RESEARCH ARTICLE
Eurasian J Bio Chem Sci, 7(2):132-138, 2024
https://doi.org/10.46239/ejbcs.1516661

EJBCS

Eurasian Journal of Biological and Chemical Sciences

Journal homepage: www.dergipark.org.tr/ejbcs

Impact of growth medium components on absorbance-viable cell count correlation and cell surface area of *Cereibacter sphaeroides* O.U.001

Kader Çakır, Gökhan Kars*
Necmettin Erbakan University, Faculty of Science, Department of Molecular Biology and Genetics, Konya, Turkey

*Corresponding author : gkars@erbakan.edu.tr
Orcid No: https://orcid.org/0000-0002-2507-2305

Received : 15/07/2024
Accepted : 07/11/2024

To Cite / Atıf için: Çakır K, Kars G. 2024. Impact of growth medium components on absorbance-viable cell count correlation and cell surface area of *Cereibacter sphaeroides* O.U.001. Eurasian J Bio Chem Sci, 7(2):132-138 https://doi.org/10.46239/ejbcs.1516661

Abstract: Today different methods are used in microbiology laboratories to monitor the growth and development of bacteria. Optical density measurement is one of the most preferred methods as being fast, practical and low cost. On the other hand, it cannot differentiate between living and non-living cells in the culture. Moreover, alteration in cell size may lead to the variations in the measurements. As a result, optical density measurements alone may cause wrong experimental results. Therefore, it is necessary to determine the absorbance-living cell number relationship for each specific culture condition. In this study, *Cereibacter sphaeroides* O.U.001 was cultured under four different culture conditions regarding the type of carbon and nitrogen sources (Malate/Glutamate, Molasses/Glutamate, Malate/N_2 and Acetate/Glutamate) and the effect of medium composition on cell size and absorbance-viable cell count relationship was investigated. Equations were obtained from curves drawn as optical density (x-axis) against CFU/mL (y-axis). Field emission scanning electron microscope was used to observe the effects of medium compositions on the size of *Cereibacter sphaeroides* O.U.001. It was revealed that the composition of the medium affected the absorbance-number of living cells relationship. Furthermore, it was evidenced that the size of the cells was changed significantly upon changing the medium composition. To conclude, it is advised that before performing experiments in which the cell numbers are significative, a comparative calibration curve for optical density measurement-the living cell number relationship should be established for more accurate results.

Keywords: *Cereibacter sphaeroides*, CFU/mL, FE-SEM, Viable Cell Count

1. Introduction

The purple non-sulfur (PNS) photosynthetic bacteria are versatile group of organisms that can grow as photoheterotrophs, photoautotrophs or chemoheterotrophs. They can switch from one mode to another depending on the environmental conditions. PNS bacteria have important morphological, biochemical, and metabolic properties, as well. Thanks to their wide range of metabolic properties, they are able to cope with different physiological or environmental conditions (Basak and Das 2007; Madigan and Jung 2009; McEwan 1994). PNS bacteria, which are widely distributed in nature, are found in mud, wastewater, lake water and pool water. Representatives of this group of bacteria are *Cereibacter sphaeroides* (recently renamed from *Rhodobacter sphaeroides*), *Rhodobacter capsulatus*, *Rhodospirullum rubrum* and *Rhodopseudomonas palustris* (Kars and Alparslan 2013; Myung et al. 2004).

Cereibacter sphaeroides, which is a gram-negative bacterium, has the ability to adapt and grow in variety of environmental conditions (Vermeglio and Joliot 1999). It has been used in the laboratories for many years for basic studies like photosynthesis, respiration, CO_2 and N_2 fixation (Kars and Gündüz 2010). Moreover, *C. sphaeroides* is a biotechnologically versatile microorganism capable of producing many biotechnologically valuable chemicals like hydrogen (Kars and Alparslan 2013), 5-aminolevulinic acid (5-ALA) (Sasaki et al. 1990), polyhydroxybutyrate (PHB) (Lee et al. 2020), B12 and Coenzyme Q10 (Sasaki et al. 2005), The type and amount of carbon and nitrogen sources are determinative for the biotechnological product that *C. sphaeroides* can produce (Basak and Das 2007). For instance, hydrogen could only be produced under anaerobic conditions preferentially using fixed nitrogen sources like glutamate (Danış et al. 2022). Ammonia can also be used but its concentration needs to be below 2 mM (Akköse et al.

