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Microalgae Cell Quantification Using Electrical Parameters

Leena Osama Fahmi Saqer

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United Arab Emirates University
College of Engineering
Department of Electrical Engineering

MICROALGAE CELL QUANTIFICATION USING ELECTRICAL PARAMETERS

Leena Osama Fahmi Saqer

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Electrical Engineering

Under the Supervision of Dr. Mahmoud Al Ahmad

May 2016
Declaration of Original Work

I, Leena Osama Fahmi Saqer, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "Microalgae Cell Quantification Using Electrical Parameters", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Mahmoud Al Ahmad, in the College of Engineering at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Electrical properties of living cells have been proven to play a significant role in understanding and characterizing the different biological activities of the cell. The objective of this work is to develop an electrical based technique for determining and estimating the number of microalgae cells in a suspension without the need for any sample treatments or pre-processing.

The proposed technique is based on the direct use of electrical capacitive model. The basic premise behind this idea is the electrical polarization of microalgae particles that get charged due to the application of an electric field. This wouldn’t cause any distortion or dissolving/diffusion of the content of the cells inside the medium. The electrical measurements of the capacitance – voltage concept is employed to determine microalgae cells counts. The microalgae cells are considered as dopants embedded inside a relevant medium. The cells count is then estimated by subtracting the intrinsic impurities of the medium from the effective ensemble impurities of the suspension inside a defined volume. Three strains of microalgae, namely Nannochloropsis, Tetra. and Scenedesmus were cultivated and examined under the proposed methodology. For validation, samples with unknown cell counts were quantified using the proposed method and compared to other techniques used for validation.

Results of the study revealed that cell count determined with the proposed electrical based methodology was done within few minutes, which is significantly shorter than all other reported techniques. The enumeration of microalgae cells count is important for their growth optimization. A real time, rapid and efficient technique is
needed for such purpose. The proposed method provided a better combination of high sensitivity, quick response, few minutes, low cost, high throughput, and ease of use.

The outcome of this work allows the development of a rapid technique for the determination of cells count of microalgae or it could be further extended to determine the cell count of other types of suspended cells of comparable size. In addition, the proposed methodology could be upgraded to be applied *in-situ* with a feedback loop that could allow for continuous monitoring of the growth conditions and rapid determination of microalgae cells count.

**Keywords:** Capacitance – Voltage measurements, cell count, electrical parameter, electrical characterization, microalgae.
تحديد عدد خلايا الطحالب باستخدام المؤشرات الكهربائية

الملخص

أثبتت الخصائص الكهربائية للخلايا الحية دورا هاما في فهم الأنشطة البيولوجية المختلفة للخلية. الهدف من هذا المشروع هو تطوير تقنية كهربائية تقوم بتقديم وتحديد عدد الخلايا الموجودة في الطحالب العائلة في وسط زراعي باستخدام طرق كهربائية دون الحاجة إلى معالجتها سابقا. تمكن أهمية المشروع في إمكانية تحسين معدل نمو الطحالب في خلال دورة حياتها.

تساعد التقنية المقترحة على الاستخدام المباشر لنموذج المكثف الكهربائي. الفرضية الأساسية وراء هذه الفكرة هو الاستخدام الكهربائي الذي يحدث لجزيئات محاكاء عندما تتعرض لتطبيق الحقل الكهربائي، وذا لن يتسبب في أي تهيج أو تذبذب في متولى الخلايا المنتشرة داخل الوسط الزراعي. لقد تم استخدام قياسات سعة الجهد الكهربائي لتحديد عدد خلايا الطحالب دون الحاجة لمياه معالجة أولية لتلك البينية تحت التجربة. أيضا يمكن اعتبار خلايا الطحالب كشواهد متواجدة ضمنيا في الوسط الزراعي الذي تنتمي به الخلايا الطحالبية يمكن تقييم عدد خلايا الطحالب عن طريق إيجاد الفرق بين الشواهد الموجودة في الوسط الزراعي وفي تلك الموجودة في البيئة تحت الاختبار مع الأخذ بعين الاعتبار النسوي حجم العينات. تم الاستعانة بثلاثة أنواع مختلفة من الطحالب نانوكلوروسبيس ونيتروسوس وسبيديوم لإجراء التجارب عليهم. لأجل التحقق من صحة النتائج، تم قياس عينات مختلفة من أنواع الطحالب التي تم ذاتها سابقا بالطريقة المقترحة وأيضا باستخدام الطرق التقليدية المذكورة في مواد البحث السابقة ومقارنة نتائجهما سويا.

أظهرت النتائج أن الطرق الكهربائية يمكن تحديدها في غضون دقائق قليلة وهو أقصر بكثير من جميع التقنيات الأخرى. تبدو أن خلايا الطحالب مهم للتنوير نموها. ولهذا السبب هناك حاجة إلى تقنية سريعة وفعالة وأيضا يتم إجراؤها في الزمن الفعلي وفي أي وقت تم أخذ عينة منها. تقدم الطرق المقترحة أفضل مزيج من الحساسية عالية والاستجابة السريعة فيما يضع دفاع من منخفضة التكلفة والانتاجية العالمية، والسهولة في الاستخدام.

تمكن أهمية تحديد عدد الخلايا في توسيع نطاق هذه التجربة يمكن استهداف إعداد عدد الخلايا لأنواع أخرى من الأحياء التي تتميز بحجم تقريبي مماثل لخلايا الطحالب تحت التجربة. بالإضافة إلى ذلك يمكن
أن يتم استخدام الطريقة المقترحة ليتم تطبيقها في الموقع و التي سوف تمهد الطريق لتحرير مباشر و سريع عن الطحالب و التي بناء على ذلك تمكن من الاختيار الأفضل لأنواع الطحالب و ظروف النمو المناسبة.

