Extracellular Endoglucanase and Exoglucanase Enzymes Production by *Trichoderma viride* Utilizing Olive Mill Wastewater (OMW) in liquid fermentation

Maram Al Tharf [1], Muhannad I. Massadeh*[1], Batol I. Dheeb [2]
Sara N. Abdulla[2]
[1]Department of Biological Sciences and Biotechnology, Faculty of Science, the Hashemite University, Zarqa, Jordan
[2]Department of Pathological Analysis, College of Applied Sciences, University of Samarra
*Correspondence email: massadeh@hu.edu.jo

Received: 25/09/2023
Accepted: 21/11/2023
Online: 16/05/2024

ABSTRACT

Background:The cellulase enzyme is one of the most industrially important enzymes. Its cost represents a significant barrier to other valuable commercial products. Reducing the cost of cellulase production is an important approach. **Objectives**: For this purpose, this work investigated the production of cellulase enzyme using olive oil production waste (olive mill wastewater (OMW). **Materials and methods**: The ability of *Trichoderma viride* to utilize OMW as a substrate for cellulase production was studied. Optimization of cellulase production was investigated to find out the optimum OMW concentration, agitation speed, aeration rate, and cellulose addition. **Results:**The results showed that 75% v/v OMW submerged shake flask culture was the most suitable culture for *T. viride* growth and cellulase enzyme production (0.82 U/ml endoglucanase activity and 0.25 U/ml exoglucanase activity). When this culture was supplemented with cellulose, the activity of endoglucanase and exoglucanase was significantly improved (10.24 U/ml and 2.17 U/ml respectively).The agitation speed of 200 rpm enhanced the production to reach 9.1 U/ml of endoglucanase and 6.38 U/ml of exoglucanase. The effect of the aeration rate on enzyme production was studied under batch cultivation. The highest cellulase activity was at 2.0 vvm, where the endoglucanase and exoglucanase activities were 55.96 U/ml and 32.62 U/ml respectively. **Conclusions:** Therefore, it is claimed that OMW is a suitable medium for cellulase enzyme production after optimization of the process.

Keywords: Cellulase, Olive Mill Wastewater, *Trichoderma viride*, Submerged culture fermentation.
DOI: https://doi.org/10.24126/jobrc.2024.18.1.768

1-INTRODUCTION

One of today's greatest challenges for scientists and technologists is to have a sustainable growing energy demand to feed industry processes, transportation, and residential uses. Renewability is the lit-motive and biological energetic resources, of which lignocellulosic biomass is a major component as a renewable nature abundant and inexpensive material and is comprised mainly of cellulose (1). There are many sources for lignocellulosic biomass such as municipal waste, agricultural residues, forestry or pulp, and by-products of food production (2). One of the most economically and technically critical steps in the production of biofuels and other important products is the enzymatic hydrolysis of raw materials, especially cellulase (3,4). Olive Mill Wastewater (OMW), a residue of the olive oil industry, is one of the major environmental problems in the Mediterranean olive-growing countries, such as Jordan, Palestine, Tunisia, Italy, Morocco, Turkey, Greece, and Spain. Mediterranean regions have an annual OMW production of about 3×10^7 m^3 (6,7). This wastewater contains organic fractions including sugar, polyalcohol, pectin, polyphenols, lipids, and tannins. Sugars and polyphenols can serve as carbon and energy sources for microbial growth. This material induces serious problems because of its high organic load, phytotoxicity, and phenolic compounds which resist biological degradation (8,9). Olive mill wastewater (OMW) pollution is becoming a serious problem, especially in the Mediterranean area since they are responsible for 95% of the worldwide olive oil

production (10,11). To resolve the environmental problems related to OMW, several physio-chemical processes, including simple evaporating, ultra-filtration, and anaerobic digestion processes were proposed to reduce the polluting effects of OMW. Alternatively, great interest focused on biological treatment using aerobic bacteria and fungi (12). Treatment of OMW is a huge challenge because it contains biological resistance compounds and both high capital and processing costs of proposed treatment. Cellulase is one of the most extensively investigated multicomponent enzyme systems, because of its ability to break down the cellulosic biomass into glucose, which in turn can be converted to other valuable chemical products (13). Cellulase can be divided into three types: endoglucanase, cellobiohydrolase (exoglucanase), and β-glucosidase.

They together with other related enzymes, such as hemicellulases and ligninases are among the most important groups of enzymes, which are employed in the processing of lignocellulosic materials (14,15,16)**.** Filamentous fungi were the best-known cellulase-producing organisms because of their ability to produce high enzyme yield, and capacity to secrete active and separable enzyme types. Studies on hypersecretory microfungi especially *Trichoderma* spp., have been extensively studied due to their strong cellulolytic activity against crystalline celluloses which results in saccharification (17). Among them *T. viride*, *T. harzianum, T. reesei* and *T. konigii* were studied (17,18, 19). However, *T. viride* was proven as an efficient candidate for biodegradation (19)**.** The high cost of cellulase enzymes represents a significant barrier to the commercial deployment of important products. Much research efforts have been directed toward improving the production of cellulolytic enzymes. There are a number of approaches that have been adopted, aiming towards reducing the cost of enzyme production by focusing on optimizing the fermentation process parameters, and improving the efficiency of known enzymes and microorganisms. These included the use of industrial wastes such as agricultural waste (20), and corn cob (21), as examples of low–cost materials**.** For this purpose, this study focuses on the use of OMW as a fermentation medium since it is a valuable source of native lignocelluloses with negligible cost for the production of cellulase enzyme by *T. viride* at different operating conditions. Therefore, the objectives of this study were to study the ability of *T. viride* to produce cellulase enzyme complex during OMW fermentation and to study the necessity of aeration and agitation in the fermentation process.