2009). PHB, for instance, could favorable be produced under high carbon to nitrogen ratios (Akpınar 2023). *C. sphaeroides* is an excellent model organism for studying nitrogen fixation, as well (Joshi and Tabita 1996). In PNS bacteria including the *C. sphaeroides*, the conversion of nitrogen gas to ammonia is catalyzed by the enzyme nitrogenase which is activated under diazotrophic conditions (under the atmosphere of nitrogen gas and in the absence of ammonia) in the absence of oxygen (Kars and Gündüz 2010). Under these conditions, the nitrogenase enzyme can convert free nitrogen gas into ammonia and then use it for cellular metabolism. The nitrogenase enzyme is known to be directly linked to the production of hydrogen. If the culture is devoid of free nitrogen gas, the nitrogenase enzyme can catalyze the conversion of protons into molecular hydrogen under anaerobic conditions in the light.

Since *C. sphaeroides* is a model bacterium for many basic metabolic activities and used as a chassis for the production of many high value-added products, it has been cultivated under different physiological conditions using various types of carbon and nitrogen sources. In these studies, starting with the correct number of cells is of great importance to get high quality data. Spectrophotometric optical density (OD) measurement at 600 nm or 660 nm is one of the mostly preferred and common methods in these studies. One of the most important reasons for the preference of spectrophotometric measurement, which is often used in laboratories to monitor the growth and development of bacteria, is that it is less time consuming and practical. Studies are being conducted by assuming that the optical density (OD) is completely proportional to the number of cells (Stevenson et al. 2016). However, this method does not directly measure viable cell number, but rather measures the turbidity of the cultures which contain both live and death cells (Beal et al. 2020; Sutton 2011). It was stated that environmental factors like temperature, water activity and pH influence the relationship between OD measurement and viable cell count (Francois et al. 2005). For example, Jorgensen et al. demonstrated that high osmotic stress resulted in elongation of *L. monocytogenes* (Jorgensen et al. 1995). Consequently, this morphological change affected the OD measurement-viable cell count relationship. The OD values do not mean the number of cells. This is because the capacity of bacteria to multiply varies with each different environment where they are found. Although this situation is already known to most researchers, it is ignored due the simplicity of the spectrophotometric measurement. However, ignoring the fact that the relation between OD values and live cell number depends on the culture conditions may lead to false results during the microbiological studies. Especially in sensitive techniques such as polymerase chain reaction (PCR), RNA-Seq and microarray, the result is strongly dependent on the initial cell number. In these techniques, live cells are generally preferred to obtain reliable and high-quality data, as they preserve the integrity of nucleic acids, allowing for the complete and accurate analysis of the desired genetic material. When working with dead cells, however, the degradation of DNA and RNA is likely, which can lead to incomplete or erroneous data. If the use of dead cells is unavoidable, special care should be taken during sample preparation to maintain nucleic acid stability and minimize degradation (Nkuipou-Kenfack et al. 2013). Although quantification and adjustment of the initial amount of RNA or DNA help to avoid false results, starting with an equal number of cells for each experimental group would be more meaningful. Furthermore, in some other biotechnological studies, the experiments are being conducted by just starting with the cultures with similar OD values to equalize conditions without further considerations.

In this study, absorbance-viable cell number relationship of *C. sphaeroides* under four different culture conditions regarding the type of carbon and nitrogen sources (Malate/Glutam ate, Molasses/Glutamate, Malate/N_2 and Acetate/Glutamate) was investigated. The sizes of the cells were also measured by a field emission scanning electron microscope (FE-SEM) under the same conditions to see if culture conditions have any effects on the morphology of the cells.

2. Materials and Method

2.1. Microorganism media and culture conditions

Cereibacter sphaeroies O.U.001 (DMS 5864) was used in this study. Four different growth media were prepared based on carbon and nitrogen content (Malate/Glutamate, Molasses/Glutamate, Malate/N_2 and Acetate/Glutamate) to investigate absorbance-viable cell count relationship of *C. sphaeroides*. The media contents are shown in Table 1.

Table 1. Media components designed regarding the carbon and nitrogen sources

Medium number	Carbon source	Nitrogen source
1	Malate (15 mM)	Glutamate (2 mM)
2	Molasses (28 g/L)	Glutamate (2 mM)
3	Malate (15 mM)	N_2 gas
4	Acetate (40 mM)	Glutamate (2 mM)

The first medium is a minimal medium often used for cultivation of *C. sphaeroides* and is called as Biebl and Pfenning minimal medium (Biebl and Pfennig 1981) medium, which uses malate (15 mM) as a carbon source and glutamate (2 mM) as a nitrogen source, was considered as a control group because it meets the requirements of bacteria at a minimum or basal level. It specifically allows the growth of PNS bacteria. In the second medium, sugar beet molasses obtained from a sugar factory (Konya Şeker, Konya, Türkiye) was used as the carbon source. Sugar beet molasses, which is obtained as a by-product in the process of sugar production, is one of the most economical carbon sources to grow bacteria (Bae and Shoda 2004). Raw molasses contains approximately 48-50% sugars, 1-3% nitrogen, a variety of organic acids (including malic, succinic, fumaric, lactic, acetic, propionic, and formic acids), as well as essential elements such as potassium, sodium, magnesium, calcium, aluminum, zinc, copper, nickel, cobalt, manganese, chromium, and boron, along

