EFFECT OF Q-SWITCHED ND: YAG LASER ON THE ACTIVITY AND SPECIFIC ACTIVITY OF *Saccharomyces cerevisiae* INVERTASE

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ABSTRACT

This study attempts to estimate the action of Q-switched Nd: YAG laser on activity and specific activity of invertase enzyme produced by Saccharomyces cerevisiae. The yeast cells were irradiated using two wavelengths (532 and 1064) nm of Q-Switched Nd: YAG laser with (1Hz) frequency; Different fluences (11.3, 22.6 and 33.9) mJ/cm^2 and different number of pulses (15, 30 and 60 pulses) were used. The irradiated cells were incubated in a liquid nutritive medium for 24 hour. After incubation, the cells were harvested and disrupted to extract the invertase enzyme and its activities were assessed. In comparison with the control, the irradiated cells with fundamental wavelength (1064 nm) showed an increase in the activity and the specific activity of invertase at energy density of 33.9 mJ/cm^2 at 15 and 60 pulses, while the irradiation with 532nm at all doses inhibited effect the activity and specific activity of invertase. It is concluded that a fluence at 33.9 mJ/cm^2 with 1064 nm wavelength stimulates the activity and specific activity of the enzyme to the maximum values.

INTRODUCTION

Interest in biostimulating effect of low-intensity laser light has increased in the eights of century. Low power laser therapy has been reported as being successful in some cases such as treating tropic ulcer (10, 5). Until now there has been little quantitative information about the

stimulative effect of light irradiation dose, wavelength, exposure time and intensity on different kinds of cells (9). Also one of practically not investigated problems in laser biostimulation is whether irradiation with monochromatic light of visible-to-near IR spectral region can cause long term effect, which appears in following cell generations (17). Therefore, it is of interest to study the interaction between laser photon and biological catalysts.

Photoactivation of enzymes is related to photocarcinogenesis, photomorphogenesis of plants, primary effects or side effects of phototherapy, deoxyribose nucleic acid (DNA) repair and many other aspects of biology and medicine. Model systems may contribute to the knowledge of protein chemistry and medicinal chemistry (8).

Recall, when cells are irradiated with monochromatic laser irradiation, Four primary action mechanisms are reviewed: changes in the redox properties of the respiratory chain components following photoexcitation of their electronic states, generation of singlet oxygen, localized transient heating of absorbing chromophores and increased superoxide anion production with subsequent increase in concentration of the product of its dismutation, H_2O_2. A cascade of reactions connected with alteration in cellular homeostasis parameters (pH, Ca^{2+}, cAMP, ATP etc) is considered as a photosignal transduction and amplification chain in a cell (secondary mechanisms) (31).

Part of M.Sc. thesis of the first author

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Enzymes by microorganisms and animal cells have aroused great interest in recent years. Yeast invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) is an attractive system in which to study biostimulation phenomenon (11).

The major portion of the enzyme activity is located outside the cell membrane (periplasmic space) and is called external invertase, but there is no agreement as to the location of the enzymes within the cell.

This enzyme is a glycoprotein containing 50% mannan and 3% glucoseamine, with a molecular weight of 270000 (11). The cytoplasmic enzyme is formed by a continuous addition (18) or removal (16) of mannose residues during enzymatic glycosylation or degradation.

Invertase catalysts α-1,4 glycosidic linkage between α-D-glucose and β-D-fructose molecules of sucrose by hydrolysis releasing monosaccharides such as glucose and fructose. It also hydrolyses β-fructans sach as raffinose in to simple sugars (4).

The enzyme activity of invertases has been characterized mainly in plants (1, 12), several filamentus fungi like Aspergillus sp, (2) and it was also expressed in different yeast strains such as Candida utilis (6), Picia anumala (23) and Rhodotorula glutinis (24). S. cerevisiae is particularly interesting microorganism, since it synthesizes two invertases: a glycosilated periplasmic protein and a cytosolic non glycosilated protein (22).

Invertase has a wide range of commercial applications including production of confectionary with liquid or soft centers and fermentation of cane molasses into ethanol. Invertase is also used in pharmaceutical industry, (as in digestive aid tablets), powder milk for infants, other infant foods and invert syrup production by microbial invertase (25).