2-MATERIALS AND METHODS

All experiments were conducted under aseptic conditions, and all media was autoclaved at 121 ºC for 15 min (HIRAYAMA, Japan). The pH was adjusted by using 1.0 M NaOH and 1.0 M HCl. All chemicals used were of analytical grade and mostly purchased from (BDH laboratory supplies, England).

Microorganism

The fungus T. *viride* (a local isolate obtained from the Department of Microbiology, Faculty of Agriculture, Jordan University of Science and Technology) was grown on potato dextrose agar (PAD) at 28ºC for 4-7 days and stored at 4ºC until use. Stock cultures were sub-cultured every 3 months.

Olive Mill Wastewater (OMW)

Throughout the study, the olive mill wastewater was used as a fermentation medium. It was collected from a three-phase centrifugal olive mill located at Na'our city of Jordan during the olive oil extraction seasons. This byproduct was stored at 4ºC to prevent biodegradation by the action of microorganisms.

Inoculum preparation

The shake culture flasks of OMW were inoculated aseptically by 3 mycelia plugs (radius of 7 mm) cut at the advancing edge of *T. viride* grown on PDA plate culture using sterile Pasteur pipettes.

Screening for the best OMW dilution for cellulolytic enzyme production

Different dilutions (25, 50, 75, and 100 %) of OMW shake cultures of 100 ml adjusted to pH 5.5 were prepared using Erlenmeyer flasks of 250 ml. After sterilization, each flask was inoculated aseptically with three mycelium plugs (radius of 7mm) cut at the advancing edge of *T. viride* and placed on an orbital shaker with agitation speed of 150rpm and 28ºC as incubator temperature for 4 days. Every day, a 5ml sample was withdrawn from the flasks in order to measure the enzyme activity and dissolved protein content.

Optimization of fermentation process:

Effect of cellulose addition on enzyme production

The shake flask cultures of 75% OMW were chosen for further studies. The effect of cellulose addition was assessed by preparing a shake culture containing 75% OMW supplied with 0.5 g of cellulose.

Effect of the agitation speed on cellulolytic enzymes production

The effect of agitation was studied in the same procedure mentioned but using different agitation speeds (100, 150, 200, and 250rpm)

Effect of aeration rate on cellulolytic enzyme production

The effect of aeration was assessed on batch operation. Shake flask cultures of 75% OMW were aerated at (0.0, 0.5, 1.5, 2.0vvm) by using air pump. The air flow rate (vvm) was calculated by counting the gas volume flow per unit of liquid volume per minute.

Enzyme extraction

A 5ml sample was obtained from flasks after each experiment, centrifuged at 6000 rpm for 15 min (NÜVE, Turkey), and filtrated using Whatman no.1 filter paper. The filtrate was used as a crude enzyme source.

Enzyme assay: The endoglucanase enzyme activity was assayed using DNS method (Miller, 1959) (22). Exoglucanase enzyme activity was analyzed according to filter paper assay (FPase) (23). One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of glucose from the appropriate substrates per minute per crude filtrate under the assay conditions.

Protein content determination: Dissolved protein content was measured according to (24).

Statistical analysis:

The standard curves for cellulase enzyme activity (endoglucanase, exoglucanase and β-glucosidase) and protein content were prepared using GraphPad PRISM (version 5.02) program. One-way analysis of variance (ANOVA) was used for testing the difference between the groups and Tukey's Multiple Comparison Test was used to determine the difference between each factor. Data were expressed as the mean ± SEM, and $P \leq 0.05$ was considered statistically significant.

3-RESULTS

Submerged shake cultures of OMW:

Shake flask cultures were conducted using OMW as the main substrate for cellulase enzyme production. OMW was used at 4 different concentrations (25, 50, 75, and 100%). It was observed that the maximum endoglucanase and exoglucanase activity were achieved in cultures employing 75% OMW. *T. viride* grown in 75% OMW cultures revealed 0.82 U/ml endoglucanase activity, 0.25 U/ml exoglucanase activity, and 1.1 mg/ml dissolved protein contents after 3 days of the fermentation process (Figure 1**)**. There was no significant difference between the four experiments conducted.

