ACTION OF ND: YAG LASER ON GROWTH OF *Saccharomyces cerevisiae*

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ABSTRACT

Irradiation with Q-switch Nd: YAG laser caused growth stimulation to the cells of *Saccharomyces cerevisiae*. The laser parameters that were used in cells irradiation include two wavelengths (1064 and 532nm) at a fixed energy density (11.3 mJ/cm^2) with different number of pulses (15, 30 and 60). After irradiated cells were incubated in liquid nutritive broth for 24h, biomass, total cell number and viability were determined. It was found that irradiation with 532nm at 30 pulses produced noticeable increasing in values of biomass and viability of the irradiated cells compared to their values in the case of irradiation with 1064nm wavelength that, it also stimulated the increasing in the value of total cell number in to a maximum value.

Introduction

The concept of "biostimulation" occurring at low energy density of laser radiation is already generally accepted, despite some controversy concerning especially the mechanisms by which the biological response appears (22).

The study of interaction between electromagnetic waves and the living systems involves many physical and biological aspects. On the one hand, it was demonstrated that there is a "threshold stimulus", in the sense that the monochromatic signal carrier becomes stimulus only if its energy exceeds a threshold value (22). On the other hand, all observed biological effects of this signal action originate from changes in cellular membrane potentials, resulting from changes in membrane permeability and microviscosity. These changes are caused by the action of the excitation that provokes charge separations, which generate concentration and potential gradients (10).

The irradiation effect, growth stimulation, was evaluated by counting the total cell number, biomass and viability (11). These were the basic measurements performed to characterize the quantitative laws of light action on the growth of microorganisms (dependences on wavelength, energy density and number of pulses) upon metabolism of eukaryotic microorganisms.

The stimulatory effects of low-fluence laser irradiation at the cellular and molecular levels have been shown by many studies. Laser light affects the mitochondrial respiratory chain by changing the electric potential of cell membranes and, consequently,their selective permeability for sodium, potassium and calcium ions, or by increasing the activity of certain enzymes such as Cytochrome oxidase and adenosine triphosphatase (11). It also increases DNA synthesis (14), collagen and pro-collagen production (1,3), and may increase the cell proliferation (23). In contrast to these stimulatory effects, some investigators have found damaging or even destructive action of soft laser radiation. Ocana-Quero *et al.* for instance, described a degenerative effect of He-Ne laser irradiation on bovine oocytes (17).

The aim of this study was to assess the effect of the fluence (energy

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density) of Q-Switch Nd: YAG laser using two wavelengths 1064 and 532nm, 19.5cm irradiation distance, with different number of pulses 15, 30 and 60 at the cellular level. Since cell proliferation is one of the basic manifestations of any living organism, insight factors affecting cell proliferation in response to laser irradiation may be important in terms of production improvement of single cell protein. *Saccharomyces cerevisiae* was selected because of its importance in baking, wine production and other food industrial application.

MATERIALS AND METHODS

Strain and culture conditions

One isolate of commercial baker's yeast (*Saccharomyces cerevisiae*) was used. It was grown on the PDA (potato-dextrose agar) slant and incubated at 28°C for 72 hours. After incubation the cells were transferred to 250- ml conical flask with 50 ml of liquid nutritive medium (2% glucose, 1%yeast extract and 0.02% ammonium sulphate, pH 5.5) and incubated in a shaker incubator (Pilot shaker) at 150rpm for 24h at 28°C. Cells were separated from the nutritive medium by centrifugation at 2000 rpm for 15 min using (Labnet international) centrifuge, then washed twice with sterile tap water. The suspension with optical density 0.06 (λ=540nm) in sodium-potassium phosphate buffer (pH=6.0) was prepared for irradiation (8). 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°C). Extraneous illumination (sunshine or artificial light, especially that from the fluorescence bulbs) was carefully avoided during preparation of the suspension and the irradiation (11).

Flasks with 1.5 cm diameter were used to irradiate yeast cells. AQ-switched Nd: YAG laser (Diamond Beauty) was used a source of irradiation. The laser was operated with 1064nm wavelength and 532 nm using KTP crystal, 6nsec pulse duration and 1 Hz frequency. The prepared cells were irradiated using 11.3mJ/cm^2 output energy density and for 15, 30 and 60 pulses, the distance between laser source and the sample was 19.5cm. Diameter of the beam was expanded by means of beam expander to 1.5 cm to provide an uniform illumination of whole area. Irradiation from the bottom was applied to avoid the (meniscus effect) which occurs when irradiating is from 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 a controlled way (8, 9, 12). The irradiated cells were incubated for 24 hours.

