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# Effects of Ferrous Ions on the *Rhodobacter sphaeroides* Growth Properties and Redox Activity

(Submitted by corresponding member of NAS RA A. H. Trchounian 9/VII 2012)

**Keywords:** *Rhodobacter sphaeroides, bacterial growth, redox potential,* $Fe^{2+}$, $Fe^{3+}$

It is well known that growth and hydrogen ($H_2$) production rate by photosynthetic bacteria vary depending on the microbial culture type, character of substrates utilized and the other factors, namely anaerobic conditions and temperature [1, 2], pH [2], light [1, 2], carbon and nitrogen sources [1-3], and metal ions [2, 4].

Various metals ions such as iron (Fe), magnesium (Mg), molybdenum (Mo), calcium (Ca) are necessary for growth and metabolism of most microorganisms. Firstly, Fe, Mg, Ca, and Mo ions were used as *Rhodobacter sphaeroides* and the other purple bacteria growth medium components [2, 4, 5]. Now, Fe, Mo and nickel (Ni) ions are known as the components of several enzymes involved in $H_2$ production, such as hydrogenase and nitrogenase [5-10]. Mg ions are one of the most abundant elements within microorganisms. Various photosynthetic pigments such as bacteriochlorophyll (Bchl) contain $Mg^{2+}$ [5]. In addition, these are also various enzymes activators [5].

The effects of different metal ions on $H_2$ production during dark-fermentation have been studied by different authors [2, 11-13], the effects of these ions on photo-fermentative $H_2$ production are less examined [5, 9, 10]. In all cases, mechanisms of these effects are not clear, one of the possible mechanisms can be connected with the influence of the metal ions on photosynthetic system formation processes.

The purpose of the present work is to study growth specificity of *R. sphaeroides* MDC6521 in the presence of different concentrations of $Fe^{2+}$ and $Fe^{3+}$. Growth properties such as specific growth rate as well as absorption spectra, medium pH and redox potential (ORP) during $H_2$ production were investigated.

**Materials and methods.** *Bacteria and preparation.* The purple non-sulfur bacterium *R. sphaeroides* MDC6521 (Microbial Depository Center, Armenia, WDCM803) used in this study was isolated from Arzni mineral springs in Armenia [4, 14]. *R. sphaeroides* was grown in anaerobic conditions in Ormerod medium at 30± 2 $^0$C under illumination of approximately 1500 lux [3, 14].Succinate (3.54 g $L^{-1}$) and yeast extract (2 g $L^{-1}$) was used as sole carbon and nitrogen sources [14]. It should be noted that the springs are related to the weak-acid (pH 6.5-7.5) carbonaceous waters containing Mg, Ca, and Fe among a wide range of chemical elements and various microelements. The concentrations of $FeSO_4 \cdot 7H_2O$ and $FeCl_3$ in the growth medium ranged from 40 to 120 μM.

*pH and ORP measurement.* The medium initial pH was adjusted to 7.0 by means of 0.1 M HCl or NaOH by a pH-potentiometer (HANNA Instruments, Portugal) with selective pH electrode (HJ1131B) [3, 4, 14]. ORP of the medium was measured by platinum electrode (EPB-1, Electrometer Equipment State Enterprise, Gomel, Belarus; PT42BNC, Hanna Instruments, Portugal) as described elsewhere [3, 14].

*Determination of bacterial growth.* Bacterial growth was monitored by a Spectro UV-Vis Auto spectrophotometer (Labomed, USA) by changes in absorbance of cell suspension at 660 nm [14]. Specific growth rate ($h^{-1}$) was determined as described [4, 14]. The absorption spectra of *R. sphaeroides* suspension were recorded at the wavelength region of 400 to 1000 nm on a programmable Spectro UV-Vis Auto spectrophotometer (Labomed, USA) [4, 14]. For obtaining comparable data the original spectra were subtracted of the scattering and normalized to the same cell concentration.

*Reagents used; data processing***.** Yeast extract (Carl Roths GmbH, Germany), succinic acid (Unichem, China), and the other reagents used were of analytical grade; used acid was neutralized by NaOH. The average data are represented from three independent experiments; the standard errors were calculated as described [3, 14] and did not exceed 5% if not indicated.