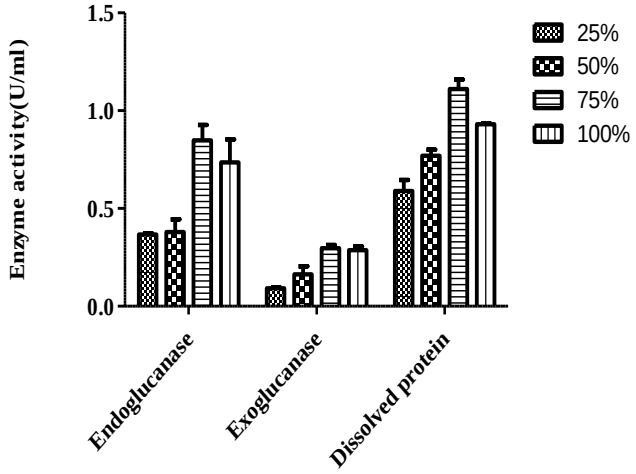


Figure (1): Enzymes activity of *T.viride* submerged cultures of different concentrations of OMW (25%, 50%, 75%, and 100%) after 3 days of the fermentation process. Error bars indicate the standard error of the mean of three replicates.

Effect of cellulose addition on enzyme production:

The cellulolytic enzyme production by *T.viride* in cultures of 75% OMW supplied with 0.5 g of cellulose was studied. Results shown in (Figure 2) revealed that cellulose addition was necessary for growth and enzyme production compared with results to previous results. The highest activity of Endoglucanase and Exoglucanase was 10.24 U/ml and 2.17 U/ml respectively after 3 days of the fermentation process. The dissolved protein content was 4 mg/ml.

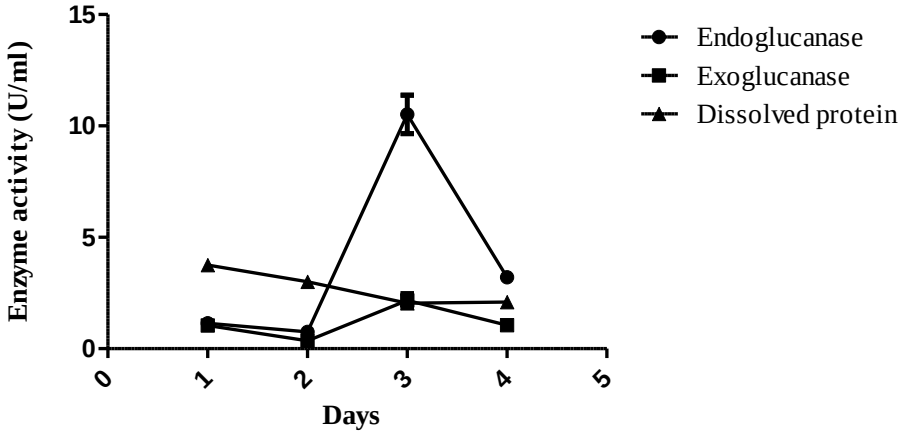


Figure (2): Enzyme activity and dissolved protein content in cultures of *T. viride.* Growing on 75% OMW after cellulose addition throughout the 4 days of fermentation. Error bars indicate the standard error of the mean of three replicates.

Effect of agitation on enzyme production:

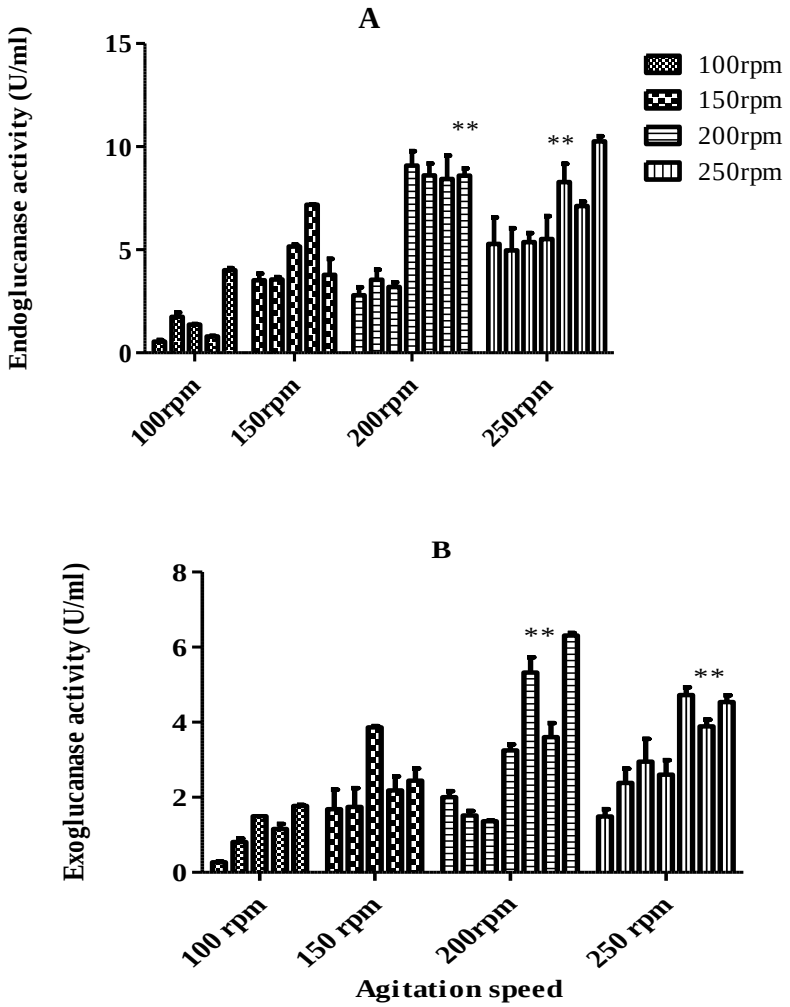
To assess the effect of agitation on cellulase production, different culture agitation speeds (100, 150, 200, and 250 rpm) were studied using 75% OMW in shake flask culture. Maximum enzyme activity was achieved at 200 rpm and 250 rpm and showed significant differences compared to 100 rpm and 150 rpm (Figure 3; Table 1). The best agitation speed for both endoglucanase and exoglucanase was 200 rpm. The highest endoglucanase activity was

