Effect of ultrasonic technology on cellulase enzyme activity produced by local bacterial isolate.

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Abstract. The research aimed to obtain new bacterial isolate producing cellulase enzyme, and ultrasonic effect on enzyme activity. Three bacterial isolates were isolated from different agricultural wastes samples. Their effectiveness in cellulolytic was detected by measurement of the clear zone diameter around bacterial growth. The enzyme was extracted after the growth of local bacterial isolate which has highest enzyme activity. The enzyme was exposed to ultrasound intensity of 40 KH, low, medium and high power, and exposure time (10, 20, 30, 40, 50, and 60) min, enzymatic activity was measured after each treatment compared with control. It was found at low level that an increase in the cellulase activity with increased ultrasonic exposure time, the higher activity at 60(min). The effect of ultrasonic treatment at mid-level, showed higher activity at 20 (min), while at high level the activity decrease with exposure time when compared with control. Protein concentration was estimated, there was no significant difference on protein content after ultrasonic treatment in samples at (10, 20, 30, 40) min while decrease in protein content in samples at (50, 60) min, compared with with control.

1. Introduction

The major component of plant biomass is the cellulose. Annually, $4 \times 10^9$ tons of cellulose is produced by plants. It is a polymer of $\beta$-1,4 linked glucose units. Enzymatic hydrolysis is difficult due to its crystalline form and insoluble nature. Microorganisms play a critical role in converting lignocellulose wastes into valuable goods such as biofuels produced through fermentation [1] Microbes have been recognized as a main source of industrial enzymes. Microbial enzymes are known to be important metabolic catalysts, which has led to their employment in a wide range of industries and applications. Microbial enzymes may be preferred over other sources of enzymes due to their ease of genetic manipulation, vast culture media, and replication. Microbial enzymes have been used in a variety of disciplines, inclusive medicine, cosmetics, technical use, animal nutrition, food production, and as tools for research and development [2].

Cellulase is an enzyme that degrades cellulose, $\beta$-glucosidases, exoglucanases, and Endoglucanases are the three important components of the cellulolytic enzyme system. Cellulases have potential applications in biotechnology and industry, including animal feed, pulp bleaching, juice clarification, malting and brewing, alcoholic beverage production, textile production, and starch processing [3]. Cellulase refers to a class of enzymes that catalyse the cellulolysis of cellulose. Enzymes are manufactured by a variety of microorganisms, typically by bacteria and enzymes application in the textile, food, detergent, leather, and paper industries. This enzyme is used in a variety of industrial
applications and is currently regarded as a prominent group of industrial enzymes [4]. Cellulases are inducible enzymes produced by a huge number of bacteria either extracellularly or cell-bound during their development on cellulosic materials [5]. Ultrasound (US) is a new technology that consists of sound waves with a vibrational energy that exceeds the human ear hearing limit (20–100 kHz). It is regarded as a "green" technology because of its environmentally adequate features and quite efficiency, and it has been widely used in a variety of areas, including the food sector: supporting high process repeatability, selective extraction, and sterilization benefits, as well as reducing processing time and costs [6].

Some studies have been examined the influence of the US on enzyme activity and structure. US has been employed as a process to an inactivation of the enzyme. Nonetheless, some researchers have found that the use of the US can help to activate and enhance the activities of enzymes. Sonication affects the biocatalyst primarily through three mechanisms: hydrogen peroxide, increasing enzyme activity, exposing more active sites, thermal effect, and other free radicals reactions. The understanding of the influence of the US on enzyme activity is quite significant. Although some effects are recognized for certain enzymes, others may have distinct ones due to distinctions in the enzyme structure and amino acid sequence. US main effects in a liquid medium are imputed to the chemical and physical phenomena, that can be generic resumed as the imploce collapse of gas micro bubbles dissolved in the liquid (cavitation), and the free radical formation, respectively. Nonetheless, because enzymes function in an aqueous context, these processes may cause changes in their functionality [6].

2. Material and method

2.1 Sample collection

Agricultural wastes samples were collected from different locations in Aljadria in Baghdadi in sterile containers.

2.2. Isolation of cellulolytic bacteria

One gram of waste sample was suspended in 9 ml of normal saline (0.85% NaCl) and serial dilutions were prepared by transferring 1ml of diluted suspension to 9ml of sterile normal saline [7]. A 0.1ml suspension of $10^4$ and $10^5$ dilution were plated on the nutrient agar incubated at 37 Cº for (18-24) hours, and purified by repeated streaking.