**Results and discussion.** The growth properties were monitored during phototrophic growth of *R. sphaeroides* in $Fe^{2+}$ and $Fe^{3+}$ containing medium. The concentration of $Fe^{2+}$ and $Fe^{3+}$ added in growth medium ranged from 0 to 120 μM. All concentrations of used ions supported the *R. sphaeroides* phototrophic growth. When $Fe^{2+}$ and $Fe^{3+}$ ions added in the culture medium cells final yield was higher than the control (no additions) (Fig. 1). This indicated that used ions could promote the growth of purple bacteria. In all cases, the optical density (OD) of the cultures reached a maximum value at 72 h, after which OD decreased.

In the presence of $Fe^{2+}$ (40–120 μM) specific growth rate was larger than that of control (Fig. 2). The highest growth rate was obtained for bacterial cells with 80 μM $Fe^{2+}$ (see Fig. 2), indicating that $Fe^{2+}$ could promote the growth of these bacteria. The value of specific growth rate with $Fe^{3+}$ was almost the same as the control in all concentrations (see Fig. 2).

Phototrophic growth of *R. sphaeroides* is known to result by synthesis of the photosynthetic apparatus, which consists of two light-harvesting (LH) complexes (B800-850 and B875) surrounding a photochemical reaction center [1,4].

The LH complexes consist of polypeptides and pigments combination, such as carotenoids and Bchl *a* [1]. The absorption spectra of *R. sphaeroides* intact cells grown with $Fe^{2+}$ and $Fe^{3+}$ were investigated in order to reveal light harvesting pigments synthesis. As shown in Fig. 3, in all absorption spectra were observed the various peaks in 400-1000 nm wavelength region, which are typical for purple non-sulfur bacteria and are indicators for their LH components [4]. These peaks could be assigned to carotenoids (450, 478 and 510 nm) and Bchl a (590, 800, 850 and 875 nm), respectively. *R. sphaeroides* pigments have been found to be sensitive to the growth medium content [4,14]. As shown in Fig. 3, the various concentration of $Fe^{2+}$ was not affected on the light harvesting pigments synthesis, such as carotenoids. However, decrease in the level of B800-850 complexes was observed with increasing $Fe^{2+}$ ion concentration.

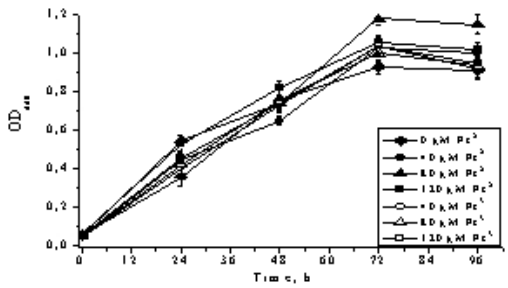


Fig. 1. Anaerobic growth of *R. sphaeroides* MDC6521 at illumination at succinate containing medium at $Fe^{2+}$ and $Fe^{3+}$ various concentrations. Growth was monitored by absorption determination (see Materials and methods).

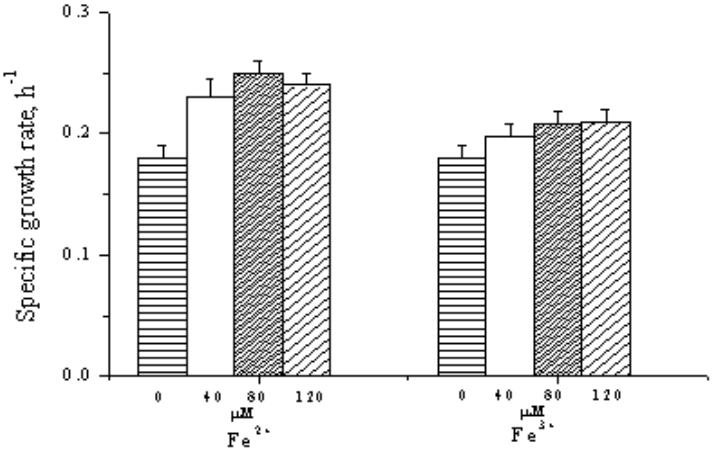


Fig. 2. The effect of $Fe^{2+}$ and $Fe^{3+}$ various concentrations on *R. sphaeroides* MDC6521 specific growth rate.

Fig. 4 shows the effects of $Fe^{2+}$ and $Fe^{3+}$ various concentrations on medium pH variation during bacterial anaerobic growth. The results obtained showed that, the final pH in the presence of all ions was higher than the initial pH,

which is important factor for the bacterial growth [3,4,14]. Increase of medium pH were shown in our previous papers [3,14], but growth time dependence change of pH and its variation for different metal ions were not reported. Various organic substrates stimulate growth of bacterial culture with alkalization of the medium [3]. As shown in Fig. 4, during the growth in the presence of used metal ions up to 72 h medium pH increased from 7.0 (initial pH) to 9.0–9.5. This result was connected with the uptake of organic acids and extrusion of $OH^-$ ions or with the formation of various products such as polyhydroxybutyrate [4]. Then, pH followed to ~9.0, which was probably connected with formation of fermentation end-products, which decay with $H_2$ production.