ادلة البحث: قياسات سعة الجهد الكهربائي، عدد الخلايا، الوصف الكهربائي، المؤشرات الكهربائية، طحالب.
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Special thanks go to my husband, parents, siblings, and family who helped me along the way. I am sure they suspected it was endless. My thanks are extended to all my friends and colleagues for their support, encouragement, and friendship.
Dedication

To my beloved husband, parents and family
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>σ</td>
<td>Conductivity</td>
</tr>
<tr>
<td>ε</td>
<td>Permittivity</td>
</tr>
<tr>
<td>CV</td>
<td>Capacitance – voltage</td>
</tr>
<tr>
<td>θ</td>
<td>Number of microalgae cells</td>
</tr>
<tr>
<td>Nₛ</td>
<td>Doping concentration of microalgae suspension</td>
</tr>
<tr>
<td>Nₘ</td>
<td>Doping concentration of medium</td>
</tr>
<tr>
<td>Dₛ</td>
<td>Debye volume of microalgae suspension</td>
</tr>
<tr>
<td>Dₘ</td>
<td>Debye volume of medium</td>
</tr>
<tr>
<td>N</td>
<td>Doping concentration</td>
</tr>
<tr>
<td>D</td>
<td>Debye volume</td>
</tr>
<tr>
<td>q</td>
<td>Electron charge = $1.60217662 \times 10^{-19}$ C</td>
</tr>
<tr>
<td>εₛ</td>
<td>Dielectric constant of the material</td>
</tr>
<tr>
<td>A</td>
<td>Overlapping area of a two plates capacitor</td>
</tr>
<tr>
<td>C</td>
<td>Capacitance</td>
</tr>
<tr>
<td>V</td>
<td>Voltage</td>
</tr>
<tr>
<td>l</td>
<td>Geometrical length of the host coaxial capacitance</td>
</tr>
<tr>
<td>L₅DCF</td>
<td>Debye length</td>
</tr>
<tr>
<td>K</td>
<td>Boltzmann constant = $1.38064852 \times 10^{-23}$ m².kg.s⁻².K⁻¹</td>
</tr>
<tr>
<td>T</td>
<td>Room temperature (Kelvin)</td>
</tr>
<tr>
<td>I₀</td>
<td>Leakage current</td>
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<tr>
<td>η</td>
<td>Ideality factor</td>
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\( I_s \) Saturation current

\( k \) Voltage coefficient

\( \gamma \) Voltage coefficient

\( \varepsilon_0 \) Vacuum dielectric permittivity = \( 8.85 \times 10^{-12} \text{ F.m}^{-1} \)

\( \varepsilon_r \) Effective suspension

\( a \) Inner radius of the coaxial cable

\( b \) Outer radius of the coaxial cable

\( \phi \) Bulk potential

\( W \) Depletion width
Chapter 1: Introduction

1.1 Motivation

Microalgae are considered a promising source for biodiesel production and have the potential to replace petro fuels [1-3]. Microalgae has received an increasing focus as a renewable energy source if production of microalgae biodiesel and fuels are to be feasible and economically sustainable [4]. Therefore, it is required to have a deep understanding of the chemical composition of algae biomass, and optimization of culture conditions is needed. The estimation of algae population is important when studying the growth kinetics that is highly affecting the biofuels production cycle, in addition to the biochemical constituents [5,6].

Cells count determination is not an easy task due to the microscopic size of algal cells. Generally, conventional protocols are available and sufficient for cell counting. Microalgae cells are enumerated with light absorption [7], and by the determination of wet/dry biomass [8]. Most conventional techniques are in most cases time consuming, tedious, indirect, require excessive sample treatments, and prone to error. Despite the development of modern techniques, some of the latest methodologies proposed require sophisticated and expensive equipment such as the inverted microscope and the chamber [9]. In addition, it is time consuming, as sometimes 24 hours are needed for a small number of algae cells existing in a sample to settle down in the chamber. The most common technique for cells count is the hemocytometer [10]. However, in spite of its usage, on-line measurement of real time cell counts is not possible using this method.

Therefore, it is desired to have a rapid based monitoring system for cell quantification that could be a leading edge to be applied in-situ, which could be then
integrated in continuous feedback control loop to adjust the growing conditions without the need for any time consuming and expensive sample preparations. To achieve this effectively, a rapid method for cell counting within less than a minute response, equipped with continuous monitoring capability is necessary in controlling and adjusting the cultivation conditions to achieve the desired objective of the growth.

1.2 Research Objectives

The main advantage of this research is the generation of electrical based methodology which has the ability to quantify cells count. Due to their free labeling capabilities electrical characterizations techniques have received increasing attentions in the last decades [11,12]. Furthermore, they are noninvasive characterization tools that can detect and quantify unlimited types of materials without the need for any sample treatments or preparations. Therefore the main research objectives of this thesis are:

1) Develop a rapid electrical based technique for microalgae cell counting.
2) Present a verified correlation to relate the electrical parameters with cells count.
3) Compare the efficiency and sensitivity of the presented approach with other methodologies proposed in earlier research work.

1.3 Thesis Significance

Although UAE is one of the largest oil exporters, the production of biodiesel falls within the country’s vision to satisfy the coming generation’s need for clean energy. The reshaping of other energy resources is needed for a sustainable fuel production. Microalgae have been utilized worldwide for biodiesel production. The UAE enjoys abundant and continuous sunshine throughout the year, combined with availability of seawater, which makes it ideal for microalgae cultivation. These
features clearly show the potential of cultivating microalgae in large scales in the UAE. Nevertheless, an economical biodiesel production from microalgae requires high biomass productivity.

The outcome of this research would allow the development of a rapid technique for the enumeration of cells count/ concentration, which is vital for the effective manipulation of the cultivation conditions to achieve the desired objective. This certainly enables the adjustment of the growth conditions in real time environment. Beside microalgae, this approach could be applied to other type of organisms as well. The lipid monitoring and quantification at the cell level could be effectively used in the optimization of the growth rate and lipids productivity.

1.4 Thesis Organization

The thesis is organized as follows:

Chapter 1 (Introduction): This chapter presents the motivation and objectives of this work. It starts with explaining the demand for microalgae cell counting and the technologies used. The chapter ends by stating the objective and the significance of this research.

Chapter 2 (Literature Review): This chapter reviews the previous research work related to the topic of the thesis. It discusses the different techniques used to determine cells concentration. It also presents the theory of characterization technique proposed in this work.