Biomass determination

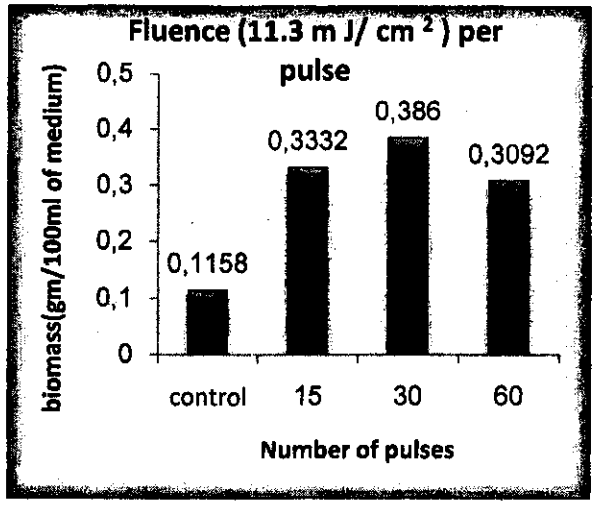
The conical flask which contained 50ml of nutritive liquid medium was inoculated with 1ml of the irradiated cells suspension at an optical density of 0.06 (540nm), then the culture was incubated in a shaker incubator with150rpm, 28°C for 24 hours. After incubation, the growth of the cells was harvested by centrifugation at 5000 rpm for 10 minutes using Beckman cooling centrifuge. The pellets were put in filter paper with a known weight and dried in the oven at 50°C for 24 hours. Weight of the biomass was taken by deducing the weight of the dried biomass and the filter paper from the known weight of the paper alone (2).

Determination of total number and viability of cells

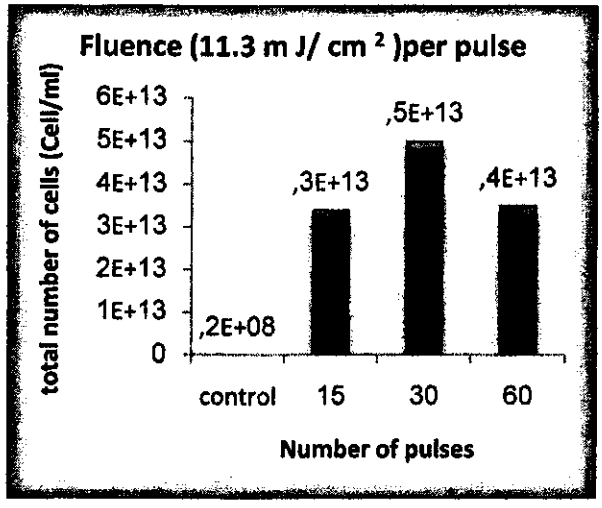
Total cell number and viability were determined according to Logothetis *et al.* (15). One ml 24 growth suspensions was diluted in 9ml with the deionized water and then 1ml of this solution dissolved into 1ml of 10% methelen blue solution. After 10 min, 0.01 ml was put on a haemocytometer. Numbers of the total cells and living yeast were determined under the lens of bright field microscope (Olympus). The living cells appeared in clear while the dead were blue to black in color (15).

Results and discussion

Biomass was proportional with total number of cells; that it means if total cell number increased biomass increased also. Figure (1 A and B) represent the effect of Nd: YAG laser irradiation at 1064nm wavelength on the biomass and total number of *S. cerevisiae* using 20mJ output energy (with fluence= 11.3mJ/cm^2), at three different number of pulses 15, 30 and 60 pulses.



A: Biomass (gm/100ml of medium)



B: Total number of cells (cell/ml)

Figure 1:Biomass and total number of *S. cerevisiae* after exposure to 11.3J/cm^2 at 1064nm.

Values of biomass and total number of cells were increased by using fluence 11.3mJ/cm^2 for different pulses. The values of biomass reached 0.3332, 0.386 and 0.3092 gm/100ml medium at 15, 30 and 60 pulses comparing to 0.1158 gm/100ml medium for the control. A noticeable increase in total cell number was observed also at 11.3mJ/cm^2 fluence when they reached 3×10^{13}, 5×10^{13} and 4×10^{13} cell/ml by using 15, 30 and 60 pulses, respectively, comparing to 2×10^{8} cell/ml for the control.

