance was $P<0.05$.
Results: The results showed that the maximum growth of Trichoderma reesei on the rice husk substrate were observed after 4 days, as fungi colonies and spores covered all of the media. Also, the activities of endogluconase enzyme and filter paper reached to its maximum level after 4 days, and decreased after that. In addition, maximum glucose production was observed after 4 days ($P<0.01$), and in 30 ($P<0.05$).
Conclusions: This study showed that the strain Trichoderma reesei (PTCC 5142) is suitable for enzymatic hydrolysis of rice husk and production of glucose by solid state fermentation.

Keywords: Management, wastes change, Glucose, rice husk, solid state fermentation.

1. Introduction

Lignocellulosic substrates, such as rice husk are the agricultural wastes and by-products of rice hulling plants. The major part of them are cellulose polymerase, hemi cellulose and lignin which can be used to domesticate animals and birds as well as for producing some of other microbial metabolites such as cellulolytic enzymes or ethanol (1-4).

In recent years, studies has been done to enhance the nutritional value of rice husk in some countries. Researches in Nigeria (2007) was shown that the fermentation of rice husk using *Trichoderma* fungi for 40 days can cause a significant increase in amount of crude protein, energy and mineral content such as sodium and potassium and it can decrease the amount of crude fiber (5). In Zaid et al., (2009) report, the amount of crude protein in the fermented rice husk increased about 97% and amount of crude fiber has reduced about 45% (6).

In fermentation of rice husk, solid state fermentation (SSF) and liquid state fermentation (LSF) or submerged fermentation (SMF) can be used, that each of them due to the type of method has advantages and disadvantages.

Solid state fermentation means control of growth of microorganism on wet solid substrate in absence of free water. This method is usually cheaper while equipment used in liquid state fermentation (L. S. F) is more expensive. Solid state fermentation (S. S. F) method does not need big space, so that, operations can be done in a pan bioreactor or even in an Erlenmeyer. The greatest advantage of solid state fermentation is that, this method does not need to soluble substrate rather enough, just it is crucial to moisten the substrate a little, in fact we do not need to free water.

The desired product is concentrated so its purification is easier. Another advantage of SSF is due to the high concentration of microorganisms and low humidity of medium which significantly reduces the microbial contamination. Also, the amount of material in SSF is less than SMF and enzymes show less susceptible to catabolic inhibition or stimulation. However, the SSF method is associated with some limitations, including limited use of microorganisms capable of growth in semi-humid environment and information about the increasing scale of production in this way is negligible (7, 8).

The purpose of this study is to optimize of growth medium of *Trichoderma reesei* and production of glucose via enzymatic hydrolysis of lignocellulosic waste by solid state fermentation.

2. Materials and Methods

For this experimental research fungal strain *Trichoderma reesei* (PTCC5142) as lyophilized ampoule was purchased from industrial scientific research organization and transferred to physiologic serum under the sterile hood. Strains were cultured in steep medium of Malt extract Agar (MEA), and incubated at 4 ° C for long-term maintenance. Rice husk was used in this study as substrate and as carbon source.

2.1. Preparation of substrate

For preparation of substrate, rice husk has been incubated at 4^0C and then grinded, the resulting rice husk particles size was less than 2 mm.

2.2. Preparation of pre culture

After elementary operation, 12.5 gr of rice husk powder was mixed with 17 cc water in the Erlenmeyer (500 ml). After preparation of solid bed, sterilization step 121^0C and 15 psi in the autoclave is necessary. After that, the temperature of the contents of the flasks should be brought to room temperature.

2.3. Fermentation culture

Spore suspension prepared from slants containing 3 strains that mentioned above with sterile distilled water, and using sterile pipet at near the flame, impregnation operation from slant to pre culture media took place and then, pre culture flasks were incubated 30^0C for 5 days.

2.4. Preparation of main culture

After the flasks were placed in the autoclave and cooled at room temperature in the completely sterile conditions, impregnation from pre culture to main culture take place. With sterile loop, a part of growth fungi colony on a solid bed was transferred to flask that contained main culture and was mixed and this process was repeated three or four times till 5% growth fungi colony was transferred. Then, after preparing fungi suspension with physiologic serum, amount of 10 micro liter of this suspension was put between lam and Lamel. Using optical microscope, the number of fungal spores in each square was counted and dilution factor that depends to the volume of physiological serum was applied. Number of spores was calculated. When the numbers of spores on a main culture reach to 8×10^5 wet weight^{-1}, again cultures were incubated at 30^0C for 5-7 days. After the mentioned time finished, the culture came out from incubator.

2.5. Separation of enzymatic solution

For separation of raw enzymatic solution, first buffer citrate at pH= 4.8 was prepared, and then, about 5 fold of solid substrate weight, buffer citrate was added to flasks (5×12.5=125 ml). Afterward, the flasks were put in the shaker for 15 mins, until the contents are equal. Then the solution was passed from Watman filter paper No.1. to assure of no presence of fungi spores, the passed solution from filter should be centrifuged at 4^0C and 4000 rpm for 20 min. This prevents turbidity in the experiment.

By this way, enzymatic solution that contains a lot of liquid sugar (glucose), can be separated. Indeed, during fermentation, oozed cellulose enzyme from *Trichoderma* fungi, hydrolyze the existed cellulose in the rice husk and convert it to glucose (lump sugar).

2.6. Measurement of glucose and protein concentration in raw enzymatic solution

For measurement of glucose concentration in raw enzymatic solution, kit of glucose oxidase (production of Man Company) that is an enzymatic method for lump sugar measurement was used. According to the existed direction in a kit, produced glucose concentration in crud enzymatic solution (mg/dl), was calculated.