In our Knowledge, there is very few researches about the action of laser irradiation on enzymes of microorganisms, especially invertase enzyme. Therefore, This study is aimed to investigate the possible action of Nd: YAG laser in pulse mode on the activity and specific activity of invertase enzyme of baker yeast by using different energy densities and different number of pulses, and determining the optimum laser parameters to increase the ability of the baker yeast for invertase production.

MATERIALS AND METHODS

Strain and culture conditions

An isolate of commercial baker's yeast (Saccharomyces cerevisiae) was used after grown on PDA (potato-dextrose agar) slant for 72 hour. The growth culture was transferred to 250- ml conical flask cotaining 50ml of liquid nutritive medium (2% glucose, 1%yeast extract and 0.02% ammonium sulphate, pH 5.5) and incubated in a shaker incubator (150 rpm) for 24h at 28°C. Cells were separated from the nutritive medium by the centrifugation 2000 rpm for 15 min by using (Labnet international) centrifuge then washed with twice sterile tap water. A suspension with an optical density of 0.06 (λ=540nm) in sodium-potassium phosphate buffer (pH=6.0) was prepared for irradiation (14). The experiments were done on December in 2010 at institute of laser for post graduate studies and department of biotechnology, college of science, university of Baghdad.

Irradiation procedure

Irradiation experiments were performed at room temperature (20-25□). Extraneous illumination (sunshine or artificial light, especially that from the fluorescence bulbs) was carefully avoided during preparation of the suspension and the irradiation (14).

Flasks with 1.5 cm diameter were used to irradiate yeast cells. AQ-switched Nd: YAG laser (Diamond Beauty) was used for as a source of irradiation. The laser was operated with 1064nm wavelength and 532nm using KTP crystal, 6nsec pulse duration and 1 Hz frequency. The prepared cells were irradiated using 11.3, 22.6 and 33.9mJ/cm^2 output energy density for 15, 30 and 60 pulse per each fluence. The diameter of the beam was expanded by means of beam expander to 1.5cm to provide a uniform illumination of whole area .Irradiation from the bottom was applied to avoid the meniscus effect which may occurs if irradiating was from the top. In this case, the meniscus of the liquid acts like a lens and the dose of the light reaching the cells (if reaching the lower layers at all) will be decreased in an uncontrolled way (9, 15, 26). The irradiated cells were incubated for 24 hours before further procedures were followed to measure the activity and specific activity of invertase enzyme.

Cell-free extract preparation

After irradiation, the irradiated yeast cells were grown overnight in 50 ml of liquid nutritive medium. The cells were harvested from the medium by centrifugation 2000 rpm for 15 min by using Labnet international centrifuge. The pellets were washed twice with distilled water and resuspended with 0.01M of sodium potassium phosphate buffer (pH 6.0) in order to obtain cell suspension with an optical density of 0.7 at 540 nm.

A portion of 2 ml of Lysozyme solution (prepared by dissolving 0.05g of lysozyme powder in 10ml of 1% glucose solution (13) was added to 8ml of the cell suspension with an optical density of 0.7(at λ=540nm) to reach a final lysozyme concentration of 1mg/ml. The suspension was incubated in shaker water bath (Jiotechnic) at 30°C for 4-6 hours. After incubation, the suspension was kept in the refrigerator at 4°C for overnight. Therefore, a smear from the pellets was prepared and stained by Gram stain to estimate the efficiency of lysozyme.

The suspension was centrifuged at 12000 rpm for 15 min by using cooling centrifugation (Beckman); the pellets were neglected while the supernatant was used for determination of enzyme activity and total protein concentration in the crude extract.