Figure (2) represents the effect of Nd: YAG laser irradiation at 1064nm wavelength on the viability of *S. cerevisiae* using 20mJ output energy (with fluence = 11.3mJ/ cm^2), at three different number of pulses 15, 30 and 60 pulses.

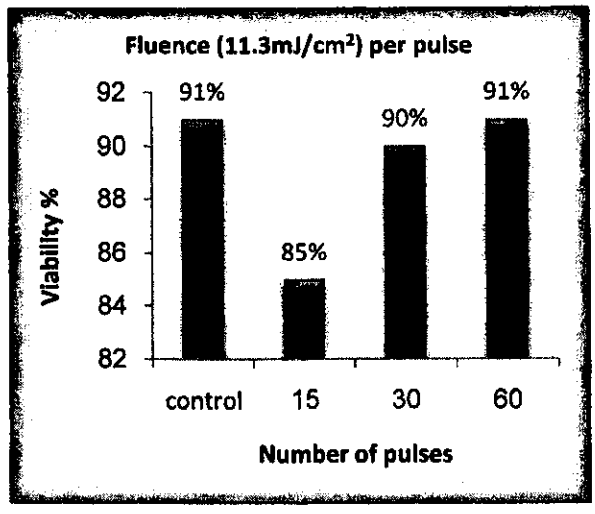
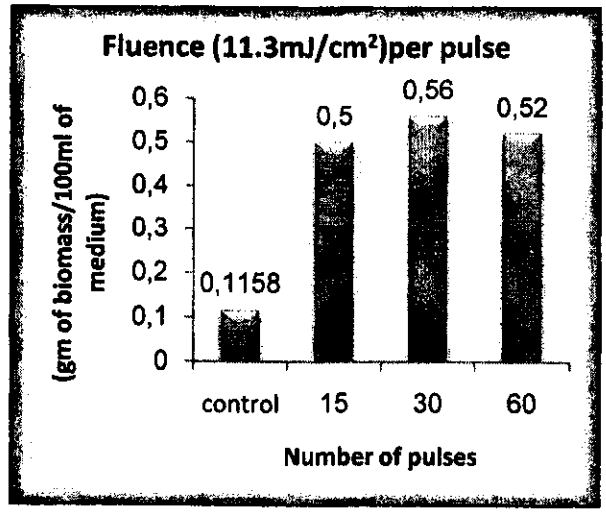


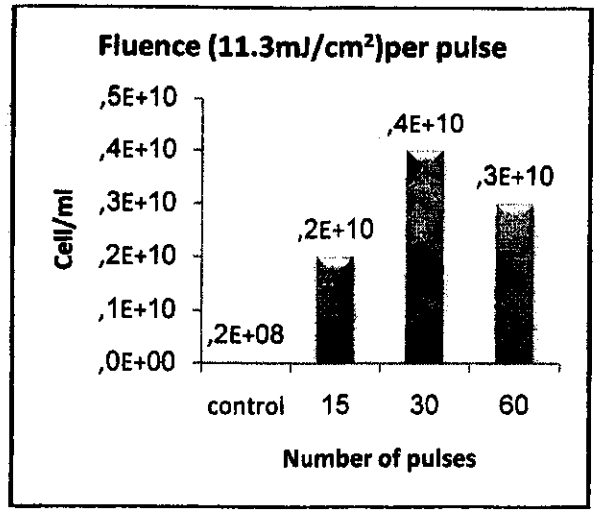
Figure 2: Viability of *S.cerevisiae* after exposure to 11.3mJ/cm^2 fluence at 1064nm wavelength.

Viability percentage decreased after irradiation at 15pulse but increased with increasing the number of pulses until reached the control level at 60pulses. The values of the viability reached 85%, 90% and 91% using 15, 30 and 60 pulses, respectively, order comparing to 91% for the control.

Figures (3) A and B represent the effect of Nd: YAG laser irradiation at 532nm wavelength on biomass and total number of *S. cerevisiae* using 20mJ output energy (with fluence = 11.3J/cm^2), at three different number of pulses 15, 30 and 60 pulses.



A: Biomass (gm/100ml of medium)



B: Total number of cells (cell/ml)

Figure 3: Biomass and total number of *S. cerevisiae* after exposure to 11.3J/cm^2 at 532nm.