For the measurement of amount solute protein, Loury method has been used. Bovine Serum Albumin (BSA) solution is as a standard solution and at the end the amount of samples absorption read at 578 wave length and with using BSA standard curve, protein concentration in the unknown sample (mg/ml) was obtained.

2.7. Measurement of enzyme activity

This research is on the basis of calculation of filter paper activity and after that calculation of endogluconase activity. With respect to the references in this field, experiments related to activity measurement done and at last, the amount of activity (unit/ml) was calculated.

2.8. Investigation of fungal growth in different period of time

Optimization of incubation time has been done by preparing 6 cultures. For this purpose, the different times (3-8 days) have been investigated.

2.9. Investigation of fungal growth in different incubation temperature

For the optimization of incubation temperature, 5 cultures have been studied in different temperatures ($26\text{-}34^0$C).

Statistical analysis: To analyze the data and comparison the amount of produced glucose in cultural groups under different incubation times and temperatures, the statistical software SPSS and ANOVA were used and $P<0.05$ was considered significant.

3. Results

The results showed that *Trichoderma* reesei in 6 cultures that grew in $30^\circ c$ and in different periods of time (3-8 day), after 96 hours (4 days), it reached to its maximum growth and fungi spores and colonies covered all the media. In these 4 days (96 hours), the activity of endogluconase enzyme and also the activity of filter papers first were reached to its maximum level and then were decreased after 4 days.

The concentration of produced glucose that was measured by enzymatic method of glucose oxidase, about 136.4 mg per dl was obtained on the fourth day that was the highest amount of glucose values and it did not have any significant difference with the amount of glucose on the third and sixth days, but it was significantly different with amount of glucose on fifth and eighth days with $P <0.05$. Also, it was significantly different with amount of glucose on the seventh day that was the lowest observed value with $P< 0.01$ (Table 1).

Table 1: Results of produced glucose in six medium of *Trichoderma* fungi after 3 to 8 days respectively and at $30^\circ c$

Time (days)	3	4	5	6	7	8
Glucose Concentration (mg/dl)	124.0	112.7	93.0	114.4	56.5	92.5

According to the previous tests, the highest level endogluconase enzymatic activity and filter paper activity was observed after four full days. In another phase study has done on the *Trichoderma reesei* fungi in 5 medium containing rice husks for 4 days at different temperatures 26, 28, 30, 32 and 34 ° C in incubator. Results have shown that in the prepared crude enzymatic solution from the cultures, the amount of glucose produced at a temperature of $30^\circ c$ (103 mg per dl) is the highest value, that did not show any significant

difference with the amount of glucose at 26 and 34 $^{\circ}c$, but it was significantly more than the amount of glucose at 28 and 32 $^{\circ}c$ (P< 0.05). The lowest amount of produced glucose was 58 mg per dl that was obtained at 32$^{\circ}c$ (Table 2).

Table 2: Results of produced glucose in 5 *Trichoderma reesei* medium at different temperatures after 4 days.

Temperature ($^{\circ}c$)	26	28	30	32	34
Glucose Concentration (mg/dl)	96.6	60.2	103.2	57.7	71.8

According to the results of previous experiments, the optimum conditions in terms of glucose production on fourth day and at 30 $^{\circ}c$ were determined. In these conditions, the amount of protein concentration was 23.4 (mg/ml) and Carboxy Methyl Cellulose activity and the paper filter activity was 680.3 and 125.7 (U/ml) respectively.

4. Discussion

Based on the findings in this research that was about solid state fermentation (SSF) of *Trichoderma reesei* fungi strain PTCC (5142) in rice husk media; it was shown that in different times and temperatures incubation, different amounts of glucose produces and the maximum amount of liquid glucose obtains at 30 $^{\circ}c$ and on fourth day (96 hours).

In Singhania et al., (2006) the report on lignocelluloses substrates with SSF method and by using *Trichoderma reesei* strains (NRRL 11460) for cellulose enzyme production, the highest activity of cellulose enzyme obtained at 28$^{\circ}c$ and on third day (72 hours) (1).

Sun et al., (2009) report using *Trichoderma .Sp* fungi strain on apple substrate with SSF method shows the maximum enzymatic activity of cellulose is in incubation condition of 32$^{\circ}c$ (10). The temperature and time difference between this study and our study is because of the difference between strain and substrate that was used. Also, our results are based on the amount of produced glucose in crude enzymatic solution. Normally glucose production occurs via acidic or enzymatic hydrolysis of starch. In America and many other parts of the world, glucose produces mainly by using enzymatic hydrolysis of grains' starch (9).

Silva and co-workers in Brazil (2010), using two microorganisms extracted from soil, *Aspergillus nigere* and *Strepotmyces. Sp*, produced glucoamilase and glucoisomerase enzymes respectively that could convert Cassava starch to glucose and fructose syrup. These researchers selected this substrate with respect to available and inexpensive substrate in that area (11). In the present study, according to the abundance and low cost of rice husk in the northern region, this material was chosen as the substrate medium. Also, the solid state fermentation (SSF) method was selected instead of liquid state fermentation (LSF) or Submerged Fermentation (SMF) because based on comparative studies in recent years this method (SSF) has more advantages (7, 8).

5. Conclusion

It can be concluded that rice husk can be suitable substrate for *Trichoderma reesei* fungal strain in order to produc liquid glucose. The optimum conditions for liquid glucose production with used mentioned substrate and strain are through solid state fermentation (SSF) and incubation of media at 30$^{\circ}c$ to 96 hours.

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