Determination of invertase activity

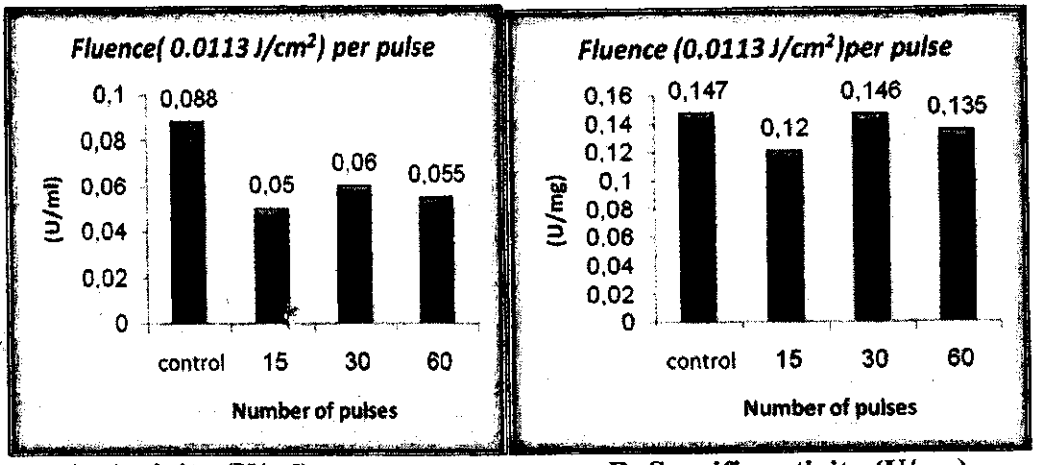
Invertase activity was determined according to Balasundaram and Pandit (3). Amount of invertase was estimated from the amount of glucose formed. Glucose can reduce 3,5-dinitrosalicylic acid (DNSA) to produce an orange color, which can be measured at 540 nm. A calibration curve with known concentrations of invertase standard was obtained to calculate the concentration of enzyme present in the sample. The unit of invertase activity is defined as the amount of enzyme which leads to release 1 μmol of reduced sugars (glucose) per one minute under experimental conditions.

Total soluble proteins were estimated by the method of Bradford (7) using Bovine Serum Albumin (BSA) as a standard. The specific activity was determined from the following equation:

$$\text{Specific activity (Unit/mg of protein)} = \frac{\text{activity of enzyme(Unit/ml)}}{\text{concentration of protein(mg/ml)}} \quad (3)$$

RESULTS AND DISCUSSION

Results of the effect of Nd: YAG laser on activity and specific activity of invertase are illustrated in figures (1, 2 and 3). Figure (1) A and B a decrease in the invertase activity and specific activity was observed by using 20mJ output energy (with fluence = 0.0339 J/ cm^2), at the three different number of pulses 15, 30 and 60 pulses.

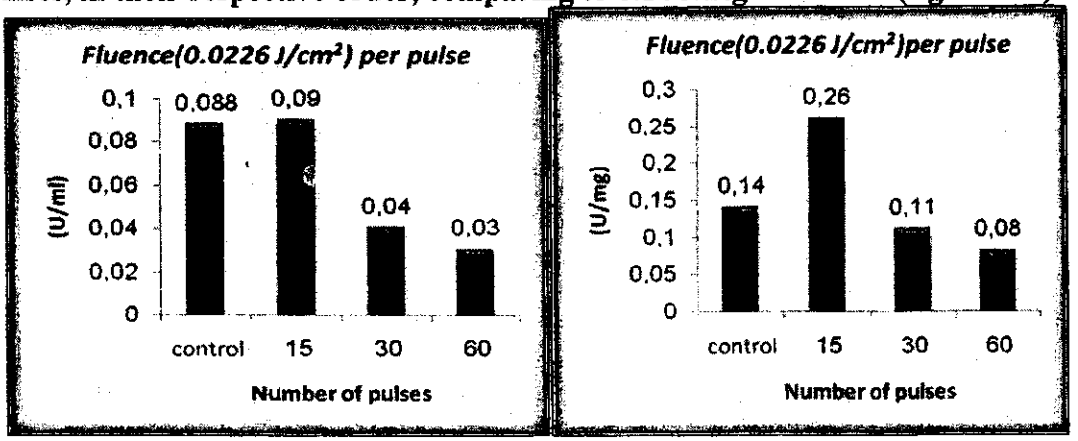


A: Activity (U/ml). B: Specific activity (U/mg).

Figure 1: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0113 J/cm^2 fluence at 1064 nm wavelength.

The values of invertase activity reached to 0.05, 0.06 and 0.055 U/ml at 15, 30 and 60pulses and 0.0113J/cm^2, respectively, comparing to 0.088 U/ml in control. The values of invertase specific activity reached to 0.12, 0.146 and 0.135 U/mg at 15, 30 and 60pulses and 0.0113J/cm^2, respectively, comparing to 0.147 U/ml in control.

A little activation in invertase activity and specific activity was observed using 0.0226J/cm^2 at 15 pulses with a noticeable inhibition at 30 and 60pulse. The values of invertase activity were 0.09, 0.04 and 0.03 U/ml at 15, 30 and 60 pulse, in their respective order, comparing to 0.088 in control (figure 2/A). While the values of invertase specific activity were 0.26, 0.11 and 0.08 at 15, 30 and 60 pulses, in their respective order, comparing to 0.14U/mg in control (figure 2/B).