with various vitamins. This rich composition positively affects bacterial growth, allowing the bacteria to reach very high cell densities (Kars and Alparslan 2013). Molasses was pre-processed before use due to its complex content. After diluting the molasses with distilled water, it was centrifuged at 9000 rpm for 20 minutes. Then, the supernatant was passed through the 0.22 µm filter to be ready for use. As the third medium, a nitrogen-fixing environment was designed. In this case, malate (15 mM) was used as carbon source and nitrogen gas was used as nitrogen source. The liquid culture was flushed with nitrogen gas for 3 min so that the bioreactor became anaerobic and saturated with the nitrogen gas at the same time (Sert 2022). The remaining content of the medium was the same as the others. In the final medium, acetate (40 mM) and glutamate (2 mM) were chosen as carbon and nitrogen source, respectively. Acetate is especially preferred in the PHB production processes since acetate catabolism uses the same pathway with PHB production metabolism in *C. sphaeroides* (Kars and Gündüz 2010). Thus, significant amounts of PHB can be formed. Acetate is accordingly chosen as a carbon source, and it was included in the study to see if PHB accumulation inside the cell leads the deviations in absorbances.

The main elements for each medium are $MgSO_4.7H_2O$ (0.2 g/L), $CaCl_2.2H_2O$ (0.05 g/L) and NaCl (0.4 g/L). KH_2PO_4 (0.5 g/L) was used as buffer. In addition, trace element, iron sulfate and vitamin solutions were put into the media as documented earlier (Kars and Ceylan 2019). Initial pH of the medium was fixed to 6.9. After sterilization, the media were transferred to 100 mL sterile glass penicillin bottles. Then, they were inoculated by 10 % (v/v) with freshly grown cultures. Argon gas was passed through the medium to provide anaerobic conditions, if needed. To set nitrogen-fixing condition, nitrogen gas was passed through the bottles for 3 minutes instead of argon gas. The light energy was provided by an incandescent lamp (100 W) which was placed at a distance of 30-40 cm. The bioreactors were kept in incubator at 29 °C (Danış et al. 2022).

2.2. Growth curves and cell counting method

In order to compare the absorbance values with the number of living cells, spread plate technique was used (Koch 2006).Simultaneous cell counting and OD measurements were performed to find the number of living cells corresponding to the OD values. The OD values of the samples were measured using a spectrophotometer (Agilent, Cary 60 UV-Vis) at a wavelength of 660 nm. After the first absorbance value was taken at the zeroth hour (t=0), the subsequent measurements were made at 24-hour intervals. After the initial absorbance value was taken at the zeroth hour (t=0), subsequent measurements were made at 24-hour intervals based on previous studies (Sert 2022). For the spectrophotometric measurements above 1, the samples were diluted and then the results were multiplied by the dilution factor to get the exact OD value. The measurements were performed at least twice and then standard error of the mean (SEM) values for each time point were calculated. At the end, OD_{660} nm versus time (h) graphs were drawn. In parallel to OD measurements, culture samples were spread onto the agar plates after several dilutions and bacterial colonies formed on petri dishes were counted. Due to the challenges of converting Molasses-Glutamate and Malate-N_2 gas liquid media to solid media, all cultures were grown on Malate-Glutamate (BP) agar plates for counting. For the total number of bacteria, the number of colonies as colony forming unit (CFU) was calculated according to the equation (Equation 1) (Koch 2006) given below and then graphs were created. The process was repeated at 24-hour intervals. Finally, OD_{660} nm versus CFU/mL graphs were drawn.

$$\frac{CFU}{mL} = number\ of\ colonies \times dilution\ factor \times {}^{1}/_{volume\ transferred\ to\ petri\ dishes\ (mL)}$$

(Equation 1)

2.3. FE-SEM analyses and measurement of cell surface area

Size and morphology of *C. sphaeroides* O.U.001 were examined by a field emission scanning electron microscope (Hitachi SU 1510). To ensure that the morphological structures of the cells were not disrupted, no chemical treatments were applied, and direct FE-SEM images were obtained from *C. sphaeroides* O.U.001 grown in all media until it reached the logarithmic phase. For this purpose, liquid samples of *C. sphaeroides* O.U.001 were collected into Eppendorf tubes and centrifuged at 7000 rpm for 5 minutes. The supernatant was discarded, and the pellet was dissolved in 1 mL of sterile distilled water. 5 µl of cell suspension was placed onto platform (stab). Sample platforms were kept in an incubator at 50 °C for 10 minutes to dry the cells. To ensure the necessary conductivity without compromising the morphological structure of the sample, it was coated with iridium at a thickness of 4.15 nm. (Zheng et al. 2017; Sert 2022). The FE-SEM images of bacteria were then used for the measurement of cell surface area by ImageJ image analysis program (Schneider et al. 2012). For this, ten bacterial cells were selected randomly (N:10) in each of the four groups and the surface area of them were calculated using the image analysis program. Then, one-way ANOVA was performed to see if growth media have any influence on the size of bacteria.