Chapter 3 (Materials and Methods): This chapter demonstrates the materials and tools used, in addition to the methodology and experimental procedures conducted in order to perform the desired measurements and analysis.
Chapter 4 (Results and Discussion): This chapter shows the results gained from performing the experiments, the processing and analysis of data, followed by a discussion of the results and the validation using a different technique.

Chapter 5 (Conclusions and Future Works): This chapter is the last one in the thesis that wraps up all the research findings, and relates them to the objectives presented in the first chapter.
Chapter 2: Literature Review

In this chapter, we present an overview of the importance of microalgae biomass as an energy source, and different technologies developed to enumerate its cell count, and then highlight their disadvantages and limitations. This chapter also introduces the theory of the electrical characterization technique presented in this work.

2.1 Microalgae Biomass as an Energy Source

Microalgae are a diverse group of photosynthetic microorganisms that can grow and live in fresh or marine water environments that convert inorganic carbon like CO₂ into algae biomass in the presence of water, light and nutrients [13]. They grow either autotrophically or heterotrophically. The autotrophic growth requires CO₂, light, and nutrients to grow, whereas heterotrophic algae needs organic carbon source like glucose which is a food source, in addition to nutrients [14]. They can grow rapidly and live in harsh conditions due to their simple unicellular or multicellular structure. They exist as colonies, filaments or ameoboids. They convert solar energy into chemical energy completing a full growth cycle every few days [15]. Microalgae stores energy in a form of its main components of lipids, carbohydrates, and proteins [16,17]. This chemical composition differs depending on the microalgae species which depends on the temperature, light intensity, salinity, PH of the medium, and the growth phase, which allows using the microalgae in different applications ranging from food products to biodiesel production. The global climate change and the rise of transportation fuels has resulted in focused interest on generating power from renewable sources to meet the increasing energy demand [18].
Many research reports demonstrated the advantages of using microalgae for biodiesel production over other available feedstocks [1,19-23]. From a practical point of view, microalgae are easy to cultivate and grow extremely rapidly and they are rich in oil [1]. They can grow anywhere with little amount of simple nutrients and sunlight, and the growth process can be accelerated by adding more nutrients and needed aeration [15]. Another advantage of microalgae biofuels is that it can replace petro-fuels as they are expensive sources and they started exploiting at the expense of environment. A variety of microalgae species are adapted to grow and live in different environmental conditions, which is not easy to find with other biodiesel feedstocks. Microalgae are a great source for different types of fuels including biodiesel, methane, ethanol, and hydrogen. The performance of algae biodiesel is similar to the petroleum diesel and does not contain any sulfur [15].

Although microalgae lipids and carbohydrates are the most valuable component in the context of biodiesel production process, the other principal biological components including proteins of the algae biomass is important to be estimated. The complete chemical composition is needed to determine the economics of the production process and the measurements of each component separately are important for cost determination [17].

2.2 Previous Work on Microalgae Cell Counting

The biovolume method is a set of mathematical equations based on geometric models that calculates the microalgae biovolume for more than 850 types of pelagic and benthic marine and freshwater microalgae. The rule of this technique is only applicable to individual cells. The mathematical model minimizes the error, inexpensive, and easy to apply. It also provides a highly systematic resolution of the
identified cells. On the other hand, this method does not cover all types of algae shapes; re-examination of the equations used is needed. Because of the variation in the microalgae life cycle that affects its cell size, the method has problems in accuracy. Therefore, depending on the average biovolume measurements is not enough. It has to be calculated for every sample taking into consideration the different depths in the same medium at each experiment. Since the general rule of this method covers individual cells only and this is difficult to apply in some cases, the shapes can be applied to the colony with each single cell measured. The biovolume technique may overestimate the size of large algal cells with a higher relative vacuole volume [24,25].

The electronic particle counter technique provides particle sizing distributions of cells. It is also referred to it as a coulter counter. The idea of this method is the use of an aperture that allow the passage of an electric current through it given that particles are suspended in an electrolyte solution. A change in resistance is a result of passing the current through the aperture which results in a change in the current and voltage correspondingly. This variation in resistance can be converted to a particle count electronically and it is proportional to the size of the particle itself. A full diagram presenting the mechanism is shown in Figure 2.1. Different ranges of particles size can be counted and this is limited by the size of the pore used in the experiment. The sizing is independent of the particle shape and it orientation in the solution. Up to 500 cells can be counted and sized per second. This methodology is straightforward and fast; however, it is vulnerable to errors due to the presence of cell clusters [26,27].
Figure 2.1: Schematic diagram of the coulter counter working principle

The qMicro is one of such existing tools as it appears in Figure 2.2. The qMicro apparatus allows one to measure the size of biological or synthetic particles in a size range between 1-300 µm in a volume of 1 µl to 1 ml (IZON, New Zealand). The principle of resistive pulse-sensing (RPS) is adapted by the qMicro device, which monitors the current flow through the pore (Figure 2.3). The aperture size is adjusted allowing a limited range size of particles to be regulated by the passage of ionic current through the pore [28].
Spectrophotometry is a method used to relate the algal density to the scattered light absorbed by particles in suspension at specific wavelengths (see Figure 2.4). The wavelength range of the electromagnetic spectrum in the visible range from 400 to 700 nm is used to determine the algae biomass. It is an accurate technique but sometimes used to find relative estimates of cell density. Since cells are disorganized and randomly distributed, if the cell density increases, less light will pass through the cuvette. Spectrophotometers are not considered to be a reliable method for cell density estimation as they do not actually count cells but rather measure absorbance, that is
affected by variable components of cell suspensions. The use of light absorption is more adequate for the estimation of the population size of microalgae rather than the determination of the actual number of individual cells. This would not allow distinguish cell types, and the assessment of cells viability [7, 29].

![Diagram of spectrophotometer](image)

**Figure 2.4:** An illustration of the working principle of the spectrophotometer methodology

Another technology presented in a device called counting chamber that determines the number of cells per unit volume of a suspension. The most widely used type of this chamber is the hemocytometer. It is a special type of microscope chamber slide illustrated in Figure 2.5 that is divided into squares of a defined area over which the volume of a suspension is distributed (Figure 2.6). Using the hemocytometer, the number of cells can be determined in a culture after staining. Some calculations need to be applied after to find the total number of cells per ml. The counting procedure requires a light or phase contrast microscope, the hemocytometer itself, and a tracking device, such as a handheld. This cost of this setup can range from a few hundred dollars to several thousand dollars. Proper washing and loading of the device are needed to start the operation. Analyzing the results is not time consuming and requires a few manual calculations. The measurement process is tedious and subject to errors due to
the cell clumping or heterogeneity of the cell size. It is also susceptible to errors due to the small number of cells counted if less than 100 [8,30,31].