Increases in the values of biomass and total number of cells were when a using fluence of 11.3 mJ/cm^2 for different pulses, was used. The values of biomass reached 0.5, 0.56 and 0.52 gm/100ml medium at 15, 30 and 60 pulses comparing to 0.1158 gm/100ml medium for the control. A noticeable increase the total cell number was observed also at 11.3J/cm^2 fluence when values reached 2×10^{10}, 4×10^{10} and 3×10^{10} cell/ml using 15, 30 and 60 pulses, respectively, comparing to 2×10^{8} cell/ml for the control.

Figure (4) represents the effect of Nd: YAG laser irradiation at 532nm on the viability of *S. cerevisiae* using 20mJ output energy (with fluence = 11.3mJ/ cm^2), at three different number of pulses 15, 30 and 60 pulses. The viability percentage decreased after irradiation at 15pulse but increased with increasing the number of pulses until reached the control level at 60pulses. Values of the viability reached 81%, 97.5% and 92% by using 15, 30 and 60 pulses, respectively, comparing to 91% for the control.

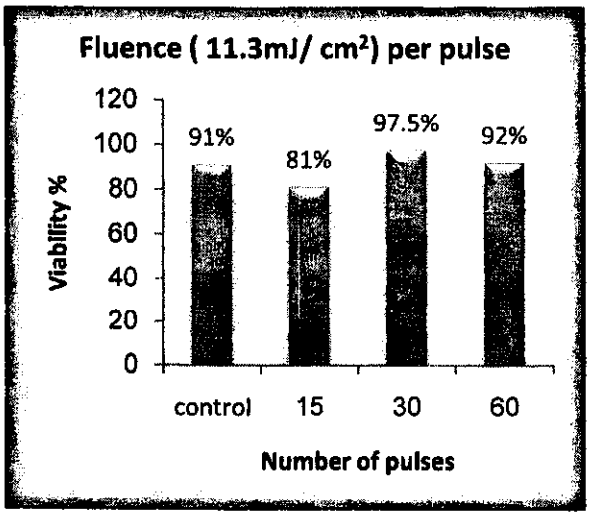


Figure 4: Viability of *S. cerevisiae* after exposure to 11.3mJ/cm^2 fluence at 532nm wavelength.

Generally, laser induced changes in the biological materials required for the absorption of the laser light energy by a specific molecule. The energy of laser irradiation may be used as stimulus for the reactions controlling cell divisions. Primary mechanism of light action after absorption of light quanta and the promotion of electrically excited states have not been established. Possible explanations include; stimulation of ascorbic acid uptake by the cells, stimulation of photoreceptors in the mitochondrial respiratory chain, changes in cellular ATP or AMP levels, and cell membrane stabilization (4).

In the mitochondria, five possible mechanisms have been discussed in previous studies by El-Batanouny *et al.* (9), Karu (12) including singlet-oxygen hypothesis, redox properties alteration hypothesis, nitric oxide hypothesis, superoxide anion hypothesis and transient local heating hypothesis. However, they were postulated for red and infra-red light operating lasers. Green laser light was not included, but some of these hypotheses may be applied to explain or discuss the results of this study.

The experimental data show low fluence 11.3 mJ/cm^2 and short periods 15, 30 and 60 pulses with pulse duration in nanoseconds of irradiation caused effect lasting for a long time (after 24 hour incubation period from irradiation). It is suggested to be a mechanism called (photosignal transduction and amplification chain). This discovered signaling (signal transduction and amplification chain) is a signaling pathway opposite to a common and well-defined pathway transforming information from the nucleus and cytoplasm to the mitochondria. The retrograde signaling sends information back to the nucleus about changes in the functional state of the mitochondria (6,21). The most investigated mitochondrial retrograde signaling pathways so far are those in the budding yeasts *Saccharomyces cerevisiae* (13) and plant cells (19).

The increase in biomass and total cell number represents main feature of stimulatory effect of laser. Maximum value of biomass after irradiation with 1064nm was reached 0.386gm/100ml medium (figure 1/A), and the total cell number was 5×10^{13} cell/ml (figure 1/B), while the maximum value of biomass after 532nm irradiation was 0.56gm/100ml medium (figure 3/A). The total cell number was 4×10^{10} cell/ml as in (figure 3/B). Maximum value of total cell number was detected at 30 pulses for 1064nm wavelength while the biomass

remains lower than that observed in 532nm irradiation at same dose. This could be due to the reduction in cell size after irradiation with 1064nm that leads to increasing in cell division rate. Such finding come in agreement with morphological studies performed by Bertoloni *et al.* (5) who showed that irradiation with He-Ne laser at 632nm increased packing of the cytoplasmic matrix and number of ribosomes, and decreased until almost complete disappearance of apical vacuoles. Electrophoretic changes did not involve proteins from the outer membrane but protein bands formed the cytoplasmic membrane were significantly intensified (5).