3. Results

3.1. Growth curves under different culture conditions

The growth of bacteria was followed by measuring the absorbances of the cultures by a spectrophotometer at 660 nm. At the time of inoculation, the time was taken as zero and then OD measurements were done at 24-hour intervals. Afterwards, these data were used to draw the growth curves as shown in Figure 1. The highest OD value obtained in each growth media was different from each other. For instance, the highest OD value (4.62) was achieved in Molasses/Glutamate media while the lowest value (1.39) was obtained in Malate/Glutamate medium. The cells attained an OD value of 2.70 in Acetate/Glutamate medium. In the case of Malate/N_2 medium, the highest OD value of the culture was 2.39. In this medium design, carbon source was malate, but the nitrogen source was molecular nitrogen different from the others. In this experimental setup,

nitrogen fixation conditions were set so that the effect of nitrogen fixation on the growth profile was assessed. The OD value achieved in this medium was still higher than that obtained in Malate/Glutamate minimal medium.

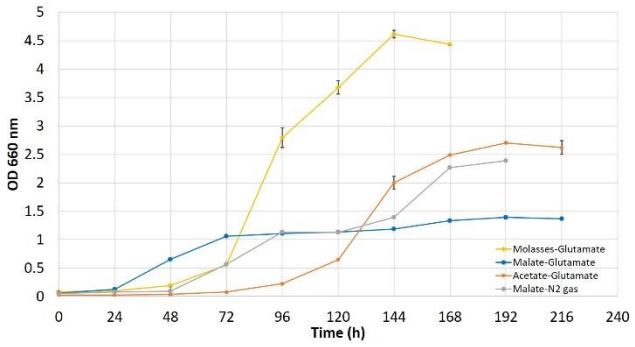

Figure 1. The growth of *C. sphaeroides* in Molasses/Glutamate, Malate/Glutamate, Acetate/Glutamate and Malate/N_2 media

3.2. Absorbance-viable cell count relationship

This section of the study looked at the link between *C. sphaeroides* O.U.001's absorbance and viable cell count in various conditions. A graph (Figure 2) was obtained, with absorbance (OD660 nm) on the x-axis and viable cell number (CFU/mL) on the y-axis. The graph illustrates how there were notable variations in the quantity of live cells with identical OD values. When the equations in the graph were used, an OD value of 1 corresponded to 5.06 x 10^8 , 8.2 x 10^8, 4.2 x 10^8, and 4.6 x 10^8 CFU/mL in Malate/Glutamate, Malate/N_2, Acetate/Glutamate and Molasses/Glutamate media, respectively. When compared to others, the highest value for viable cells per milliliter of culture was achieved in that where nitrogen source was supplied as molecular N_2. On the other hand, when acetate was used as the carbon source, the smallest value for viable cell count per milliliter of culture was achieved.

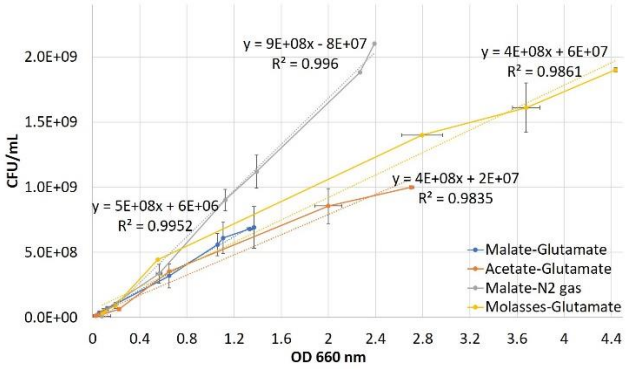

Figure 2. Absorbance-viable cell count relationship of *C. sphaeroides* O.U.001. Error bars indicate standard error of the mean

3.3. Field emission scanning electron microscope analyses and measurement of cell size

Field Emission Scanning Electron Microscopy (FE-SEM) was used to visualize the changes in the cell morphology of *C. sphaeroides* growing under different culture conditions. Moreover, the FE-SEM images (Figure 3) were used to measure cell sizes using ImageJ image analysis program (Schneider et al. 2012). In order not to give rise to any deformities in the cell structure, fixatives were not used. Cells were only treated with distilled water before FE-SEM analyses. No distinctive differences in cell morphologies were observed upon examining FE-SEM images (Figure 3). Further analyses with higher magnification might help to unveil the detailed morphological structures in the future studies.