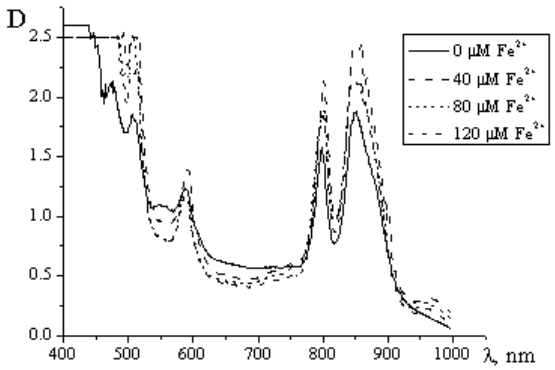


Fig. 3. Dependence of the absorption spectra of *R. sphaeroides* MDC6521 suspension on $Fe^{2+}$ various concentrations. The absorption spectra were recorded as described in Materials and methods. D was optical density.

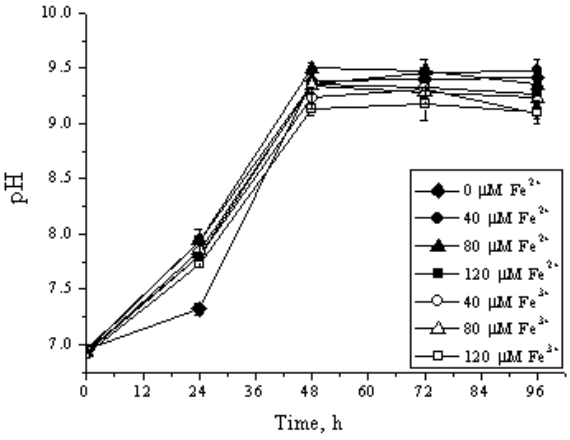


Fig. 4. The change of pH of *R. sphaeroides* MDC6521 during the anaerobic growth at illumination in succinate containing medium at $Fe^{2+}$ and $Fe^{3+}$ various concentrations. The pH was measured at regular intervals by using a pH-potentiometer (see Materials and methods).

The ORP can be considered as the key factor determining bacterial anaerobic growth, which is connected with falling of ORP from positive to negative values [4,14]. Decreasing ORP indicates to enhancement of reduction processes related to formation of fermentation end-products, production of amino acids and synthesis of proteins and other compounds which apparently is typical for the metabolic processes during the cell anaerobic growth [4,14].

Various concentrations of $Fe^{2+}$ ions affected on ORP of *R. sphaeroides* during the anaerobic growth upon illumination (Fig. 5). Actually, ORP of *R. sphaeroides*, measured by a Pt electrode, gradually decreased during the anaerobic growth with $Fe^{2+}$ ions (0-96 h). This decrease was more intensive in the presence of 80 μM $Fe^{2+}$: ORP decreased to –720±20 mV (see Fig. 5). Such decrease might indicate enhanced $H_2$ production [3,4,14]. In contrast in the presence of $Fe^{3+}$ ORP of medium was not changed much, and its variation was similar to the control (no additions); and $H_2$ production was not observed (Fig. 5). It is suggested that $Fe^{2+}$ can increase nitrogenase or hydrogenase expression in suitable concentration. Note, ORP was not changed much, and $H_2$ production was not observed in the absence of $Fe^{2+}$ (see Fig. 5).

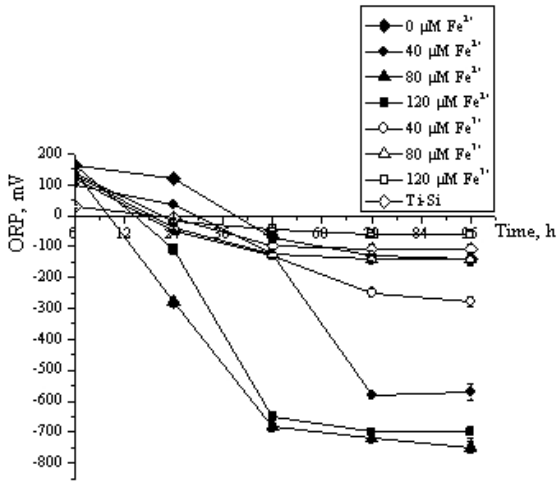


Fig. 5. The change of ORP of *R. sphaeroides* MDC6521 during the anaerobic growth at illumination in succinate containing medium at $Fe^{2+}$ and $Fe^{3+}$ various concentrations. ORP of bacterial growth medium was measured at regular intervals using a Pt and Ti-Si electrodes (see Materials and methods).