Figure 2.5: Hemocytometer slide

Most conventional techniques for the microalgae cells enumeration are time consuming and in most cases are require tedious steps and require sample treatments and preparations steps. In addition to this, the availability of sophisticated equipment
in the previously mentioned techniques adds more cost and decrease the opportunity of using them. Furthermore, on-line measurement of real time cell counts is not possible using such methods.

2.3 Theory of Electrical Characterization

Electrical characterization and specifically dielectric measurements is the most interesting technique because it’s a straightforward, measures cell counts directly, continuously, and in real-time. A material that has the ability to charge without conducting it to a significant degree is dielectric material. The different materials would have variation in their capacitance that supports the induced charge [32].

Every material has two types of charges that are positive and negative particles. Under normal conditions, the total charge in any area of the material is equal to zero because of the strong bond and mutual attraction that keep these different charges together. When the material is exposed to the application of electric field, charges experiences electrical forces that drives the charges to move freely in the material [33]. The movement of charges is basically dependent on the strength of the bonds between the two particles. If it was weak, then they are free to move through the material, and the material is called electric conductor. However, for an electric insulator, charges are allowed to move slightly from their positions as their bonds are strong. As a result, the dielectric material can be conductive as long as it polarizes [34].

The polarization of any material is possible provided that electric dipoles exist in it. When the material is placed between two electrodes, and an electric field is applied, one surface of the electrodes develops a net negative charge while the other
surface accumulated the positive charge. This is illustrated in Figure 2.7 below.

![Diagram of polarization effect](image)

**Figure 2.7: Polarization effect of the material when subject to electric field**

An electric dipole or impurity is a result of the presence of two opposite charges separated by some distance [35]. When the dielectric material is placed in an electric field, the dipoles of the material align in the electric field as shown in Figure 2.8. So within the material, the electric dipoles will cancel each other but at the surface the dielectric will attain the net positive charge \((+Q)\) on the negative electrode and the net negative charge \((-Q)\) on the positive electrode [36].

The polarization mechanism is dependent on the nature of the material under test. Generally, material polarization doesn’t occur instantaneously once the material is subject to electric field application; it takes some time to react to changes [37]. And due to this, conductivity, permittivity, and other factors are frequency dependent [38].
Figure 2.8: A parallel-plate capacitor with a dielectric material in between causes a charge separation in the internal electric field

The electrical properties of material are a correspondence on how the material reacts when it is exposed to electric field application. The response of the material is described by different electrical properties such as the conductivity ($\sigma$), and permittivity ($\varepsilon$) [39]. The conductivity parameter measures the level of charge conduction through the material, and permittivity parameter measure the charge storage level due to the polarization. It is important to note that both parameters are irrespective of the material’s dimensions [40,41].

In order to measure these parameters, the material should be placed in a capacitor. A capacitor is described as any device that stores charge, but usually it consists of two conducting plates with a dielectric material in between [34,41]. Parameters can be determined by conductance and capacitance measurements. Conductance is measured from the magnitude of the electric current passing through the material as a function of the voltage applied, whereas capacitance is defined by the amount of charge stored on the plates as a function of the voltage applied.
Chapter 3: Materials and Methods

This chapter demonstrates the materials used and the experimental procedures conducted in this thesis work in order to determine the cells count for different microalgal strains.

3.1 Microalgal Cultivation

The cultivation approach was done for different strains of microalgae which are *Nannochloropsis*, *Tetra* and *Scenedesmus sp*. They were allowed to grow in nutrient – rich medium (F/2) and (3-N-BBM) shown in appendix A [42, 43, 44] respectively for two weeks in a 5L bubble column bioreactor to enhance the biomass productivity. The prepared medium was purified in an autoclave (Hirayama HV-50, Japan) for 15 min at 121 °C and cooled to room temperature before using it [45].

The 5L bubble column photobioreactor has an outer diameter of 10 cm, an inner diameter of 5 cm, and 40 cm height. It was illuminated with one 50 cm, 60 watts white fluorescent light at an intensity of 120 μmol m\(^{-2}\) s\(^{-1}\). All the cultivations in this work were autotrophic, meaning that CO\(_2\) is the sole carbon source in the system and naturally presented in air bubbled through the system [46]. A photograph of the bioreactor is illustrated in Figure 3.1.
3.2 Microalgae Electrical Polarization

The work in this thesis demonstrates the use of semiconductor theory and its basics and principles to achieve the main objective which is the enumeration microalgae cells. The principle behind the idea was the polarization of the microalgae cell as a result of exposing it to the application of electric field [47]. Microalgae cell is basically composed of proteins, carbohydrates, lipids, nucleic acids and other minor constituents [47, 48]. The polarization depends on the composition of the cell itself and its interaction with the cultivation medium polarity [49]. Generally, microalgae cells are randomly distributed in the medium as shown in Figure 3.2 (a). The cells could be redistributed according to their polarization; the negatively charged cells will be attached or get closer to the positive electrode surface, while the positively charged cells will be attached or get closer to the negative electrode surface; Figure 3.2 (b) demonstrates this.
To demonstrate the polarization of microalgae cells, a sample of microalgae suspension was loaded into an electrical analyzer (Gamry Reference 3000) and measured its charging profile. The result is shown in Figure 3.3. Based on these results, the microalgae particle could be presented as an electrical dipole that has two pairs of electrical charges of equal magnitude but opposite sign, separated by some distance (d) [50] as depicted in Figure 3.4. Therefore, it is suggested to consider a single microalgae cell as an "impurity" or "dopant" that exists in a non-intrinsic semiconductor material. A schematic of the charge distribution inside the cell is depicted in Figure 3.5.
Figure 3.3: Charging profile of a sample of microalgal suspension