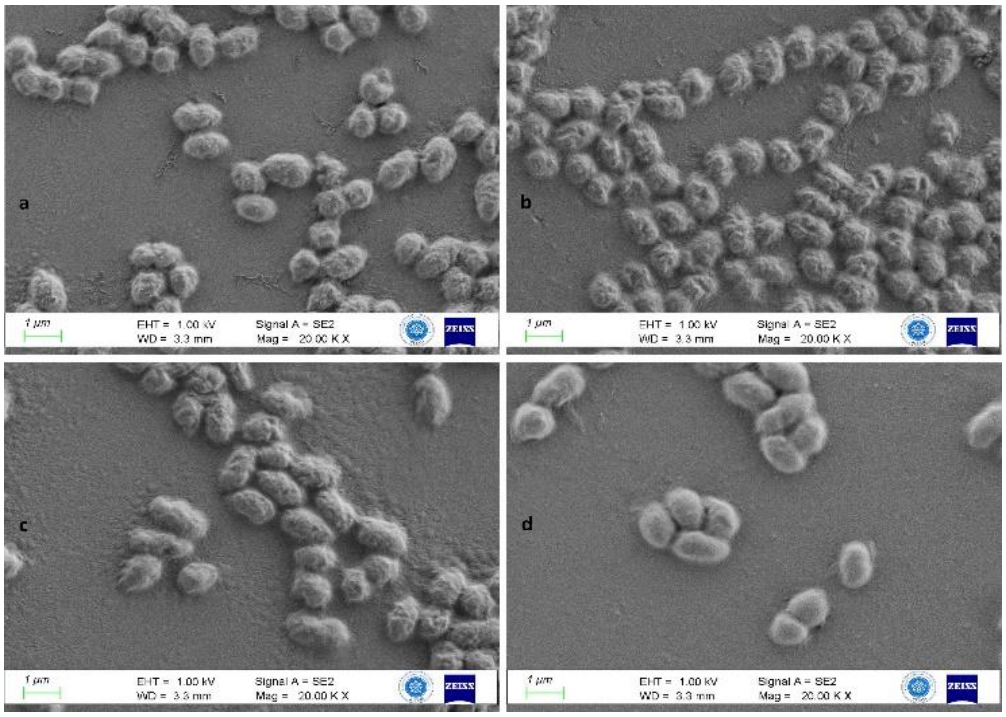

Figure 3. FE-SEM images of *C. sphaeroides* grown in Malate-Glutamate (a), Molasses-Glutamate (b), Malate-N_2 (c) and Acetate-Glutamate media (d)

FE-SEM images of *C. sphaeroides* grown in different growth media were used to analyze cell surface area (Figure 4). Surface area measurements of the cells in each growth media and subsequent one-way ANOVA analysis revealed that there was a statistically significant difference in test scores between at least two groups ($F(2, 36) = 3.777$, $p = 0.019$). Tukey's test for multiple comparisons found that mean surface areas were significantly different between the growth media Malate/Glutamate and Acetate/Glutamate ($p = .031$, 95% C.I. = [-.34637, -.01243]) and Molasses/Glutamate and Acetate/Glutamate ($p = .043$, 95% C.I. = [-.33797, -.00403]). There was no statistically significant difference between the other media ($p > .05$).

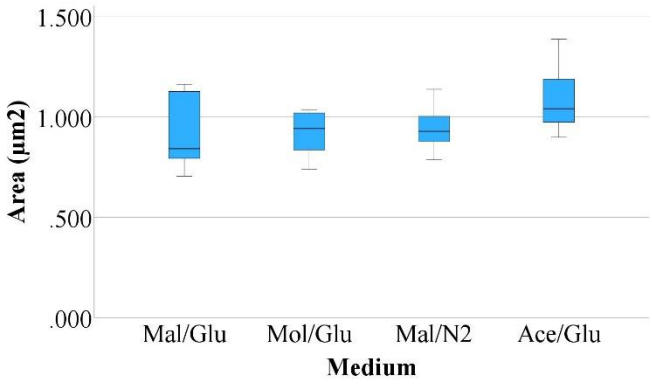

Figure 4. Cell surface area of *C. sphaeroides* grown in different media

4. Discussion

When working with microorganisms, assessing growth is often crucial, and the most commonly used method for measuring growth/growth inhibition is absorbance measurement (Gabrielson et al. 2002). However, it is incorrect to directly relate absorbance values (OD) to live cell number without taking into account the physiological state of the medium, the type of microorganism, and the metabolic products produced. In this study, it was found that the OD value-viable cell count relationship of bacteria grown under different physiological conditions varied significantly.