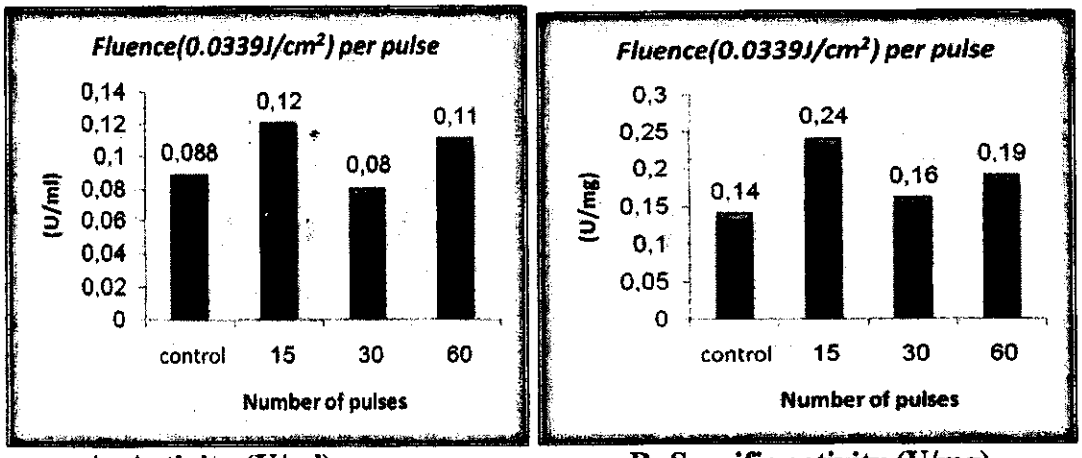


A: Activity (U/ml). B: Specific activity (U/mg).

Figure 2: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0226 J/cm^{2}fluence at 1064 nm wavelength.

Figure (3) shows the effect of laser using 0.0339J/cm^2 energy density. Invertase activities were increased at 15 and 30 pulses reaching to 0.12 and 0.11 U/ml, in their respective order, while the value of invertase activity was 0.08 at 30 pulses comparing to 0.088 U/ml in the control.

Figure (3) B illustrate the effect of 0.0339J/cm^2 energy density on the values of specific activity of invertase. A noticeable increase in the specific activity value (0.24 U/mg) at 15 pulses, while at 30 and 60 pulses the values reached to 0.16 and 0.19 U/mg in their respective order comparing to the control (0.14 U/mg).

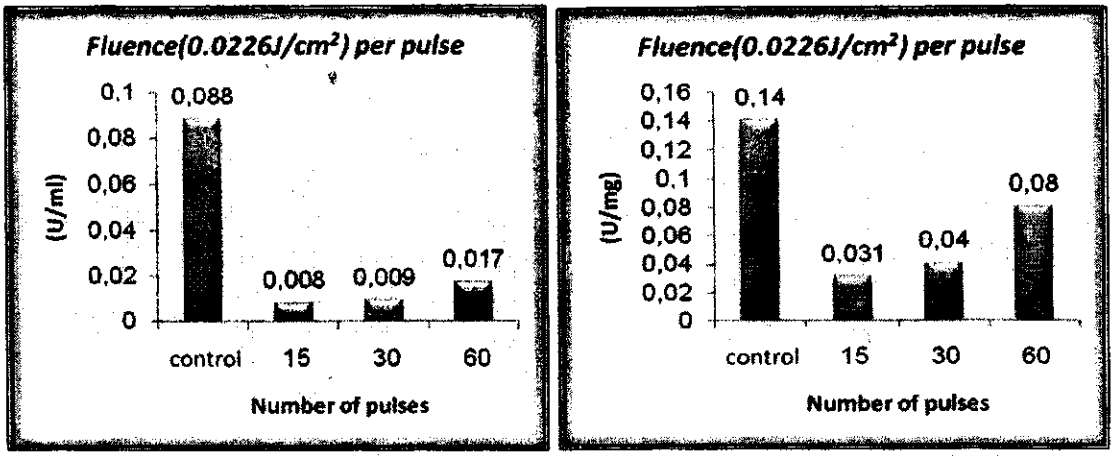


A: Activity (U/ml). B: Specific activity (U/mg).