Figure 3.4: Polarized microalgal cells inside an alternating current electric field
3.3 Principle of Operation

The principle of the proposed electrical approach is to calculate the effective dopant concentration of microalgae suspension, which represents the microalgae dopants summed with the intrinsic dopants of the cultivation medium. The cultivation medium intrinsic dopants are embedded and can be found by subtracting it from the effective dopant concentration value. The number of cells in a suspension (θ) is estimated using Eq. (3.1) [51]

$$\theta = |(N_S - N_M)(D_S - D_M)|$$  \hspace{1cm} (3.1)

where, \((N_S)\) and \((N_M)\) are the doping concentrations of the suspension containing the microalgae particles and that of the cells free medium, respectively, and \(D_S\) and \(D_M\) are the Debye volumes of both suspension and medium, respectively. The doping concentrations \((N)\) and the Debye volumes \((D)\) are determined from the capacitance–voltage (CV) measurements, as given in Eqs. (3.2) [52] and (3.3), respectively:

$$N = \left[0.5q\varepsilon_S A^2 \left(\frac{d(1/\varepsilon^2)}{dv}\right)^{-1}\right]^{-1}$$  \hspace{1cm} (3.2)

$$D = L_D^3$$  \hspace{1cm} (3.3)
where, \( q \) is electron charge, \( \varepsilon_s \) is the dielectric constant of the suspension (or medium), \( A \) is the overlapping area, \( C \) is the measured capacitance, \( V \) is the applied voltage, and \( L_D \) is Debye electrical length, determined by Eq. (3.4) [53]

\[
L_D = \sqrt{\frac{\varepsilon K T}{q^2 N}}
\]  

(3.4)

where, \( K \) is Boltzmann constant \((1.38 \times 10^{-23} \text{ J/K})\), and \( T \) is the kelvin temperature [54].

### 3.4 Experimental Setup

To conduct the experiment, different microalgae strains were used and analyzed in this study. The first step in this approach is to have the microalgae strain and cultivate it in a photo-bioreactor with its corresponding medium based on the type of algae used under specific conditions of light and nutrients. To perform the electrical measurements, samples of microalgae suspension were collected from the bioreactor over predetermined periods of time, as well as samples from the control medium. They were loaded into an open-ended coaxial capacitance structure, and modeled as a dielectric material. The coaxial capacitance adaptor was used as a the host structure and it has an inner and outer conductors with dimensions of 2 mm and 5 mm, respectively, and a length of 7mm.

The main advantages behind using this topology is that the radio frequency signal propagations are protected from the outside interferences that could lead to noise results and the signals do not escape between the inner and outer conductors [55]. The host structure is directly connected to the electrical analyzer (Gamry Reference 3000).

The different set of electrical measurements for both the microalgae suspensions and their relative media were performed including impedance, capacitance–voltage (CV), and current–voltage (IV) measurements. The full diagram
of the experimental setup of the proposed electrical based technique is depicted in Figure 3.6.

The main component of this experiment which is the electrical analyzer (Gamry Reference 3000) that is used to make measurements on electrochemical cells that has the conductivity property. The electrical analyzer has a current USB potentiostat with 11 current ranges from 3 amps to 300 picoamps of high performance, and a voltage that reaches up to 32 volts. The measurements are conducted over a range of frequency from 10 μHz to 1 MHz using an electrochemical impedance spectroscopy. Performing impedance measurements was done over a range of 100 mHz to 100 KHz with an oscillation applied at a voltage of 15 mVpp. Frequency range selection was based on measurements conducted earlier for different microalgal strains at high frequency from 1 MHz to 13.5 GHz. A better identification of microalgae frequency responses was found on the range 100 mHz to 100 KHz. This will make identification and characterization of microalgae much easier using its
specific "signature", as well as exploring its viability for titer quantification. Power signals are produced at a range of frequencies using the radio frequency generators.

The device is also used to measure current–voltage, capacitance–voltage, polarization as well as charging/discharging profiles with the ability to change some parameters based on the measurement requirements. The system was calibrated before the starting of the experiment using the provided manufacturer calibration kit to guarantee that the measurements actually present the samples under test. Any losses or phase shifts are excluded in order to avoid adding noise to the measured signal, hence: the reference was moved to the test cables ends [56].
Chapter 4: Results and Discussion

To demonstrate the concept and operational principle, different microalgal strains were used and to be tested and analyzed in this study, namely *Nannochloropsis* sp. and *Tetra*, which are a marine strains, and *Scenedesmus* sp., which is a fresh water strain. This chapter discusses the results obtained from the conducted experiments on microalgae for cell quantification.

A set of electrical measurements were performed on the microalgae samples and relative media. They were collected at predetermined periods of times and loaded into a coaxial capacitance cable to perform the experiment. The setup was calibrated before measurement to ensure that it actually presents the sample under test. The calibration excludes the effect of losses and phase shifts due to the cables and host structure which could add noise to the measured signal.

The impedance magnitude and phase of the all the tested microalgae suspensions and their corresponding blank medium were measured at mid-band frequency of the capacitive region to ensure the capacitive behavior of the sample under test. A sample of *Nannochloropsis* suspension and control medium measurements conducted is depicted in Figures 4.1 and 4.2. These graphs represent the phase and the magnitude of the impedances of the control medium and the suspension, respectively. This shows that the DC field polarizes the algae due to the induced charging effect. Figure 4.3 (a) and (b) show how the impedance magnitude and phase change over time for measurements on the same type of strain *Scenedesmus* over a period of time. The microalgae cell exhibits a capacitive behavior and it increases with time. This is shown from the impedance phase degree that ranges from $-60^\circ$ to $-90^\circ$. 
Figure 4.1: Electrical measurements of *Nannochloropsis* magnitude measurements versus frequency of microalgae particles compared to the medium profile.

Figure 4.2: Electrical measurements of *Nannochloropsis* impedance phase versus frequency of microalgae particles compared to the medium profile.
Figure 4.3: Impedance measurements: (a) phase versus frequency and (b) magnitude versus frequency over different periods of time.
The IV and CV measurements are represented in Figures 4.4 and 4.5 respectively. The IV profile exhibited a schottky-like diode performance of typical p-semiconductor material since the “turn on” voltage is of positive values. The CV measurements revealed that the microalgae particles exhibited a higher dielectric constant than their relative blank medium. The host structure used in these DC voltage measurements was an open-ended coaxial cable that forms a capacitance wherein the microalgae suspension acts as its dielectric material. The CV profile displays a smooth frequency behavior with a higher capacitance of the suspension compared to the cultivation medium.