The growth profiles changed depending on the culture conditions used. For example, the maximum OD value (4.62) was obtained in Molasses/Glutamate media (Figure 1), whereas the lowest (1.39) was obtained in Malate/Glutamate medium (Figure 1). This result was very plausible and reasonable when considering the composition of the medium. The highest cell density was obtained in the medium having the richest composition (Molasses/Glutamate) while the lowest cell density was obtained in the medium with minimal composition (Malate/Glutamate). The result was also consistent with previous findings. In a previous study, the highest cell density for *C. sphaeroides* O.U.001 (OD_{660}: 9.26, 4.54 g cdw/L) was reported to be obtained when using molasses as substrate (Kars and Alparslan 2020). This might be due to the fact that sugar beet molasses has a very rich composition, and it includes around 50% sucrose by weight. Moreover, several types of organic acids and elements were found to be present in molasses (Kars and Alparslan 2013). In the study conducted by Palmonari et al. (2020) the detailed chemical composition of molasses includes sugars, elements, and vitamins, as well as nitrogen sources (such as protein and nitrate). Therefore, it is believed that the highest growth values of *C. sphaeroides* in molasses medium may be attributed to other nitrogen sources. It has been concluded that additional nitrogen sources likely enhance growth and significantly support bacterial development. Molasses is not only a rich substrate but also a cheap carbon source when compared to other commercially available sources. Thus, it is frequently used as a substrate in microbiology studies. Unlike molasses, the Malate/Glutamate medium which was named as minimal medium supported the cell growth at the basal level. As a result, obtaining the lowest cell density while utilizing malate/glutamate medium is highly likely and predictable. This medium, named as Bieble and Pfennig minimal medium, selectively allows the growth of PNS bacteria so that it has specific uses. For instance, *Escherichia coli* strains cannot grow in this minimal medium so that it can be used to select single recombinant *C. sphaeroides* after conjugation in site directed mutagenesis studies (Kars et al. 2008).

In the Acetate/Glutamate medium, the cells reached an OD value of 2.70 (Figure 1). The acetate assimilation pathway differs among bacterial species. Regarding acetate assimilation pathways in PNS bacteria, glyoxylate cycle, citramalate cycle and ethylmalonyl-CoA pathways were declared to exist (Kars and Gündüz 2010). *C. sphaeroides* lacks isocitrate lyase, the enzyme that provides the renewal of C4 acids in glyoxylate cycle. However, studies indicated that another acetate assimilation pathway called as ethylmalonyl-CoA (EMC) was operating in *C. sphaeroides* to metabolize acetate (Kars and Gündüz 2010; Shimizu et al. 2019). This pathway is linked to the production of PHB. Thus, acetate is known to positively affect the production of PHB. In the experiments, it was observed that acetate sufficiently supported the growth of *C. sphaeroides* so that the cells attained considerably high cell density. It is also highly probable that excess amount of acetate was stored in the form of PHB granules in the bacterial cells. The stored PHB granules can then be used as carbon sources and may cause late arrival to the stationary phase as witnesses in this study. The maximum OD value of the culture in the Malate/N_2 media was 2.39 (Figure 1). Malate served as the carbon supply and molecular nitrogen served as the nitrogen source in this particular medium design. The nitrogen fixation settings in this experiment were chosen to evaluate the impact of nitrogen fixation on the growth profile. Even with this medium, the OD value was greater than in the Malate/Glutamate minimum media. Consequently, it was discovered that nitrogen fixation promoted bacterial growth more effectively than glutamate, suggesting that nitrogen gas was a superior nitrogen source to glutamate.

When the association between the absorbance and viable cell count of *C. sphaeroides* O.U.001 in various medium was examined, significant variations in the quantity of live cells with identical OD values were observed, as the graph

illustrates (Figure 2). These variations might result from each cell's unique capacity to absorb light depending on the growth conditions. The ability of cells to absorb light can be influenced by a variety of factors including pigments, internal membrane architecture, granules, and the types of metabolites found inside the cells. For example, when anaerobic conditions are met, a photosynthetic intracytoplasmic membrane (ICM) system forms in the cell (Kars and Gündüz 2010). This internal structure is required for the light reactions of photosynthesis since it houses the photosynthetic machinery that contains carotenoids and bacteriochlorophylls. Owing to the presence of carotenoids and bacteriochlorophylls, these bacteria may display distinct light absorption characteristics. Furthermore, the cells convert excess carbon sources into PHB granules for later use when they are provided with a high amount of carbon source with limited amount of nitrogen or elements. Changes in the cells' ability to absorb light may also result from presence of such granules. Additionally, the growth medium's characteristics might have an impact on the cultures' ability to absorb light. This is particularly true when employing vibrant growth media, such as molasses made from sugar beets. These feedstocks' vibrant colors or particles invariably change the culture's capacity to absorb light. Consequently, the aforementioned discussions should be considered to understand the fluctuations in the absorbance-viable cell number relation of a bacterium under various physiological settings.