9.1 U/ml whereas exoglucanase activity was 6.38 U/ml after 4 days of fermentation (Figure 3). Figure (3C) shows the effect of the 4 agitation speeds on dissolved protein contents and the highest dissolved protein contents was 3.8 mg/ml at 200 rpm.

Table(1): Effect of agitation speed on enzyme production; endoglucanase, exoglucanase, and dissolved protein throughout the fermentation days. *

Agitation speed(rpm)	Enzyme activity (U/ml)		Dissolved proteins (mg/ml)
	Endoglucanase	Exoglucanase	
100 rpm	1.69±0.61ns	1.10±0.26ns	1.43±0.30ns
150 rpm	4.64±0.70	2.38±0.39	1.56±0.24
200 rpm	6.32±1.12**	3.34±0.72**	2.97±0.17**
250 rpm	6.68±0.75**	3.23±0.45**	2.10±0.16ns

* Each value is represented as mean± SEM of replicates for 5 days at 100 and 150 rpm, 7 days at 200, 250 rpm .** indicate correlations significances at $p < 0.05$. ns indicates no significant differences. Significance was compared with values acquired at 150 rpm.



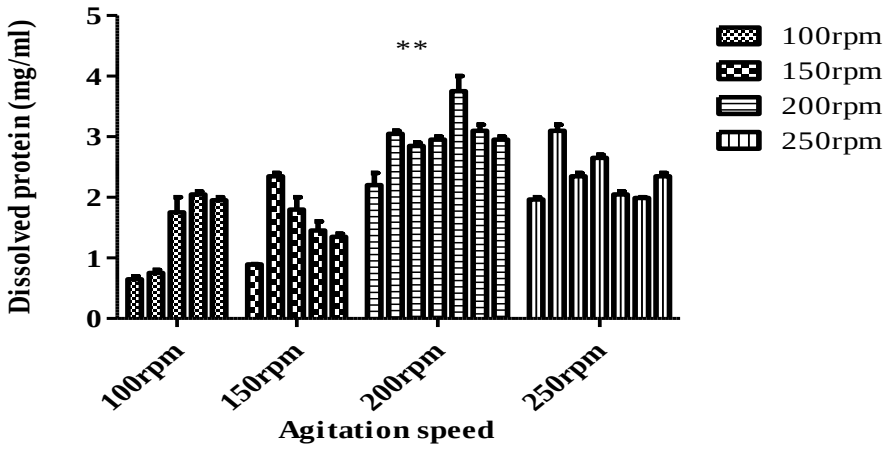


Figure (3):Effect of agitation speed on enzyme activity and dissolved protein content throughout the time profile of the experiment. (A) Endoglucanase (B) Exoglucanase (C) dissolved protein. ** indicate correlations significant differences at $P \leq 0.05$ on enzyme activity and protein content. Each column represents the time after which a sample was withdrawn for analysis. Error bars indicate the standard error of the mean of three replicates. Significance was compared with values acquired at 150 rpm.

Effect of process operation mode

In order to study the effect of operation mode on enzyme production, a fed-batch process and a semi-continuous process were performed using a 75% OMW shake flask with 200 rpm agitation speed at a temperature of 28 ℃ and pH of 5.5. Maximum enzyme activity of endoglucanase and exoglucanase was achieved at semi-continuous mode (7.5 U/ml and 3.15 U/ml respectively). The highest endoglucanse activity at semi-continuous mode was after seven days of fermentation whereas the highest endoglucanase activity was after eight days of fermentation at fed-batch mode (Data not presented). The dissolved proteins were 3 mg/ml in semi-continuous mode (Table 2). There were no significant differences comparing fed-batch and semi-continuous processes to batch mode for cellulase enzyme complex production. Therefore, we decided to continue our investigation using batch cultures.

Table (2): Effect of operation mode on cellulase enzyme production and dissolved protein throughout the fermentation days of each mode.*

Mode of process operation	Enzyme activity (U/ml)		Dissolved proteins (mg/ml)
	Endoglucanase	Exoglucanase	
Batch	3.20±0.63	2.01±0.35	2.25±0.19
Fed batch	3.09±0.13ns	1.48±0.07ns	2.06±0.46ns
Semi-continuous	3.91±1.63ns	2.31±0.57ns	2.95±0.13ns

* Each value is represented as mean± SEM of replicates of fermentation days at each mode. ns indicates no significant differences. Significance was compared with values acquired in batch mode.

