Kinetic model of aerobic Agro Strain Growth under Constant Magnetic Field in Batch system

النظام الحركي لنمو عزلات Agro الهوائية تحت الحقل المغناطيسي الثابت في مزارع الدفعة الواحدة

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Abstract

The specific growth rate is a key control parameter in the industrial production of any microorganism. In this study we investigated an effect of the direct impact constant magnetic field in aerobic batch system of microorganism kinetic growth. Agro type was used in this study, with motor oil as a substrate compound in mineral salts solution. Analysis regarded the impact of constant magnetic field for the Agro growth in specific growth rate in batch system. The experiment was carried out in the laboratory scale with use of technological system comprised of magnetic activator of culture. By experiment, Cultures of Agro were grown in a magnetic field (330 Gauss) for 12 hr; where an obvious increasing in quantity of viable count of cells was achieved against time with impact constant magnetic field. This value was found equal to 14250 cfu with degradation in substrate from 0.439 to 0.338 ml/150ml during 7 hours; on the other hand control media was found 7125 cfu with degradation in substrate form 0.439 to 0.428 ml/150ml during 7 hours. Monod equation was applied to determine the Monod's constant value (K_s) for exposure experiments and was found equal to 0.388 ml/150ml at maximum specific growth rate constant 0.142 h^{-1}. The doubling time of growth for agro Strain was determined and was found equal 4.6 hours.

المستخلص

معدل النمو النوعي هو مؤشر السيطرة لأي كائن مجهري . في هذه الدراسة تم ملاحظة التأثير المباشر للحقل المغناطيسي الثابت على حركيات النمو للكائن المجهري في التنمية الهوائية لنظام الدفعة الواحدة . تم أستخدام Agrobacterium في هذه الدراسة مع زيت المحركات كمادة أساس في وسط الأملاح المعدنية . وقد أعتمدت الدراسة على تأثير المجال المغناطيسي الثابت على معدل النمو النوعي للـ Agrobacterium في مزارع الدفعة الواحدة . الدراسة تمت على المستوى المختبري بأستخدام تقنيات تضمنت المغنطة المنشطة للمزارع . تم تنمية مزارع الـ Agrobacterium تحت تأثير مجال مغناطيسي 330 كاوس لمدة 12 ساعة مع ملاحظة زيادة للعدد الحي للبكتريا مع مرور الوقت بأستخدام المجال المغناطيسي الثابت ؛ حيث وجدت هذه القيمة تعادل 14250 cfu مع تفكيك للمادة الأساس من 0.439 الى 0.338 مل/150 مل خلال 7 ساعات ، من جهة اخرى وجد أن القيمة لوسط السيطرة 7125 cfu مع تفكيك للمادة الاساس من 0.439 الى 0.428 مل/150مل خلال نفس الوقت . طبقت معادلة Monod لتحديد ثابت Monod (K_s) في تجارب التعريض فوجد أنه يعادل 0.388 ml/150ml مع معدل نمو نوعي ثابت يساوي h^{-1} 0.142 . كذلك حدد الوقت اللازم لمضاعفة النمو للـ Agro ووجد أنه يعادل 4.6 ساعة .

Keyword: batch culture, magnetic treatment of Agro, magnetic field, Monod's *constant*

Introduction

Among the earth's complex conditions affecting the life and development of populations is the sphere of electromagnetic interactions extending from the magnetosphere to the centrospheres. Living organisms have electrical and magnetic characteristics, but it is difficult to present them in biochemical terms. Electromagnetic interactions influence living organisms, which may modify their structures and processes under the influence of electromagnetic waves. Recent years have brought increased interest in the action of the electromagnetic field upon living organisms, especially animals as well as man. Only a few studies deal with the influence of the magnetic field on plants or microorganisms [1].

The action of electromagnetism is a complex Phenomenon to study. It is convenient to start with one of its components, magnetism. When microorganisms are grown in a batch reactor certain phases of growth can be detected. A typical growth characteristic is shown in Figure (1) [3]. The appearance and the length of each phase depend on the type of organisms and the environmental conditions.