Field Emission Scanning Electron Microscopy (FE-SEM) was used to visualize changes in cell morphology of *C. sphaeroides* growing under different conditions. Cell sizes were measured using ImageJ image analysis program. No distinctive differences were observed in cell morphologies, but further analyses with higher magnification could reveal detailed structures. Surface area measurements showed a statistically significant difference in test scores between at least two groups, with significant differences between growth media Malate/Glutamate and Acetate/Glutamate and Molasses/Glutamate and Acetate/Glutamate. Similar to polyhydroxybutyrate biosynthesis, *C. sphaeroides*'s acetate assimilation mechanism follows the ethylmalonyl-CoA pathway. Stated differently, PHB synthesis is a necessary consequence of using acetate as the carbon source. As a result, in the Acetate/Glutamate medium, acetate stimulates the growth of PHB granules inside the cell, allowing the cells to attain a much larger surface area. This might be the reason why the cells in the Acetate/Glutamate medium had the largest surface area.

5. Conclusion

Bacterial cell cultures progress through distinct life stages, namely the lag phase, log phase, stationary phase, and death phase. To track this progression over time, the conventional method employed is spectrophotometric measurement. When using a spectrophotometer for measurement, the absorbance value is influenced not only by the cells but also by the composition of secretory substances and the properties of the growth medium, including its color. Consequently, the relationship between absorbance and the number of living cells varies depending on the specific environment. Furthermore, cells exhibit distinct metabolic activities and produce different metabolites in various conditions, which can also impact absorbance values. Metabolites and bacterial structures resulting from diverse metabolic activities, such as membrane folds formed under photosynthetic conditions, are believed to influence absorbance. Physiological conditions not only determine the relationship between absorbance and viable cell count but can also lead to variations in cell size. As a result of this study, it was proved that cell surface area of *C. sphaeroides* varied significantly upon changing growth media.

In conclusion, despite the inherent limitations of spectrophotometric measurements due to variations in culture conditions, this method can still be useful following adequate control and calibration of the procedure. It is advisable to establish calibration protocols that compare absorbance with viable cell counts before utilizing a spectrophotometer to measure the density of cell suspensions. Once these calibration protocols are tailored to specific culture conditions, spectrophotometric studies can be conducted.

Acknowledgements

This study was supported by TÜBİTAK 2209-A research project support program.

Authors' contributions: KÇ performed the experimental studies and prepared the draft of the manuscript. GK managed the project, performed the experimental studies, made the analyses and prepared the manuscript.

Conflict of interest disclosure

There is no conflict of interest.

References

Akköse S, Gündüz U, Yücel M, Eroğlu I. 2009. Effects of ammonium ion, acetate and aerobic conditions on hydrogen production and expression levels of nitrogenase genes in *Rhodobacter sphaeroides* O.U.001. Int J Hydrogen Energy. 34:8818-8827.

Akpınar BN. 2023. Asetat ortamında büyütülen *Cereibacter sphaeroides* O.U. 001, *Rhodopseudomonas palustris* 7850 ve *Cupriavidus necator* H16 ile polihidroksibütirat (PHB) üretimi ve üretilen polimerlerin karakterizasyonu. N. E. Ü. Fen Bilimleri Enstitüsü, MSc Thesis.

Bae S, Shoda M. 2004. Bacterial cellulose production by fed-batch fermentation in molasses medium. Biotechnol Prog. 20:1366-1371.

Basak N, Das D. 2007. The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art. World J Microbiol Biotechnol. 23:31-42.

Beal J, Farny NG, Haddock-Angelli T, Selvarajah V, Baldwin GS, Buckley-Taylor R, Gershater M, Kiga D, Marken J, Sanchania V, Sison A, Workman CT. 2020. Robust estimation of bacterial cell count from optical density. Commun Biol. 3:512.

Biebl H, Pfennig N. 1981. Isolation of Members of the Family Rhodospirillaceae. In: Starr MP et al (eds.) The prokaryotes: a handbook on habitats, isolation, and identification of bacteria, Springer Berlin Heidelberg, Berlin.

Danış K, Bingöl BN, Kars G. 2022. Production of biological hydrogen and bacterial carotenoids with *Rhodobacter sphaeroides* O.U.001 in a biorefinery concept. Eurasian J Bio Chem Sci. 5:56-61.

Francois K, Devlighere F, Standaert AR, Greeraerd AH, Cools I, Van Impe JF, Debevere J. 2005. Environmental factors influencing the relationship between optical density and cell count for Listeria monocytogenes. J Appl Microbiol. 99:1503-1515.

Gabrielson J, Hart M, Jarelöv A, Kühn I, McKenzie D, Möllby R. 2002. Evaluation of redox indicators and the use of digital scanners and spectrophotometer for quantification of microbial growth in microplates. J Microbiol Methods. 50:63-73.