2.3. cellulolytic bacteria Screening

The purified bacterial colonies were grown on nutrient agar media and checked for cellulolytic activity by growing bacterial colonies on cellulosic medium without Congo red for 48 h at 37 Cº, then the medium was flooded with an aqueous solution of Congo red (1mg/ml) for 15 min to allow to reach to cellulose. The excess Congo red solution was washed by NaCl 1M [8].

2.4. Preparation of crude enzyme

After incubation at 37 Cº for (18-24) hours, the cultures were centrifuged at 4,000 rpm for 20 min. and supernatants were used as source of crude enzyme [9]. The crude enzyme solution was utilized for determination of enzyme activity, protein estimation and effect of ultrasonic.

2.5. Enzyme activity assay

Cellulase activity was measured by the DNS (3,5-dinitrosalicylic acid) method [10], by calculating the amount of reducing sugars.

carboxy methyl cellulose (CMC) solubilized in 50 mM citrate buffer, pH 4.8. enzyme solution was added, the mixture incubated for 60 min at 37 °C, the reaction was stopped by the addition of DNS solution. The optical density of the treated samples was measured at 550 nm after they were boiled for 10 minutes and cooled in water for color stabilization. The amount of enzyme that released 1 mol of glucose per minute was defined as one unit of enzyme activity [6].

2.6. Effect of sonication on cellulase activity

Crude enzyme solution sonicated at ultrasonic intensity of Treatment time was varied from 10 to 60 min [11]. The equipment used to all experiments was an ultrasonic water bath at ultrasonic frequency of 40 kHz and the total ultrasonic power low, mid, high (39, 104,175) W respectively.

2.7. Protein Estimation

protein concentration in the fermentation broth and after ultrasonic treatment was quantified by Lowry’s method with bovine serum albumin (BSA) as a standard [2].
3. Result and discussion

3.1. Isolation of cellulolytic bacteria

A total number of three bacterial isolates were isolated and purified from wastes samples as shown in figure (2).

3.2. Screening of cellulolytic bacteria

Three bacterial isolates have cellulytic activity as shown in figure (3), by appearance of clear zone round bacterial growth. The measurement of enzyme activity of cellulose lysis by determined of diameter of clear zone divided by diameter of bacterial colony [3], as shown in table (1) the bacterial isolate number (1) has higher activity compared with other isolates. Bacterial ability of cellulose lysis is because of production of cellulytic enzymes which degrade (β-1-4) linkage in cellulase polymer [12, 13, and 14].

Figure 1. Standard curve of protein concentration.

Figure 2. Pure bacterial isolates on nutrient agar.
**Figure 3.** Screening of cellulase producing bacterial isolates  
a: before staining with Congo red  
b: after staining with Congo red

**Table 1.** Screening of cellulytic activity of bacterial isolates.

| Isolate no. | Diameter of clear zone (mm) | Colony diameter (mm) | Clear zone/Colony |
|-------------|-----------------------------|----------------------|-------------------|
| 1           | 12                          | 2                    | 6                 |
| 2           | 7                           | 2                    | 3.5               |
| 3           | 5                           | 2                    | 2.5               |

3.3 **Effect of sonication on cellulase activity**

After enzyme extraction, the activity was determined as control, then crude enzyme treated with ultrasonic at low, mid and high level at 40 KH and exposure time (10, 20, 30, 40, 50, 60) min. Enzymatic activity was measured after each treatment, the effect of ultrasonic treatment on cellulase activity at low level power has been shown in figure (4) the graph shows that an increase in ultrasonic treatment time significantly increased the cellulase activity, the higher activity at 60 (min). Figure (5) shows the effect of ultrasonic treatment at mid-level of ultrasound power, the graph shows higher activity at 20 (min), and the rest treatment lower than control, while at high level the activity decrease with time when compared with control, as shown in figure (6). The ultrasonic mechanisms that change the enzyme activity are specific to the enzyme under investigation and depends on its amino acid composition and the formational structure. The reasons of increase of enzyme activity are activation of latent isoenzymes or changes in the three-dimensional structure, low intensity ultrasound help to disintegrate the enzyme molecular aggregates into smaller fragments exposing more active sites, contributing to increase enzyme activity [6]. While the inactivation of enzyme activity is protein denaturation change the protein structure leading to partial or irreversible denaturation resulting in loss of activity [13].