Different ways to enhance $H_2$ production by photosynthetic bacteria are including not only choosing of effective substrates, but also exploring novel pathways in hydrogen metabolism and regulation of electrons transfer within the cell membrane and activity of membrane-associated enzymes [4,14]. This might delight novel ways in regulating $H_2$ production by different bacteria.

**Conclusions.** The $Fe^{2+}$ ions affected the growth of *R. sphaeroides* MDC6521, and effects had a concentration-dependent manner. The highest

growth specific rate was obtained with 80 μM $Fe^{2+}$ ions. At the same time $Fe^{3+}$ ions was not affected on the *R. sphaeroides* growth properties. The various concentration of $Fe^{2+}$ was not affected on the photosynthetic apparatus structural components synthesis, such as light harvesting pigments. The obtained results show that $H_2$ production was detected in all concentrations of $Fe^{2+}$ at 48 h.

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## Effects of Fferrous Ions on the *Rhodobacter sphaeroides* Growth Properties and Redox Activity

The $Fe^{2+}$ and $Fe^{3+}$ ions effects on growth properties and external oxidation-reduction potential (ORP) variation during bacterial growth under anaerobic conditions upon illumination were examined of *R. sphaeroides* strain MDC6521 isolated from mineral springs in Armenia were established. These effects had a concentration-dependent manner. The highest growth specific rate was obtained with 80 μM $Fe^{2+}$ ions. In the presence of different concentrations of $Fe^{2+}$ ions ORP decreased up to negative values (–720±20 mV) indicating $H_2$ production. At the same time the various concentration of $Fe^{3+}$ was not affected on the *R. sphaeroides* growth properties and photosynthetic apparatus structural components synthesis, such as light harvesting pigments.

Լ. Յ. Հակոբյան

## Երկաթի իոնների ազդեցությունը *Rhodobacter sphaeroides* բակտերիաների աճման և օքսիդավերականգնողական ակտիվության վրա

Ցույց է տրվել Հայաստանի հանքային աղբյուրներից անջատված *R. sphaeroides* բակտերիաների MDC6521 շտամի աճման և միջավայրի օքսիդավերականգնողական պոտենցիալի (ՕՎՊ) վրա $Fe^{2+}$ և $Fe^{3+}$ իոնների ազդեցությունը, որը դրսևորել է կոնցենտրացիոն կախվածություն: Առավելագույն աճման տեսակարար արագություն է նկատվել 80 մկՄ $Fe^{2+}$ իոնների առկայությամբ: $Fe^{2+}$ իոնների տարբեր կոնցենտրացիաներում դիտվել է միջավայրի ՕՎՊ-ի անկում մինչև բացասական արժեքներ (–720±20 մՎ), ինչը վկայում է $H_2$-ի արտադրության մասին: Միևնույն ժամանակ $Fe^{3+}$ իոնները չեն ազդել ո՛չ բակտերիաների աճման, ո՛չ էլ դրանց ֆոտոսինթեզային համակարգի կառուցվածքային բաղադրամասերի՝ լուսահավաք պիգմենտների սինթեզի վրա:

**Л. Ю. Акопян**

## Влияние ионов железа на характеристики роста и редокс активность *Rhodobacter sphaeroides*

Показано влияние ионов $Fe^{2+}$ и $Fe^{3+}$ на параметры роста *R. sphaeroides* MDC6521 и окислительно-восстановительный потенциал (ОВП) среды во время роста бактерий, изолированных из минеральных источников Армении. Воздействие имело концентрационную зависимость. Наибольшая удельная скорость роста бактерий наблюдалась в присутствии 80 мкМ $Fe^{2+}$. В присутствии разных концентраций $Fe^{2+}$ наблюдалось падение ОВП до отрицательных значений (–720±20 мВ), что свидетельствует о производстве $H_2$. В то же время ионы $Fe^{3+}$ не влияли на рост и синтез структурных компонентов фотосинтетического аппарата, таких как светособирающие пигменты.

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