![Figure 4.4: Electrical measurements of Nannochloropsis Current-Voltage (IV) versus bias profile](image-url)
The difference between the measured capacitances is due to the volume of microalgae particles suspended in the medium and their intrinsic properties. As a result, the microalgae demonstrate a semiconductor behavior, which could help in determining the cells count of the microalgae in a medium. Along with the electrical measurements conducted, the cell count and the size distribution of microalgae particles were determined using the qMicro.

The qMicro tool allows one to measure the size of biological or synthetic particles in a size range between 1-300 μm in a volume of 1 μl to 1 ml (IZON, New Zealand). The cell concentration versus size distribution is depicted in Figure 4.6. The basic premise of the qMicro tool is the principle of resistive pulse sensing (RPS) which monitors the current flow through a pore, allowing the ionic current passing through the pore and particles to be regulated by adjusting the pore size. The cost of this device
is high, and requires qualified and well-trained people to handle the experiments and understand the analysis of the result which is done automatically by the software. qMicro provides the distribution of cell sizes and counts directly. It measures the individual microparticles sizes in a solution and counts the number of particles in the loaded analysis volume to calculate the cell concentration. There is a linear relationship exists between the change in the electric resistance that results from the passage of the ionic current, and this value is correspondent to the different particles volume. A linear calibration curve to a series of calibration particles of various diameters is created and then applied to calculate the size of "unknown" microparticles.

The procedure is a bit lengthy; it requires around 30 minutes for each sample to be measured because at each experiment, the device need to be calibrated with the conductive solution and then measure the suspension sample. The real-time scanning of the pore conductivity at different stretches enables the detection and discrimination of microparticles in a mixed multimodal suspension. However, when using a single micropore in an experiment, the size distribution cell detection is limited by the pore size. As shown in Figure 4.6, the total number of microalgae particles, which is the sum of all existing different sized particles, is of $10 \times 10^6$ particles per 1 ml. The micropore filter size used was of 25 $\mu$m size which only allowed the size in the range of 1 to 50 $\mu$m to pass [28, 57].
The electrical measured data was then used with the help of equations 3.1 – 3.4 to determine the microalgaecell's count. Before this step, the measurements conducted on each sample were processed with a developed program in MATLAB based on the theory explained earlier. As mentioned previously, the proposed methodology utilizes the use of semiconductor model. The extractions of the semiconductor parameter set associated with each measurement are carried out through fitting the resulted Current-Voltage (IV) and Capacitance-Voltage (CV) curves. The fitting procedure is based on constructing a curve using a mathematical formula that best fits the set of plotted data.

The simple result of IV measurements can be used to provide a large amount of useful information. By fitting the forward characteristics of the IV curve, two types of fitting were used in order to extract the first set of parameters: with a third degree polynomial represented by Eq. (4.1) the leakage current \( I_0 \) and conductivity \( \sigma \) are
extracted while fitting the IV measurements with exponential fitting is represented by Eq. (4.2) [58], respectively, the ideality parameter ($\eta$) is determined. The mobility could be determined correspondingly using Eq. (4.3) [59].

\[ I = I_0 + \sigma V + kV^2 + \gamma V^3 \]  
(4.1)

\[ I = I_0 \exp(q V / \eta k T) \]  
(4.2)

\[ \mu = \sigma / q \eta \]  
(4.3)

where $I_0$ is the saturation current, $K$ is Boltzmann constant of $1.3806488 \times 10^{-23}$ J/K, $T$ is the test temperature of 300K, and $q$ is the electron charge of $1.60217657 \times 10^{-19}$ C. $k$ and $\gamma$ are voltage coefficients.

CV measurements are performed in forward bias with a limited DC voltage bias from $-1$V to $+1$V. Rather than plotting dC/dV, it is sometimes desirable to view the data as $1/C^2$ vs. voltage because some parameters are related to this type of data. For example, the doping density ($N$) was derived from the slope of the linear region of the CV curve, and with the help of Eqs. (4.4 - 4.6) [53.60], the doping concentration ($N$) and the dielectric constant ($\varepsilon_r$) are then computed.

\[ N = 2/q \varepsilon_r A^2 [d(1/C^2)/dV] = 2/q \varepsilon_r A^2 (\text{slope}) \]  
(4.4)

\[ C = 2\pi \varepsilon_l l / \ln(b/a) \]  
(4.5)

\[ \varepsilon_r = \varepsilon_r \varepsilon_0 \]  
(4.6)

where $A$ is the capacitor area ($94.2478$ mm$^2$), and $C$ is the represented coaxial cable capacitance. $\varepsilon_0$ is the vacuum dielectric permittivity ($8.85 \times 10^{-12}$), $\varepsilon_r$ is the effective permittivity of the suspension, $l$ is the cable length, $a$ and $b$ are the inner and outer radiuses, respectively. Next, the Debye Length ($L_D$), bulk potential ($\phi$) and the
depletion width ($W$) are computed using Eqs. (4.6 and 4.7) [54] and Eq. (4.9) [61], respectively.

$$L_D = \sqrt{\frac{\varepsilon, K T}{q^2 N}}$$  \hspace{1cm} (4.7)

$$\phi = K T \ln\left(\frac{N}{\eta}\right)/q$$  \hspace{1cm} (4.8)

$$W = \sqrt{\frac{4\varepsilon, \phi}{N q}}$$  \hspace{1cm} (4.9)

For the data presented in Figures 4.1 - 4.2, and 4.4 - 4.5, the corresponding cell count is found to be of $8.7 \times 10^6$ cells in a volume of one milliliter. This difference between the qMicro and our electric technique is most likely due to the fact that any "impurities" comparable to electron sizes ($\approx 2.82 \times 10^{-13}$ cm) were calculated by our method, while qMicro detection has a size limitation of the micropore filter used.