Effect of aeration rate on the fermentation process:

Different aeration rates (0.0, 0.5, 1.5, and 2.0 vvm) were studied for their effect on cellulase enzyme production using 75% of fresh OMW shake flask cultures with the previous optimum condition obtained. Maximum enzyme activity was achieved at 1.5 and 2.0 vvm (Table 3). The highest cellulase enzyme activity was at 2.0 vvm. The endoglucanase and exoglucanase activities were 55.96 U/ml and 32.62 U/ml respectively after 4 days of fermentation (Figure 4). In the case of dissolved proteins content, there was a significant difference compared to 0.0 vvm as a control whereas there was no significant difference when compared to 0. 5 vvm.

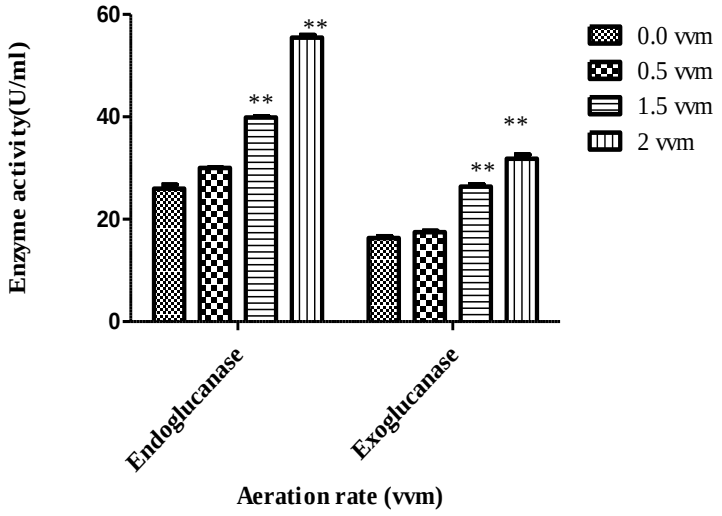


Figure (4): Effect of aeration rate on cellulase enzyme activity after 4 days of fermentation. ** indicate correlations significant differences at $P \leq 0.05$ on enzyme activity. Significance was compared with values acquired at zero vvm as a control.
Error bars indicate standard error of the mean of two replicates.

Table (3): Effect of aeration rate on enzyme production; endoglucanase, exoglucanase and dissolved protein.*

Aeration rate(vvm)	Enzyme activity (U/ml)		Dissolved proteins (mg/ml)
	Endoglucanase	Exoglucanase	
0.0 vvm	30.78±2.50	12.79±1.41	1.37±0.31
0.5 vvm	30.35±0.21ns	11.19±2.20ns	1.40±0.29ns
1.5 vvm	40.62±4.51**	21.41±3.39**	2.46±0.88ns
2.0 vvm	42.31±5.72**	21.49±3.97**	2.50±0.81ns

* Each value is represented as mean± SEM of replicas of 4 days of the fermentation at each aeration rate. ****** indicate correlations significant differences at $P \leq 0.05$ on enzyme activity. ns indicates no significant differences. Significance was compared with values acquired for the control at zero vvm.

4-Discussion

Cellulase enzyme was produced using olive mill wastewater by *T. viride*. The suitable dilution of olive mill wastewater (OMW) for cellulolytic enzyme production is important for *T.viride* metabolism, growth, and enzyme production system, So submerged shake cultures of *T.viride* were investigated for the best dilution. In this study, it was observed that the endoglucanase, exoglucanase, and dissolved protein contents were at the maximum levels when 75% OMW was employed. The enzyme activities were 0.82 U/ml for endoglucanase activity, 0.25 U/ml for exoglucanase activity, and 1.1 mg/ml dissolved protein contents (Figure 1). These low values of enzyme activity and protein content might be explained form the data of dissolved protein where it indicates that the microorganism was utilizing the cellulosic portion of OMW slowly. When cellulose was added, the endoglucanase, exoglucanase activity, and dissolved proteins rose to become 10.24 U/ml and 2.17 U/ml and 4.0 mg/ml respectively (Figure 2). Abu Mie, (2009) (25,26,27,28) reported that 10, 25, 50, 75, 100% were employed to investigate the suitable dilution for hydrolytic enzyme production. It was observed that the maximum endoglucanase and exoglucanase activity was achieved in cultures employing 50, 75 and 100% OMW cultures, but from general observation and biomass growth, it was highest at 50% OMW cultures (29,30,31,32,33). This might be due to the high concentration of carbohydrates presented in the OMW. The highest endoglucanase and exoglucanase activities were (16.33 U/ml and 8.76 U/ml respectively). The dissolved protein content was 5.99 mg/ml. Azbar *et al.* 2004 (9) reported that the quality and