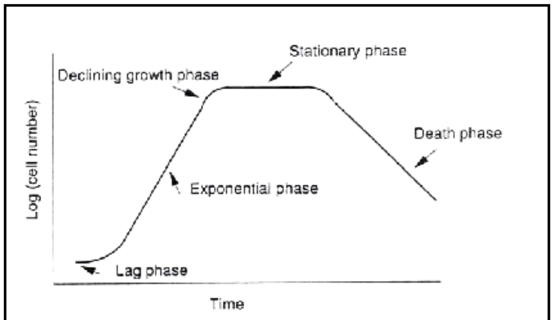


Fig (1): Growth phases in a batch process

The first phase in the growth, where the growth rate stays almost constant, is the lag phase. The lag phase is caused for many reasons. For example, when the cells are placed in fresh medium, they might have to adapt to it or adjust the medium before they can begin to use it for growth. Another reason for the lag phase might be that the inoculum is composed partly of dead or inactive cells. If a medium consists of several carbon Sources, several lag phases might appear [1, 2]. This phenomenon is called diauxic growth. Microorganisms usually use just one substrate at a time and a new lag phase really results when the cells adapt to use the new substrate. When a substrate begins to limit the growth rate the phase of the declining growth begins. The growth rate slows down until it reaches zero and the stationary phase begins. In the Stationary phase the number of the cells remains practically constant, but the phase is important because many products are only produced during it. The last phase is called the death phase. During the death phase the cells begin to lye and the growth rate decreases [3].

The aim of the present study is to assess the effect of constant magnetic field on specific growth rate of Agro strain grown in aerobic batch culture.

Material and Methods

A pure Agro Strain was isolated from Iraqi soil and supplying from genetic engineering department - Baghdad University, which used in this study. Bacterial cells were grown to stationary phase in 250 ml shaken flask cultures on mineral medium adjusted to pH 7.0 and containing 0.439 ml/150ml motor oil [4, 5].

Media

The defined mineral medium contained the following (per liter):

$(NH_4)2SO_4$, 5gr; KH_2PO_4, 3gr; $MgSO_4.7H_2O$, 0.5gr; $ZnSO_4.7H_2O$, 4.5mg; $CaCl_2.6H_2O$, 0.3 mg; $MnCl_2.4H_2O$, 1 mg; $CuSO_4.5H_2O$, 0.3 mg; $CaCl_2.2H_2O$, 4.5 mg; $FeSO_4.7H_2O$, 3 mg.

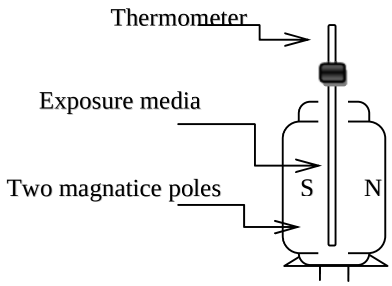
The medium was prepared and sterilized for batch cultivation [6].

Spectrophotometer test

The batch experiment was carried out to measure the consumed substrate at 400 nm in supernatant and optical density variation of Agro Strain growth at 600 nm with time by using spectrophotometer [6].

Magnetic Unit

A cylindrical permanent ferrite magnet 5 cm in diameter was placed around 250 ml culture glass tube as shown below, containing 0.439 ml/150 ml of a suspension of motor oil with subculture Agro strain cells in the mineral salts liquid medium. Constant Magnetic Field and homogeneity of 330 Gauss were checked using a Teslameter (Hall Effect Teslameter digital) [7, 8]. The intensity of static magnetic field used in our experiments was chosen on the basis of [9] findings,they had used more or less 330 gauss of a constant Magnetic Field to induce the growth and development of trichomanas vaginalis. For this type of exposure, no shielding against the natural variations of terrestrial Magnetic Field was required; the value of approximately 0.050 mT is negligible with respect to the Magnetic Field intensities applied [9].



Exposure Unit

Experiment using cells not exposed to Magnetic Field was simultaneously performed as the control, which was placed at a distance of about 100 cm from the exposed unit [6, 7]. In the absence of magnets, the earth Magnetic Field was 0.05±0.01 mT. The bacterial sample was exposed to Magnetic Field for 12 hour. After Magnetic Field exposure, individual samples were tested in visible-spectrophotometer at 600 nm.

Microbial activities

Microbial activities are allowed the mineralization of some petroleum components into carbon dioxide and water, and microbial transformation is considered a major route for complete degradation of petroleum components. The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants. Many scientific approaches have been used in the in situ and ex situ biodegradation of organic pollutants. However, the extent of biodegradation is critically dependent on salinity, temperature, pH, heavy metals surfactants, nutrients and presence of readily assailable carbon sources [10, 11].

Microbial Growth kinetics

The relation between the specific growth rate (µ) of a population of microorganisms and the substrate concentration (S) is a valuable tool in biotechnology [12].