The average time taken for microalgae cell counting using the electrical technique was about 5 minutes. This is potentially less than all other conventional techniques, and was attained without any time consuming sample preparation or treatment steps. It is worthwhile to mention that the time needed to perform the qMicro was about 30 minutes as it needs to first perform a calibration on each sample and then the sample measurements, while with the current proposed technique, there was no need for sample preparation.

The accuracy and reproducibility of the presented method was validated by repeating the electrical measurements both the current-voltage and capacitance-voltage for multiple microalgae strains prepared at different times. For further validation and statistical analysis, a total of 16 samples were considered in this study. Table 1 shows the types of microagal cells tested along with their properties. The cell size range, size average, and physical count were measured using the qMicro equipment. Next, the corresponding IV and CV measurements for each sample were
conducted using the Gamry 3000. Figures 4.7 and 4.8 show a set of current-voltage (IV) and capacitance-voltage (CV) profiles for the 16 microalgae samples, respectively. As can be seen, the measurements for each sample differed from the others and these results were strongly dependent on the intrinsic property of the material tested. This led to different IV and CV sample profiles.

Table 4.1: Description of the microalgae samples tested and their results using the qMicro tool

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Microalgal Strain Tested</th>
<th>Size Range (μm)</th>
<th>Size Average (μm)</th>
<th>Electrical Cell Count</th>
<th>qMicro Cell Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Nanochloropsis</em></td>
<td>7.85 - 36.11</td>
<td>21.98</td>
<td>426</td>
<td>1707</td>
</tr>
<tr>
<td>2</td>
<td><em>Nanochloropsis</em></td>
<td>2.05 - 24.11</td>
<td>13.06</td>
<td>4043</td>
<td>968</td>
</tr>
<tr>
<td>3</td>
<td><em>Nanochloropsis</em></td>
<td>2.99 - 23.01</td>
<td>13.00</td>
<td>1716</td>
<td>1303</td>
</tr>
<tr>
<td>4</td>
<td><em>Nanochloropsis</em></td>
<td>2.77 - 17.32</td>
<td>10.05</td>
<td>10133</td>
<td>3273</td>
</tr>
<tr>
<td>5</td>
<td><em>Tetraselmis</em></td>
<td>2.16 - 18.32</td>
<td>10.24</td>
<td>1143</td>
<td>792</td>
</tr>
<tr>
<td>6</td>
<td><em>Tetraselmis</em></td>
<td>3.96 - 27.14</td>
<td>15.55</td>
<td>1723</td>
<td>410</td>
</tr>
<tr>
<td>7</td>
<td><em>Scenedesmus</em></td>
<td>2.47 - 14.02</td>
<td>8.25</td>
<td>1723</td>
<td>758</td>
</tr>
<tr>
<td>8</td>
<td><em>Scenedesmus</em></td>
<td>2.29 - 21.67</td>
<td>11.98</td>
<td>1723</td>
<td>8314</td>
</tr>
<tr>
<td>9</td>
<td><em>Scenedesmus</em></td>
<td>3.87 - 36.42</td>
<td>20.15</td>
<td>1723</td>
<td>3764</td>
</tr>
<tr>
<td>10</td>
<td><em>Nanochloropsis</em></td>
<td>1.44 - 15.24</td>
<td>7.84</td>
<td>1806</td>
<td>10018</td>
</tr>
<tr>
<td>11</td>
<td><em>Nanochloropsis</em></td>
<td>6.54 - 14.49</td>
<td>10.52</td>
<td>1806</td>
<td>863</td>
</tr>
<tr>
<td>12</td>
<td><em>Nanochloropsis</em></td>
<td>7.73 - 45.13</td>
<td>26.43</td>
<td>8453</td>
<td>8289</td>
</tr>
<tr>
<td>13</td>
<td><em>Nanochloropsis</em></td>
<td>7.78 - 33.37</td>
<td>20.58</td>
<td>30063</td>
<td>41324</td>
</tr>
<tr>
<td>14</td>
<td><em>Nanochloropsis</em></td>
<td>7.53 - 23.45</td>
<td>15.49</td>
<td>873</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td><em>Nanochloropsis</em></td>
<td>7.78 - 49.52</td>
<td>28.65</td>
<td>1063</td>
<td>3654</td>
</tr>
<tr>
<td>16</td>
<td><em>Nanochloropsis</em></td>
<td>8.81 - 57.61</td>
<td>33.21</td>
<td>333</td>
<td>176</td>
</tr>
</tbody>
</table>
Figure 4.7: Current-voltage (IV) measurement curves for the 16 samples presented in Table 1 conducted at 10 Hz. The y-axis (logarithmic scale) unit is amperes and the x-axis (linear) unit is volts.
Figure 4.8: Capacitance-voltage (CV) measurement curves for the 16 samples presented in Table 1 conducted at 10 Hz. The y-axis (logarithmic scale) unit is Farad and the x-axis (linear) unit is volts.
A comparison of the extracted cell count with the electrical method and the qMicro are shown in Figure 4.9(a). To determine the limit of detection of the proposed method, a known concentration of the *Nannochloropsis* microalgae cells were diluted to different concentrations in their relevant cultivation control medium. The diluted samples were measured using both the qMicro and the proposed method. Figure 4.9(b) shows that the limit of detection using the Gamry 3000 was excellent with 20 cells per milliliter volume. The sensitivity and the limit of detection could be further improved by using microfluidic channels which would allow one to quantify a single cell.
Figure 4.9: Comparative analysis of the microalgal cell count performed by qMicro and electrical methods: (a) qMicro vs. Gamry (b) Test of the sensitivity of detection of the electrical method using Gamry
To further validate the presented approach, the cell count of the *Nannochloropsis* microalgae suspension reported earlier was counted also using the light spectrophotometer and the well-known hemocytometer method. The obtained results from the different techniques, qMicro, current approach, spectrophotometer and hemocytometer for the same suspension are presented in Figure 4.10. A summary of the comparison between the performances of the mentioned techniques is presented in Table 4.2.