quantity of the constituents of olive mill wastewater (OMW) are dependent on many factors i.e. type of olives, type of soil, cultivation system, and production process. These factors and others could play an important role in demonstrating the suitable concentration of OMW for microorganisms' growth and cellulolytic enzymes' production. Dissolved oxygen and agitation speed are important parameters for performing a desired fungal fermentation with a given strain and medium (26). The availability of oxygen is a major parameter to be considered for effective microbial cell growth rate; agitation is directly related to oxygen transported from the gas phase to the liquid phase followed by oxygen uptake by individual microbial cells (27). Therefore, the effect of different aeration and agitation rates on enzyme activity and growth was investigated. Agitation is important for proper oxygen transfer and homogenous mixing of nutrients in the fermentation process. To create an optimal environment in a culture, agitation is required for cells to have access to all substrates including oxygen in an aerobic culture (27). At 100 rpm and 150 rpm, low cell growth and enzyme activity compared to results of other agitation speeds was observed. This could be attributed to improper mixing which led to oxygen limitation. 200 rpm of agitation speed was found to be most conducive for cell growth, enzyme production, and stability for both endoglucanase and exoglucanase activity. A further increase to 250 rpm showed a reduction effect in endoglucanase stability and exoglucanase activity, which could be attributed to shear stress and heterogenous mixing effects. Similar results were also reported in other studies as (Nadeem *et al.,* 2009) (28). Present results supported by Gunjikar *et al* (2001) (29) who studied cellulase deactivation in a stirred tank bioreactor and concluded that: (1) the extent of deactivation increased with an increase in agitation speed, (2) the extent of deactivation for cellulase and its three components differed significantly and that (3) exoglucanase contributes to the major decrease in cellulolytic activity in the initial stage of shearing. Maximum enzyme activity was achieved at 4-7 days depending on the experiment's conditions. Comparing the results of batch shake cultures to fed-batch and semi-continuous shake cultures enzyme activity from general observation and biomass growth was highest at semi-continuous mode. The highest enzyme production at semi-continuous might be due to the availability of nutrient requirements and reducing of accumulated waste which were more suitable for growth and enzyme production. Whereas a comparison between batch and fed-batch showed that batch had slightly higher enzyme activity and dissolved protein than fed-batch mode. Fed-batch seems to be not good for cellulase production compared to others of operation mode. This may be due to the insufficient availability of nutrients for microbial growth. Statistically, there was no significant difference compared the fed-batch and semi-continuous process to batch mode for cellulase complex enzyme production. To understand the importance of batch production of cellulase and to enhance the whole process, oxygen was supplied at varied flow rates. The agitation rate was fixed at 200 rpm while aeration was varied. Dissolved protein content (which indicates growth) at 1.5 and 2 vvm showed a rapid rise followed by slower growth. This observation could be the result of rapid conversion of nutrients in the medium to cell mass, corresponding to the rapid growth in the first 2 days of culture. In this study, the elevated aeration rate was accompanied by a corresponding increase in cellulase enzyme production, with the highest enzyme activity of endoglucanase and exoglucanase observed at 2.0 vvm. As stated by Xu and Yun (2004) (30), aeration results in better mixing of the exopolysaccharides from *Paecilomyces tenuipes* C240 in a stirred-tank fermenter. This in turn helps to maintain a concentration gradient between the interior and exterior of the cell, allowing better diffusion of nutrients to the cells. Increasing the aeration rate leads to an increase in dissolved oxygen and then raises the growth. Fadzilha and Mashitah (2010) (31,34) studied the effect of aeration and agitation rate on cellulase enzyme production in a submerged culture of *Pycnoporus sanguineus* in a 2.5 L stirred-tank bioreactor using palm oil mill effluent as a substrate. They noticed that maximum cellulase activity was obtained at an aeration rate of 1.0 vvm and agitation speed of 300 rpm.

5-Conclusion:

This study confirmed the remarkable cellulase production potential of *T. viride* using olive mill wastewater. The utilization of OMW for the biological production of valuable products may have a good effective impact on the environmental problem of OMW management since it can also be as a first step of effluent treatment. Attempts were made to find the optimum fermentation conditions for successful cultivation of *T. viride*, and also for enhancing the production of one of the most industrially important enzymes with lower cost. Aeration and agitation rates affect cell growth and cellulase production. Therefore, the suitable aeration rate and agitation speed were essential for optimum cell growth and cellulase production. 200 rpm of agitation speed and 2.0 vvm of aeration rate were the most suitable for cellulase production. 2.0 vvm enhanced the enzyme activity up to 55.0 U/ml of endoglucanase and 32.0 U/ml of exoglucanase. When operation mode processes (batch, fed-batch, and semi-continuous) were studied, there were no significant differences in enzyme activity between these operation mode processes. The ability of *T. viride* to grow on relatively high phenolic-content OMW and produce notable quantities

of cellulase, make this fungus worthy of further investigation. Specifically, the utilization of OMW as a carbon source in order to avoid the supplementary addition of nutrients to reduce the overall process cost, as well as the study of a potential process scale-up.