Figure 3: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0339 J/cm^{2}fluence at 1064 nm wavelength.

The obvious results show that maximum values of invertase activity and specific activity reached to 0.12 U/ml and 0.24 U/mg, respectively, at the dose of 15pulses and 0.0339J/cm^2 fluence. Also the lower value of invertase activity and specific activity reached to 0.03U/ml and 0.08 U/mg, respectively, at the dose of 60 pulses and 0.0226 J/cm^{2}fluence.

Results of the effect of Nd: YAG laser on the activity and specific activity of invertase are illustrated in figures (4, 5 and 6). In figures (4) A and B a decrease in invertase activity and specific activity was observed by using 20mJ output energy (with fluence = 0.0339 J/ cm^2), at the three pulses 15, 30 and 60.

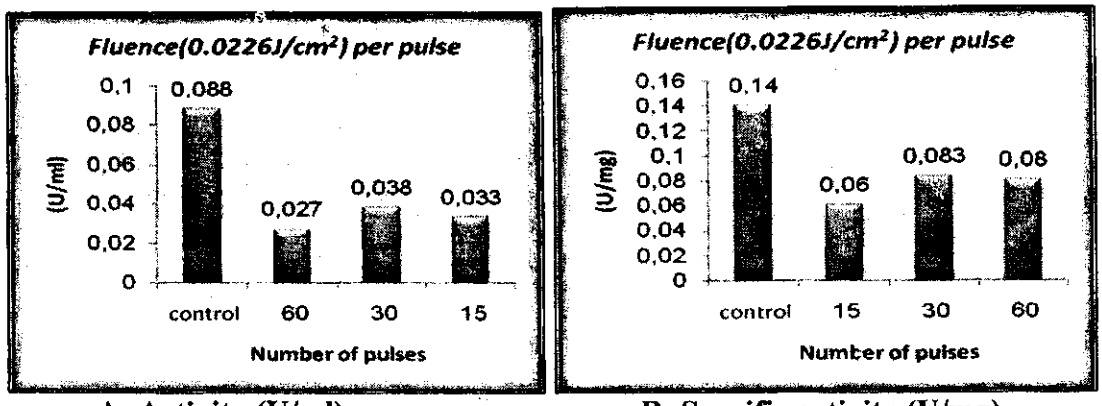


A: Activity (U/ml). B: Specific activity (U/mg).

Figure 4: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0113J/cm^2 fluence at 532 nm wavelength.

The values of invertase activity reached to 0.008, 0.009 and 0.017 U/ml at 15, 30 and 60pulses and fluence of 0.0113J/cm^2, respectively, comparing to 0.088 U/ml for the control. The values of invertase specific activity reached to 0.031, 0.04 and 0.08 U/mg at 15, 30 and 60pulses and fluence of 0.0113J/cm^2 respectively comparing to 0.147 U/ml in control.

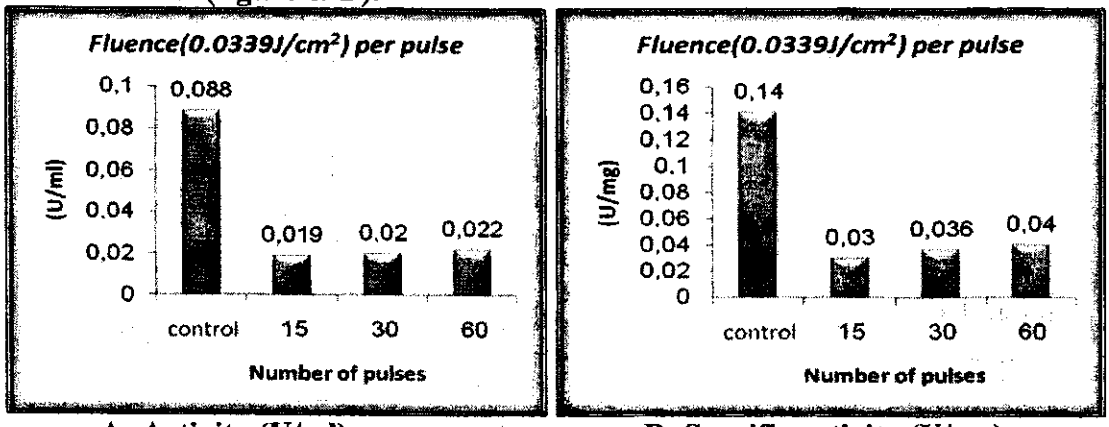
The values of invertase activity were 0.027, 0.038 and 0.033 U/ml at 15, 30 and 60 pulse, respectively, comparing to 0.088 in the control (figure 5/A). While the values were 0.06, 0.083 and 0.08 at 15, 30 and 60 pulse, respectively, comparing to 0.14U/mg in the control (figure 5/B).