This relationship is represented by a set of empirically derived rate laws referred to as theoretical models. These models are mathematical expressions generated to describe the behavior of a given system. The idea of microbial growth kinetics has been dominated by an empirical model (Equation 1) originally proposed by Monod (1942) [13]. The Monod model introduced the concept of a growth limiting substrate [13].

$$\mu = \mu_{\max} \frac{S}{S + K_s} \qquad (1)$$

Where:

μ = specific growth rate h^{-1},

μ_{max} = maximum specific growth rate h^{-1},

S = substrate concentration *ml/150ml,*

K_s = substrate saturation constant *ml/150ml* (i.e. substrate concentration at half μ_{max}).

In Monod's model, the growth rate is related to the concentration of a single growth-limiting substrate through the parameters (μ_{max}) and (K_s).

Derivatives of the Monod kinetic model in 1912, [13] proposed the first kinetic principle for microbial growth. They stated that the relationship between (µ) and (S) is best described by a "saturation" type of curve where at high concentration of substrate, the organism grows at a maximum rate (μ_{max}) independent of the substrate concentration. Monod's model satisfies this requirement, but it has been criticized particularly because of derivations of (µ) at low substrate concentration [14].

Results

In batch cultures, the maximum specific growth rate of Agro strain in motor oil media in a defined mineral medium was 0.029 h^{-1}, while these values increasing to 0.182 h^{-1} at exposure process.

To prove this result in specific growth rate, first optical density of growth cell was measured at 600 nm by using spectrophotometer as shown in Figure (2), which clearly appears the increasing of optical density against time, then each media were tested to estimate the actual viable count which appear clear difference between exposure and control media as shown in Figure (3).

In Figure (4), the increasing in viable cell lead to increasing in motor oil degradation with time, which give maximum consuming when exposed to 330 gauss.

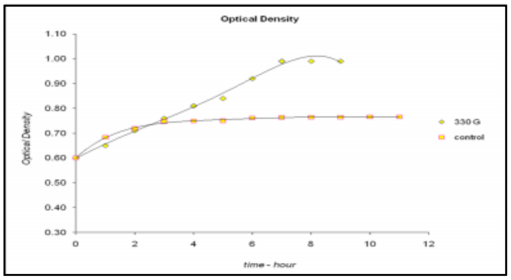


Fig (2): Relation of optical density of agro strain after 12 hours of magnetic exposure and not exposure for magnetic field (control media)

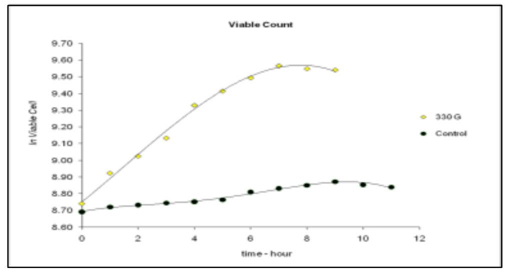
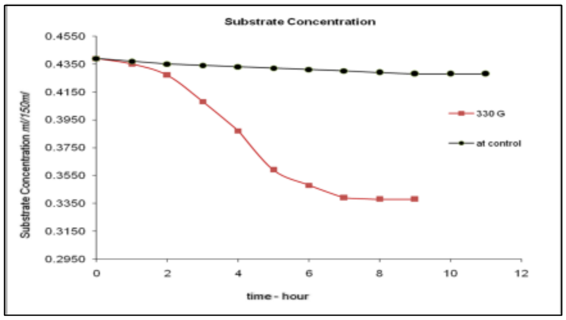


Fig (3): Relation of counted viable cells after 12 hours of magnetic exposure and not exposure for magnetic field (control media)



Fig(4): Motor oil degradation after 12 hours of magnetic exposure and not exposure for magnetic field (control media)

Monod Kinetic modeling

The main result of exposure effect on agro strain can be express in Monod model, this model have two main parameter, (K_s) and (μ_{max}) needed to calculate because these values are important to use in bioreactor design. Monod model calculation depended on exponential growth phase of agro strain as shown in Figure (1). Figures (5, 6) are shown the exponential growth phase for exposure and control media,

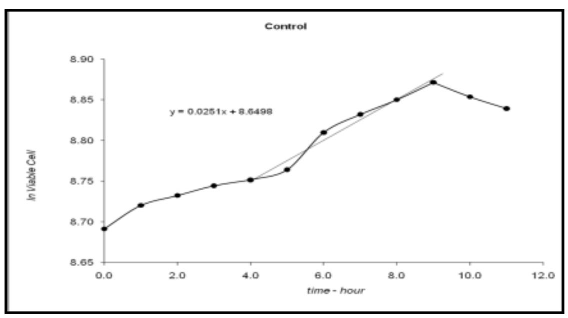


Fig (5): viable count against time for control media, shown exponential growth phase [3]

As shown in Figure (5), the exponential phase [3] starting after 4 hour, because the cell adaptation to mineral salt media required this period then start to growth to reach to maximum value after 9 hours, while in magnetic exposure media as shown in Figure (6), the adaptation point disappeared, because the exposed cells starts healthy and active to new effect.