REFERENCES

1. Maki M.L, Broere M, Leung K.T, Qin W. Characterrization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizer. International J Biochem Molecular Biology, (2011); 2(2): 146-154.
2. Greene, N. Growing energy. How biofuels can help end America's oil dependence. Nat Res J Def Council Rep. (2004); 1-86.
3. Ojumu, TV., Solomon, BO., Betiku, E., Layokun, SK. and Amigun, B. Cellulase production by *Aspergillus Flavus* Linn isolate NSPR 101 fermented in sawdust, bagasse and corncob .African J Biotechnology. (2003); 2: 150-152.
4. Abdulbaqi, NJ. and Dheeb, BI. and Irshad, R. Expression of Biotransformation and Antioxidant Genes in the Liver of Albino Mice after Exposure to Aflatoxin B1 and an Antioxidant Sourced from Turmeric (Curcuma longa) Jordan J of Biological Sciences (2018);. 11(2): 89 – 93.
5. Eroglu, E. Eroglu, I. Gunduz, U. Turker, L. Meralyucel. Biological hydrogen production from olive from olive mill wastewater with two-stage processes. International J of Hydrogen Energy. (2006); 31: 1527-1535.
6. Massadeh, MI. and Modallal, N. Ethanol Production from Olive Mill Wastewater (OMW) Pretreated with *Pleurotus sajor-caju*. Energy and Fuels. (2008); 22: 150-154.
7. Asses, N. Ayed, L. Bouallagui, H. Ben Rejeb, I. Garhouri, M. and Hamdi, M. "Use of *Geotrichum candidum* for olive mill wastewater treatment in submerged and static culture Bioresource Technology. (2009); 100: 2182-2188
8. Azbar, N. Bayram, A. Ayes, F. Ayesn, M. Fusun, S. Ozer, A. A review of waste management options in olive oil production. Environmental J of Science and Technology. (2004); 34:209-247
9. Papanikolaou, S. Fakasb, S. Fick, N. Chevalot, I. Galiotou-Panayoton, M. Komaitis, M. Marc, I. Aggelisc, G. Biotechnological valorization of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil. Biomass J of and Bioenergy. (2008); 32: 60-71.
10. Ugurlu, M. and kula, I. Decolourization and removal of Some Compounds from Olive Mill waste water by Advanced Oxidation process and Lime Treatment. Enveronmental Science J and Pollution. (2007); 14:319-325.
11. Papafotiou, M. Phsyhalou, M. Kargas, G. Chatzipavlidis, I. and Chronopoulos, J. Olive-mill wastes compost as growing medium component for the production of poinsettia. Scientia J of Horticulturae. (2004); 102: 167-175.
12. Domingues, FC. Queriroz, JA. Cabral, J.MS. Fonseca, LP. The influence of culture conditions on mycelia structure and cellulase production by *Trichoderma reesei Rut C30* .J of Enzyme and Microbial Technology. (2000); 26:394 – 401.
13. Beguin, P. Molecular biology of cellulose degradation . J of of Microbiology. (1990); 44: 219-248.
14. Howard, RL. Abotsi, E. Jansen van Rensburg, EL. and Howard S. "Lignocelluulose biotechnology: issues of bioconversion and enzyme production . African J of Biotechnology. (2003); 2: 602-619.
15. Turpeinen B. Lignocellulose degradation and humus modification by the fungus *Paecilomyces inflatus*MSc Thesis, Academic Dissertation in Microbiology, University of Helsinki, Finland (2007).
16. Hashmi S.S, Mahmood Z.F, Abdulateef S.F, Dheeb B.I. Evaluation Cytotoxicity Effects of Centaurea Cineraria Extracts Against some of Cancer Cell Lines. Biomed Pharmacol J.(2023); 16(1).
17. Salih I.I., Seddiq S.H., Hashim S.S., Dheeb B.I. Application of Omics and Proteomics in Fungi. AIP Conference Proceedings.(2022).
18. Abdullah H.I, Hammadi S.Y, Hussein A.S, Dheeb B.I. Investigation of genetic diversity and relationships among the clinical candida species using random amplified polymorphic DNA (RAPD) analysisResearch J of biotechnology. (2019); 14(Special Issue 1): pp. 6–13.
19. Awad F.M , Al-Samarrai A.H.M, Dheeb B.I. Study of the inhibitory effects of rheum ribes extracts on a pathogenic fungi and cancer cell line. Plant Archives J.(2020); 20(1): pp. 3161–3168.
20. Hawary F.I., Mostafa Y. S. Factors affecting cellulases production by *Trichoderma KoningiiActa Alimentaria*. (2001);30(1):3-13.
21. Abed.S.M, Farhan M.G, Madhloom N.K, Dheeb B.I. Magnetic Field Exposure to Clinical Isolates of *Acinitobacter baumanii*. J of Biomed Pharmacol.(2022); 15(4).
22. Liming, X. Xueliang, S. High-yield cellulase production by *Trichoderma reesei ZU-02* on corn cob residue. J of Bioresour Technology. (2000); l 91: 259–262.

23. Dheeb B.I, Al-Halbosiy M.M.F, Al Lihabi R K, Khashman B.M. The effects of Rubus idaeus extract on normal human lymphocytes and cancer cell line .J of BMC genomics. (2016); 17 (6): 18.

24. Mandels, M. Andreotti, R. Roche, C. Measurement of saccharifying cellulose. Biotechnology and Bioengineering Symposium. (2022); 6: 21-23.

25. Bradford M.M. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. J of Analytical Biochemistry. (2022); 72: 248-255.