A: Activity (U/ml). B: Specific activity (U/mg).

Figure 5: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0226 J/cm^{2}fluence at 532 nm wavelength.

When 0.0339 J/cm^2 energy density was used, invertase activity and specific activity values were inhibited at all three pulses. The values of invertase activity were 0.019 at 15 pulses and 0.02 at 30; 0.022 U/ml at 60 pulses comparing to 0.088 for the control (figure 6/A). Invertase specific activity values were 0.03, 0.036 and 0.04 U/mg at 15, 30 and 60 pulses, respectively, comparing to 0.14U/mg for the control (figure 6/ B).



A: Activity (U/ml). B: Specific activity (U/mg).

Figure 6: Activity and specific activity of invertase enzyme of S. cerevisiae after exposure to 0.0339 J/cm^{2}fluence at 532 nm wavelength.

From the above results, effect of Nd: YAG laser irradiation on protein concentration has reflected on the results of enzyme activity and specific activity (production). Enzymes are considered to be secondary metabolite products and the production occur in general at the end of logarithmic phase and the beginning of stationary phase (G_0) throughout the cell cycle; i.e. after nutrients starvation of the medium where the cell populations growth. Walker (30)

mentioned that the cell cycle arrest, at checkpoints in S. cerevisiae due to nutrient starvation may be thought of as a survival mechanism under adverse conditions, and this induces a response that includes cessation of growth, decreasing protein synthesis and an increase in the resistance to stress (30). In term of invertase activity and specific activity, Myers et al. (19) hypothesized that when glucose concentration became less than 1%, the synthesis of invertase enzyme increased at the level of transcription and translation. Concentration of glucose in the biomass production broth, was 2% ; this concentration will be decreased gradually during progressing in cell growth and because of the effect of Nd: YAG laser irradiation at 1046nm in increasing cell populations (total cell number after 24 hour) comparing to the population of irradiated at 532nm and preirradiated one (control), the increasing in cell number resulting in glucose starvation and then increasing in the activity and specific activity of invertase enzyme as in figure (3 A and B) that showed the maximum value of invertase activity was reached to 0.12U/ml and specific activity 0.24U/mg of protein at 0.0339J/cm^2 fluence and 1064nm. Figure (7) represents the comparison among the growth curves at both wavelengths (1064nm and 532nm). It had noticed that the volume of cell population, after 24hour of incubation period after irradiation with 1064nm and 0.0339J/cm^2, is more than the cell population volumes at other doses and at the same period of incubation, and this prove our hypothesis.

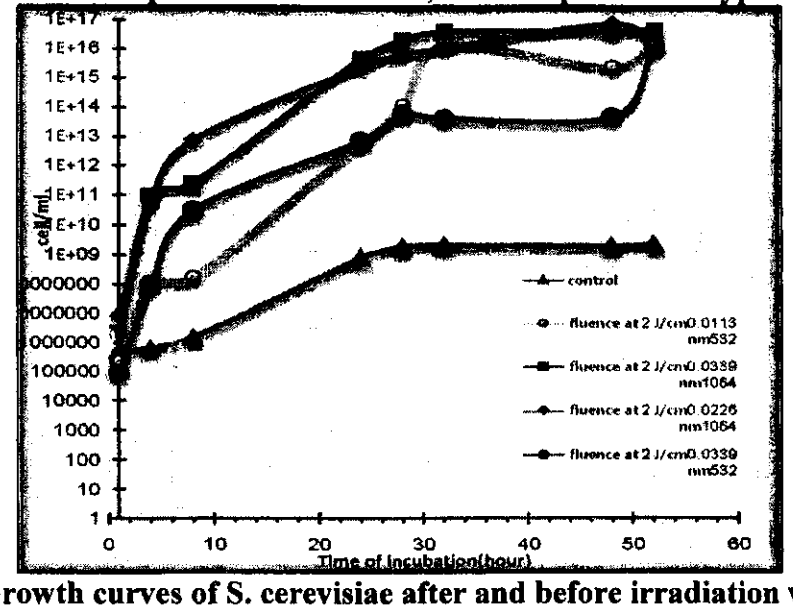


Figure 7: Growth curves of S. cerevisiae after and before irradiation with λ=532 nm and 1064 nm.