![Comparative of the cell count using different methods](image)

*Figure 4.10: Comparative of the cell count using different methods*
<table>
<thead>
<tr>
<th>Technique</th>
<th>Sample preparation</th>
<th>Time for result</th>
<th>Cost</th>
<th>Accuracy and precision</th>
<th>Result analysis</th>
<th>Training / skilled personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemocytometer</td>
<td>Easy</td>
<td>7-10 min</td>
<td>cheap but requires light microscope (~ 2000 $)</td>
<td>No, SD is very high</td>
<td>Simple calculation</td>
<td>Training for precision</td>
</tr>
<tr>
<td>Spectrophotometer</td>
<td>Easy</td>
<td>Reading 30 seconds</td>
<td>100 $ and higher for better specifications</td>
<td>99.9% For this application-controls needed calculate the relative cell density</td>
<td>Compare to the control graph</td>
<td>No</td>
</tr>
<tr>
<td>Electrical method</td>
<td>Not needed</td>
<td>5 min</td>
<td>Low Cost</td>
<td>Yes</td>
<td>Simple calculations</td>
<td>No</td>
</tr>
<tr>
<td>qMicro</td>
<td>Easy</td>
<td>30 min</td>
<td>Expensive</td>
<td>Yes, for a specific size range depending on the size of the micropore</td>
<td>Done automatically by the software</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Chapter 5: Conclusions and Future Work

5.1 Conclusions

The aim of this work described in the thesis was to explore and demonstrate the use of an electrical based technique based on capacitance-voltage measurements to enumerate the microalgae cell counts in a suspension. The methodology characterizes microalgae cells using the dielectric spectroscopy and semiconductor theory.

The basic idea of the presented approach is that microalgae suspension that includes cells and the medium get polarized when they are exposed to the electric field application. The strength of polarization is mainly dependent on the composition medium as well as the composition of the cells: the chemical constituents of the microalgae particle. Each of the microalgae suspensions under test were exposed to three types of measurements which are impedance spectroscopy, Current-Voltage (IV), and Capacitance-Voltage (CV). The application of voltage to the suspension would lead to the alteration of the electric field in both magnitude and phase. The cells count was then estimated by calculating the dopant concentration of the suspension, and de-embedding the contribution of the cultivation medium. The difference between both dopant concentrations was found and multiplied by the different of the corresponding volumes for both the suspension and medium.

This technique provides a better combination of high sensitivity and quick response and overcomes the problems of the previous technologies. When compared with other conventional counting techniques, the developed approach was found to be the fastest and cheapest. The characterization experiment potentially gives extra information about each single particle of microalgae which are difficult to get in real
time with other methods. This would include information about the lipids, carbohydrates, and proteins content in the cell. In addition to that, measurements can be conducted with no preprocessing or pretreatments for the samples under test.

The applied technique is suggested to be applicable for all types of microalgae and other types of biological cells as long as the cells get polarized after the application of an electric field. The sensitivity of the method was demonstrated by diluting a known concentration of cells with the corresponding cultivation medium solution, revealing a detected limit of ~20 cells per milliliter. Hence, this reveals that the proposed technique is sufficiently sensitive to allow the detection of cells present during the early stage of growth cycle. Multiple experiments demonstrated good accuracy and prediction.

Overall, the project was successful and has been shown that cell counting measurements using the electrical characterization technique is done rapidly and the processing was achieved using relatively simple mathematical relations.

5.2 Future Work

Electrical characterization technique of microalgae cells count is solidly based on theory. There are several potential applications that could be suggested as a future work for this project. The recommendations are as follows and include:

- Upgrade the implementation of the system to be applied in-situ with a feedback control loop which will not only pave the way for direct and rapid cell counting, but also enable the continuous monitoring and optimization of the microalgae growth process.
- Implement the proposed methodology to determine lipids, proteins, and carbohydrates contents.


List of Publications


- L. Saqer, M. Al Ahmad, H. Taher, S. Al-Zuhair and A. Al Naqbì. "Novel Electrical Based Technique for Microalgae Lipid Content Quantification", in UAE Graduate Research Conference (UAE GSRC) 2015, Abu Dhabi, UAE.

- L. Saqer, M. Al Ahmad, UAEU Annual Research and Innovation Conference 2015 (Abstract #83).

Appendix

Microalgae Strains and Culture Medium

Both freshwater and marine strains of microalgae were tested in this study. The fresh water strain *Scenedesmus* sp. culture was obtained from Algal Oil Company, Philippines, and cultivated in a modified Bassel medium (+3N-BBM) composed of (mM) 0.17 CaCl$_2$·2H$_2$O, 0.3 MgSO$_4$·7H$_2$O, 1.29 KH$_2$PO$_4$, 0.43 K$_2$HPO$_4$, 0.43 NaCl, 1 mLL$^{-1}$ of Vitamine B$_{12}$, and 6 mLL$^{-1}$ of P-IV solution that consisted of 2 Na$_2$EDTA·2H$_2$O, 0.36 FeCl$_3$·6H$_2$O, 0.21 MnCl$_2$·4H$_2$O, 0.37 ZnCl$_2$, 0.0084 CoCl$_2$·6H$_2$O and 0.017 Na$_2$MoO$_4$·2H$_2$O.

The marine strains *Nannocloropsis* and *Tetra* were obtained from a local marine environment Umm Al-Quawain Marine Research Center, UAE. They were grown and obtained in the F/2 medium (32 ppt salinity) consisting of the following major nutrients (in μM): 880 NaNO$_3$, 36 NaH$_2$PO$_4$·H$_2$O, 106 Na$_2$SiO$_3$·9H$_2$O, 1 (mL$^{-1}$) of: vitamin B$12$, biotin vitamin, and thiamine vitamin solutions, and 1 (mL$^{-1}$) of trace metals solution that consisted of (μM): 0.08 ZnSO$_4$·7H$_2$O, 0.9 MnSO$_4$·H$_2$O, 0.03 Na$_2$MoO$_4$·2H$_2$O, 0.05 CoSO$_4$·7H$_2$O, 0.04 CuCl$_2$·2H$_2$O, 11.7Fe(NH$_4$)$_2$(SO$_4$)$_2$·6H$_2$O, and 11.7 Na$_2$EDTA·2H$_2$O. The prepared media were sterilized in an autoclave (Hirayama HV-50, Japan) at 121°C for 15 min and cooled to room temperature prior to use.