Physiologically, Nedelina *et al.* (16) mentioned that irradiation of cells with a wide-band visible light of $\lambda \geq 400$ nm, enhanced the activity of ATP synthetase, and caused rising in the intracellular H^+ concentration that controls the activity of the Na^+/H^+ antiporter actuated in the cellular membrane (18,24). This enzyme plays a key part in the alkalization of the cytoplasm. A short term increase in the intracellular pH is one of the necessary components involved in the transmission of mitogenic signals in the cell (16). The increase in the intracellular hydrogen ion and ATP concentrations in eukaryotic cells caused activation of other membrane ion carriers as well, such as Na^+, K^+-ATPase. Activation of this enzyme leads to an increase in $[K^+]_i$ (intracellular concentration of K^+) and a decrease in $[Na^+]_i$ (intracellular concentration of Na^+) and E_m (membrane potential). The variations in these parameters are necessary components in the control of the proliferation activity of the cells (7,20) (Figure 5).

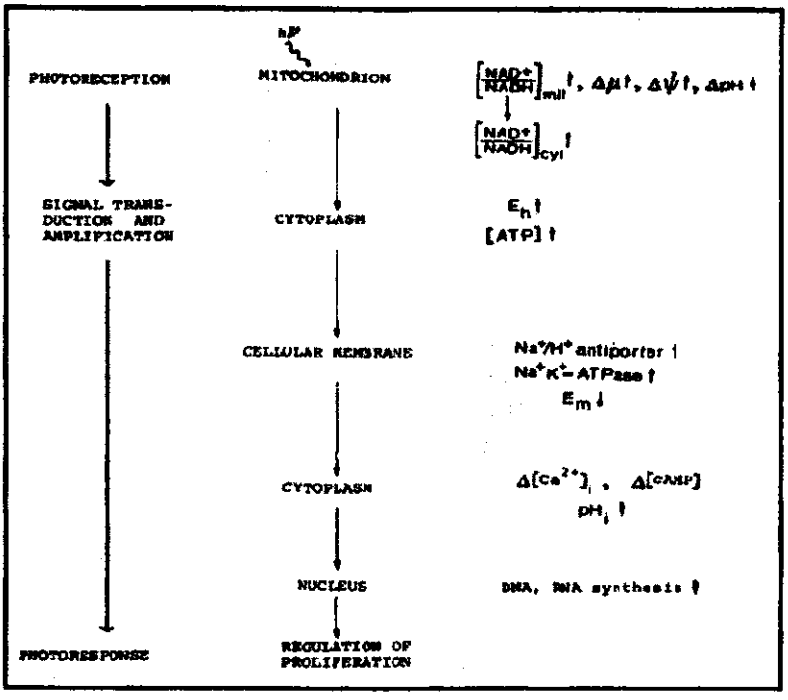


Figure 5: Possible photosignal transduction chains for proliferation stimulation by monochromatic bands of light (20).

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تأثير ليزر النيديميوم- ياك في نمو خميرة *Saccharomyces cerevisiae*

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الملخص

ادى التشعيع باستخدام الQ-switch نيديميوم-ياك الى تحفيز نمو خلايا الـ *Saccharomyces cerevisiae*. تضمنت المعايير الليزرية المستخدمة بالتشعيع استخدام طولين موجيين 532 و1064 نانوميتر، بثبوت كثافة الطاقة وبعدد مختلف من النبضات 15, 30 و 60. حُضنَتْ الخلايا المشعة في الوسط الغذائي السائل مدة 24 ساعة، وبعد الحضانة تم تقدير الوزن الجاف، العدد الكلي للخلايا والحيوية. استُنتج بان التشعيع باستخدام 532 نانوميتر عند عدد النبضات 30 يسبب زيادة ملحوظة في الوزن الجاف والحيوية للخلايا المشعة بالمقارنة مع قيمها الوزن الجاف والحيوية عند التشعيع ، 1064 نانوميتر والذي هو في الوقت نفسه ادى الى زيادة قيم العدد الكلي للخلايا لاعلى قيمة ممكنة.

جزء من رسالة ماجستير للباحث الاول.

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