**Figure 4.** Ultrasonic effect on cellulase activity at low level.
3.4. Protein estimation
After treatment of enzyme preparation with ultrasonic at high level at (0, 10, 20, 30, 40, 50, 60) min, protein concentration was estimated as shown in figure (7). It can be clearly seen that there was no significant difference on protein content after ultrasonic treatment in samples at (0, 10, 20, 30, 40) min were ranged (0.046–0.0436) mg/ml while decrease in protein content in samples at (50, 60) min was (0.0365 and 0.0332) mg/ml respectively. The differences in initial concentration of soluble protein in the samples generated different changes in spatial configuration of protein molecules, although the ultrasonic treatment conditions were similar [11, 16].

Figuer 5 Ultrasonic effect on cellulase activity at mid-level.

Figuer 6. Ultrasonic effect on cellulase activity at high level.
4. Conclusions

Three local bacterial isolates that produced cellulase enzyme were isolated. Cellulase enzyme activity was increased by ultrasonic treatment, higher enzyme activity obtained in exposed time 60 (min). There was no significant difference on protein content after ultrasonic treatment.

5. References

[1] Irfan, M.; A. Safdar; Q. Syed, and Nadeem M.(2012). Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. Turk J Biochem. 37 (3) : 287–293.

[2] Adeleke B. S ; Ojo1 S. O.; Oluwafemi Y. D. and Olaniyi O. O.(2017). Chemical Mutagenesis of Bacillus subtilis for Improved Mannanase Biosynthesis. J. Advan. Microbiol. 3(2): 1-6.

[3] Saini, A.; Aggarwal, N. K; Yadav, A. (2017). Isolation and Screening of Cellulose Hydrolyzing Bacteria from Different Ecological Niches. Bioengin. and Bioscienc. 5(1): 7-13

[4] Sadhu S. and Maiti T. K. (2013). Cellulase Production by Bacteria: A Review British Microbiol. Res. J. 3(3): 235-258.

[5] Shanmugapriya K.; Saravana P.S.; Manoharan M; Mythili A. and Joseph S. (2012). Isolation, screening and partial purification of cellulase from cellulase producing bacteria. Int. J Adv. Bioengin. and Bioscienc. 5(1): 7-13

[6] Dalagnol L. M. G.; Silveira V.C.C. and . Silva H. B. (2017). Improvement of pectinase, xylanase and cellulase activities by ultrasound: Effects on enzymes and substrates, kinetics and thermodynamic parameters. Process Biochem.( 61): 80-87.

[7] Ashwani K.; Saida L.and Reddy K.V. (2014). Isolation, Screening and Characterization of Cellulolytic bacteria from forest soil sample. Int.J.Curr.Microbiol.App.Sci. 3(10): 679-685.

[8] Hunag Sh. , Sheng. P. and Zhang H. (2012). Isolation and identification of cellulolytic bacteria from the gut of Holotrichiaparallelalarvae(coleoptra: Scarabaeidae). Int. J.Mol.Sci.13(3): 2563-2577.

[9] Kotchoni O.S.; Shonukan O. O.; Gachomo W. E. (2003). Bacillus pumilus BpCRI 6, a romising candidate for cellulase production under conditions of catabolite repression. Afr. J. Biotechnol. 2(6): 140-146.

[10] Miller GL. (1959). Use of dinitrosalicylic acid for the determination of reducing sugar. Anal Chem 31: 426-428.

[11] Nguyen, T.T.T. and Le, V.V.M. (2013). Effects of ultrasound on cellulolytic activity of cellulase complex. Intern. Food Res. J. 20(2): 557-563.

[12] Pratiksha, P. and B. Gireesh (2012). Isolation and identification of methanogenic bacteria from cowdung. 4(7): 28.

[13] Liang Y.-L., Zhang Z., . Wu M. , Wu Y, and . Feng J.-X. (2014). Isolation, Screening, and Identification of Cellulolytic Bacteria from Natural Reserves in the Subtropical Region of China and
Optimization of Cellulase Production by *Paenibacillus terrae* ME27-1. *BioMed Res. Inter.* 2014. 13 pages.

[14] Ram L., Kaur K., and Sharma S. (2014). Screening Isolation and Characterization of Cellulase Producing Micro-Organisms from Soil. *Intern. J. Pharm. Scien. Inven* 3(3):12-18.

[15] Rojas M. L.; Trevilin J. H and Esteves P. (2016). The ultrasound technology for modifying enzyme activity. *Scientia Agropecuaria* 7 (2): 145 – 150.

[16] Sinisterra, J. V. 1992. Application of ultrasound to biotechnology: an overview. *Ultrasonecs* 30: 180-185.

[17] Kautish, S., Peng, S.-L., & Obaid, A. J. (2021). Computational Intelligence Techniques for Combating COVID-19. Springer International Publishing.