26. Abu Mie, RA. Simultaneous production of citric acid, hydrolytic enzymes and reducing sugars in a multistage process using olive mill wastewater as a substrate. Msc Thesis. Department of biology. Hashemite University. Jordan(2009).

27. Cui Y.Q, Ouwehand J.N, W. Vanderlans R. G.J.M , Lunben K.C.A.M. Effects of dissolved oxygen tension and mechnical forces on fungal morphology in submerged fermentation. J of Biotechnology Bioengineering. (2020); 57: 409-419

28. Najafpour G.D, "Chapter3: Gas and liquid system (Aeration and Agitation). J of Biochemical Engineering and Biotechnology (Ed): Elsevier, Netherlands, ISBN(2010); 978-0-444-52845-2, pp: 22-68.

29. Salih I.I, Seddiq S.H, Hashim S.S, Dheeb B.I. Application of Omics and Proteomics in Fungi. AIP Conference Proceedings(2022).

30. Nadeem M, Qazi J.I, Baig S. Effect of aeration and agitation rates on alkaline protease production by *Bacillus Licheniformis* UV-9 mutant . Turkish J of Biochemistry.(2009); 34: 89-96.

31. Gunjikar T.P, Sawant S.B, Joshi J.B. Shear deactivation of cellulase, exoglucanase, endoglucanase and β-gluvosidase in a mechanically agitated reactor .Biotechnology Progress. (2001); 17:1166-1168.

32. Xu C.P, Yun J.W. Influence of aeration on the production and the quality of the exopolysaccharides from *Paecilomyces tenuipes* C240 in a stirred-tank fermenter. Enzyme Microbial Technol. (2004);35: 33-39.

33. Fadzilah K., Mashitah M.D. Cellulase production in palm oil mill effluent: Effect of aeration and agitation . J of Applied Sciences. (2010); 10(24): 3307-3312.

34. Hussein H.S, Dheeb B.I, Hamada T.A. Studying the *candida* resistance and sensitivity for some antifungals. Journal of Biotechnology Research Center.(2019); 13 (2):25-34.

انتاج إلانزيمات الداخلية والخارجية المحللة للكلوكان من Trichoderma viride باستخدام مياه الصرف الصحي لمعصرة الزيتون (OMW) في التخمير السائل

مرام الظرف[1] ، مهند مساعده[1*] ، بتول عمران ذيب[2]، ساره نجم عبدالله[2]

[1]قسم العلوم الحياتية و التكنولوجيا الحيوية، كلية العلوم، الجامعة الهاشمية، الزرقاء-الأردن
[2] جامعة سامراء، كلية العلوم التطبيقيه ، قسم التحليلات المرضيه

الخلاصة

خلفية عن الموضوع :يعد إنزيم السليوليز أحد أهم الإنزيمات الصناعية. تمثل تكلفتها عائقًا كبيرًا أمام المنتجات التجارية القيمة الأخرى. يعد تقليل تكلفة إنتاج السليوليز نهجًا مهمًا. **الهدف من الدراسة**: قام هذا العمل بدراسة إنتاج إنزيم السليوليز باستخدام مخلفات إنتاج زيت الزيتون (مياه الصرف الصحي لمعصرة الزيتون (OMW). **المواد وطرائق العمل** : تمت دراسة قدرة T.viride على استخدام OMW كركيزة لإنتاج السليوليز. وتم دراسة تحسين إنتاج السليوليز من أجل معرفة التركيز الأمثل لـ OMW، وسرعة التقليب، ومعدل التهوية، وإضافة السليلوز.**النتائج** :أظهرت النتائج أن زراعة 75% حجم/حجم OMW المغمورة في دورق الرج كانت الأكثر ملائمة لنمو T. viride وإنتاج إنزيم السليوليز (0.82 وحدة/مل). نشاط الانزيم المحلل للكلوكان الداخلي و 0.25 وحدة / مل من نشاط إكسوجلوكاناز). عندما تم استكمال هذه المزرعة بالسليلوز، تحسن نشاط و الانزيم المحلل للكلوكان الداخلي بشكل ملحوظ (10.24 وحدة / مل و 2.17 وحدة / مل على التوالي). سرعة التحريك البالغة 200 دورة في الدقيقة عززت الإنتاج ليصل إلى 9.1 وحدة / مل الانزيم المحلل للكلوكان الداخلي و 6.38 وحدة / مل إنزيم الانزيم المحلل للكلوكان تمت دراسة تأثير معدل التهوية على إنتاج الإنزيم تحت الزراعة الدفعية وكان أعلى نشاط للسيليولاز عند 2.0 فولت في الدقيقة، حيث بلغ نشاط الانزيم المحلل للكلوكان الداخلي والخارجي 55.96 وحدة / مل و32.62 وحدة/مل على التوالي. **الاستنتاجات:** أن OMW هي وسيلة مناسبة لإنتاج إنزيم السليلوز بعد تحسين العملية.

الكلمات المفتاحية: السليوليز، مياه الصرف الصحي لمعصرة الزيتون، *Trichoderma viride* ، التخمير بالمزارع المغمورة