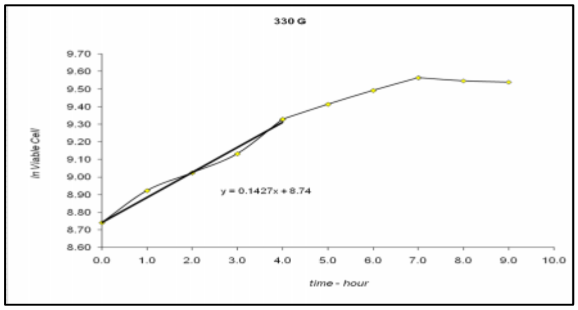


Fig (6): viable cells count against time for exposure media, shown exponential growth phase [3]

Depending on experiment data for exponential growth phase, the slop line will represent the specific growth rate constant for each experiment; the results are listed in Table (1).

Table (1): Specific growth rate constant and doubling time obtained from each experiment

Media	$\mu\ h^{-1}$	Doubling time - hour
Exposure	0.1504	4.67
Control	0.0234	26.5

Depending on Equation (1), the Monod parameter can be found by plotting Specific growth rate constant against substrate concentration, as shown in Figures (7 , 8). These values are listed in Table (2):

Table (2): Monod model parameter required for each experiments

Media	$\mu_{max}\ h^{-1}$	Ks ml/150ml
Exposure	0.142	0.3650
Control	0.0292	0.4273

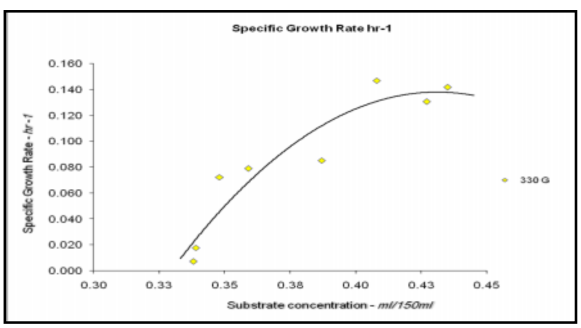


Fig (7): Specific growth rate constant against substrate concentration for exposure media, exponential growth phase

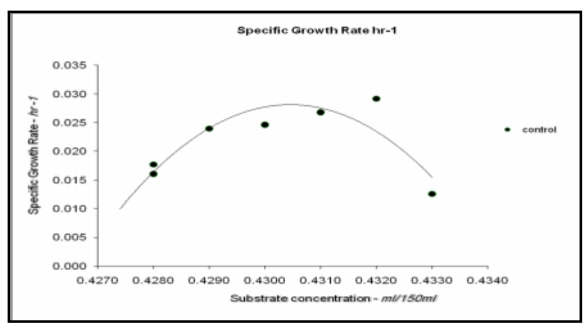


Fig (8): Specific growth rate constant against substrate concentration for control media, exponential growth phase

Discussion

Static magnetic fields may have some potential to activate or inactivate microorganisms in mineral slat [12, 14]. This effect causes a chain of processes in the colonies, and that lead to improvement of health. A cell produces its own energy, called ATP (Adenosine Tri-phosphate). This energy is necessary for the movement of the body, building up and breaking down molecules, and transporting substances across cell membranes. This phenomenon was approving from experimental result, which appeared healthy cell increasing in short time [15].

Conclusion

In the present paper, motor oil in the mineral salt was determined by ultraviolet spectrophotometer at (400 nm). The exposure media appear good response to consume the motor oil concentration in short time at 330 gauss, at the same time; viable count cell became healthier along this treatment compared with the control media (non exposure media). Monod equation applied to obtain the half saturation of substrate concentration (K_s) for each experiment. The effect of Constant Magnetic Field on agro strain gives good result in lab treatment which can easily applied in industrial operation to treat other hydrocarbons in short time.

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