A study by Tsivunchik (27) about the effect of Ar-laser found effect on the level of α-amylase in dry grains was decreased as well as in germinated one, She reported also a contrary result when He-Ne and YAG-laser were used. Such effect is difficult to be explained with the theory of light absorbancy because the maximum of absorption of α-amylase lies quit far away from wavelengths of the lasers (20, 30). Tsivunchik (27) presented a probability to explain such results were the factor of enzyme activation can be conformational locations in space of molecule structure (namely alpha-amylase) under laser light; and mechanism of this phenomenon is realized with the activation of genetic control systems of metabolic processes in plant cells (29, 28). This opinion may be true to explain changing of α-amylase level because the experiment was subjected to dry grain. While in case of experiment of this study, the effect of Nd: YAG laser was

subjected to yeast cells that were irradiated and incubated for 24h in liquid nutritive medium. In case of increasing in invertase activity and specific activity was a result from the effect of laser at 1064nm wavelength that caused decreasing in the generation time leading to produce a high population volume of cells which compete to decrease the concentration of glucose to the threshold concentration that trigger to invertase synthesis.

Inhibition of invertase activity and specific activity after irradiation with 532nm wavelength at all fluencies may be related to two allosteric reasons; First, irradiation at visible wavelengths causes immediate acidification in cytoplasm (rise of intracellular H^+ concentration), asa result of activation of the respiratory chain, which controls allosterically the activity of Na^+/H^+ antiporter situated in the cytoplasmic membrane acting to rise again the intracellular pH (21, 32). This alkalization causes inhibition in activity of invertase (the optimum pH for invertase activity 4.5). These events occur as a result of activation action of 532nm wavelength to the respiratory chain. Secondly, the releasing of O_2^-, after irradiation with 532nm, may interfere with the mechanisms of synthesis of this enzyme at transcriptional and translational level; so the soluble form of invertase will decrease in cytoplasm. Also, the partial dysfunction in ER-MT associations and cytoskeleton as a result of laser irradiation effect may block the packing of mature enzyme (large molecule) to the plasma membrane where the enzyme located (17).

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تأثير ليزر الـ Q-switched نيديميوم–ياك في الفعالية و الفعالية النوعية لانزيم الانفرتيز في خميرة الـ *Saccharomyces cerevisiae*

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الملخص

أجريت الدراسة للتحري عن تأثير ليزر الـ Q-switch نيديميوم–ياك في الفعالية والفعالية النوعية لانزيم Invertase المُنتج من خميرة Saccharomyces cerevisiae. فقد تم لهذا الغرض تشعيع خلايا الخميرة بطولين موجيين (1064 و532) نانوميتروباستخدام جهاز الـ Q-switch نيديميوم–ياك، تردد 1هيرتز،كثافة طاقات مختلفة 11.3، 22.6 و33.9 mJ/cm^2 وعدد مختلف من النبضات 15، 30 و 60. بعد ان حُضنَتْ الخلايا المشعة في الوسط الغذائي السائل مدة 24 ساعة. حُصدتْ الخلايا و حُطّمت لاستخلاص انزيم الانفرتيز وقياس فعاليته. بالمقارنة مع معادلة السيطرة، ان الخلايا المشعة بالطول الموجي الاصلي لليزر (1064) نانوميتر، فقد ظهرت زيادة في الفعالية والفعالية النوعية لانزيم الانفرتيز(Invertase) عند كثافة الطاقة 33.9 mJ/cm^2 والنبضات 15 و60، فيما ادى التشعيع بالطول الموجي 532 نانوميتر وبالجرع جميعها الى حصول تاثيراً تثبيطياً في الفعالية والفعالية النوعية للانزيم، ويمكن الاستنتاج بان كثافة الطاقة (33.9mJ/cm^2) وبالطول الموجي 1064 نانوميتر قد حفزت الفعالية والفعالية النوعية للانزيم لتصل الى اعلى القيم.

جزء من رسالة ماجستير للباحث الاول

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