Jorgensen F, Stephens PJ, Knochel S. 1995. The effect of osmotic shock and subsequent adaptation on the thermotolerance and cell morphology of *Listeria monocytogenes*. J Appl Bacteriol. 79:274-281.

Joshi HM, Tabıta FR. 1996. A global two component signal transduction system that integrates the control of photosynthesis, carbon dioxide assimilation, and nitrogen fixation. Proc Natl Acad Sci. 93:14515-14520.

Kars G, Alparslan Ü. 2013. Valorization of sugar beet molasses for the production of biohydrogen and 5-aminolevulinic acid by *Rhodobacter sphaeroides* O.U.001 in a biorefinery concept. Int J Hydrogen Energy. 38:14488-14494.

Kars G, Alparslan Ü. 2020. Evaluation of high concentrations of sugar beet molasses as substrate for hydrogen and 5-aminolevulinic acid productions. Int J Adv Eng Pure Sci. 32:398-404.

Kars G, Ceylan A. 2019. Hydrogen generation by *Rhodobacter sphaeroides* O.U.001 using pretreated waste barley. Cumhur Sci J. 40:414-423.

Kars G, Gündüz U. 2010. Towards a super H2 producer: Improvements in photofermentative biohydrogen production by genetic manipulations. Int J Hydrogen Energy. 35:6646-6656.

Kars G, Gündüz U, Rakhely G, Yücel M, Eroğlu I, Kovacs KL. 2008. Improved hydrogen production by uptake hydrogenase deficient mutant strain of *Rhodobacter sphaeroides* O.U.001. Int J Hydrogen Energy. 33:3056-3060.

Koch AL. 2006. Growth. In: Reddy CA et al. (eds.) Methods for General and Molecular Microbiology. American Society for Microbiology.

Lee YR, Nur Fitriana H, Lee SY, Kim MS, Moon M, Lee WH, Lee JS, Lee S. 2020. Molecular profiling and optimization studies for growth and PHB production conditions in *Rhodobacter sphaeroides.* Energies. 13:6471.

Madigan MT, Jung DO. 2009. An overview of purple bacteria: systematics, physiology, and habitats. In: Hunter et al (eds.) The purple phototrophic bacteria, Springer, Dordrecht.

McEwan AG. 1994. Photosynthetic electron transport and anaerobic metabolism in purple non-sulfur phototrophic bacteria. A Van Leeuw J Microb. 66:151-164.

Myung KK, Choi KM, Yin CR, Lee KY, Im WT, Ju HL, Lee ST. 2004. Odorous swine wastewater treatment by purple non-sulfur bacteria, Rhodopseudomonas palustris, isolated from eutrophicated ponds. Biotechnol Lett. 26:819-822.

Nkuipou-Kenfack E, Engel H, Fakih S, Nocker A. 2013. Improving efficiency of viability-PCR for selective detection of live cells. J Microbiol Methods. 93:20-24.

Palmonari A. Cavallini D, Sniffen CJ, Fernandes L, Holder P, Fagioli L, Fusaro I, Biagi G, Formigon A, Mammi L. 2020. Characterization of molasses chemical composition. J Dairy Sci. 103:6244-6249.

Sasaki K, Tanaka T, Nishizawa Y, Hayashi M. 1990. Production of a herbicide, 5-aminolevulinic acid, by *Rhodobacter sphaeroides* using the effluent of swine waste from an anaerobic digestor. Appl Microbiol Biotechnol. 32:727-731.

Sasaki K, Watanabe M, Suda Y, Ishizuka A, Noparatnaraporn N. 2005. Applications of photosynthetic bacteria for medical fields. J Biosci Bioeng. 100:481-488.

Schneider C, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 9:671-675.

Sert M. 2022. Diazotrofik koşullarda çoğaltılan *Rhodobacter sphaeroides* O.U 001 ile hidrojen üretimi ve Nifh gen ifade analizi. N.E.Ü. Fen Bilimleri Enstitüsü, MSc Thesis.

Shimizu T, Teramoto H, Inui M. 2019. Introduction of glyoxylate bypass increases hydrogen gas yield from acetate and l-glutamate in *Rhodobacter sphaeroides*. Appl Environ Microbiol. 85:1-17.

Stevenson K, McVey AF, Clark IBN, Swain PS, Pilizota T. 2016. General calibration of microbial growth in microplate readers. BioRxiv. 6:32888.

Sutton S. 2011. Microbiology Topics. Measurement of microbial cells by optical density. J Valid Technol. 17:46-49.

Vermeglio A, Joliot P. 1999. The photosynthetic apparatus of *Rhodobacter sphaeroides*. Trends Microbiol. 7:435-440.

Zheng Y, Cosgrove DJ, Ning G. 2017. High-resolution field emission scanning electron microscopy (FESEM) imaging of cellulose microfibril organization in plant primary cell walls. Microsc Microanal. 23:1048-1054.