DEVELOPMENT OF HELICAL TUBULAR REACTOR FOR HYDROGEN PRODUCING PHOTOSYNTHETIC BACTERIA

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DEVELOPMENT OF HELICAL TUBULAR REACTOR FOR HYDROGEN PRODUCING PHOTOSYNTHETIC BACTERIA

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ABSTRACT

DEVELOPMENT OF HELICAL TUBULAR REACTOR FOR HYDROGEN PRODUCING PHOTOSYNTHETIC BACTERIA

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Photobiological hydrogen production from organic materials occurs with the help of illumination and under aerobic conditions within photobioreactors. Novel designs are needed in order to increase the light conversion efficiency and to improve the biological hydrogen production. In this thesis, purple non sulfur bacteria *Rhodobacter sphaeroides* O.U. 001 was employed as the hydrogen producing microorganism. Two different types of photobioreactors, namely oscillatory helical photobioreactor and recycling helical bioreactor, were devised and successfully operated for bacterial growth and hydrogen production.

Total liquid capacity of the pneumatically driven oscillatory flow helical tubular photobioreactor was 11.5 L, and 4.5 L of which was occupied by the bacterial culture. The bacteria grew very well both in malate-based and acetate-based media

under nitrogen atmosphere. The bacteria sustained their vitality 24 days before the system was shut down.

The recycling helical tubular photobioreactor, which was developed for hydrogen production, had a fully occupied total volume of 6.5L. The bacteria produced approximately 1.9L of hydrogen in four days on malate-based media. The hydrogen production rate was $0.009L_{H2}/L_{culture}$.h.

The effects of molecular nitrogen gas and the sodium glutamate concentration on the growth of hydrogen producing photosynthetic bacteria *Rhodobacter sphaeroides* O.U.001 in the reactor were also examined in 500ml-bottles. The bacterial growth curves did not show any difference at the control medium containing 15mM of acetate and 10 mM of sodium glutamate. However, other bottles containing a lesser amount of N-source was found to grow earlier under the nitrogen atmosphere. Besides, even a 15/2 acetate/sodium glutamate ratio was observed to be sufficient to grow the bacteria for inoculation, and to spend extra sodium glutamate was not necessary.

The novel designs developed in this study aim to improve the biological hydrogen production by photosynthetic bacteria, and to provide new ways in adaptation of photobiological systems to outdoor conditions for large-scale applications.

Keywords: Photobioreactor, photosynthetic bacteria, photofermentative hydrogen production

HİDROJEN ÜRETEN FOTOSENTETİK BAKTERİLER İÇİN HELEZONİK BORU TİPİ REAKTÖR GELİŞTİRİLMESİ

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Fotobiyolojik hidrojen üretimi, anaerobik koşullar altında aydınlatmaya tabi tutularak organik maddelerin parçalanması yoluyla gerçekleştirilebilir. Ancak, ışığın hidrojene daha etkin olarak dönüşebilmesi ve biyolojik hidrojen üretiminin artırılabilmesi için yeni tasarımlara ihtiyaç vardır. Bu çalışmada hidrojen üreten mikroorganizma olarak sülfürsüz bir pembe bakteri olan *Rhodobacter sphaeroides* O.U. 001 suşu kullanılmıştır. Birisi salınımlı helezonik fotobiyoreaktör ve diğeri geri-dönüşlü helezonik fotobiyoreaktör olmak üzere iki çeşit reaktor geliştirilmiş, ve sırasıyla bakteri büyütülmesi ve hidrojen üretiminde başarıyla çalıştırılmıştır.

Hava yardımı ile çalışan salınımlı helezonik fotobiyoreaktörün toplam sıvı kapasitesi 11.5 L idi ve bunun 4.5 L'si bakteri kültürü tarafından işgal edilmekteydi. Bakteriler azot atmosferi altında hem malat-içerikli, hem de asetat içerikli besiyerinde oldukça iyi büyüdüler, ve sistem kapatılana dek canlılıklarını 24 gün süreyle muhfaza ettiler. Hidrojen üretimi için tasarlanan geri-dönüşlü helezonik boru tipi reaktör tümü kullanılan 6.5L'lik bir sıvı hacmine sahipti. Bakteriler malat içerikli besiyerinde dört gün boyunca yaklaşık 1.9L hidrojen ürettiler. Hidrojen üretim hızı $0.009L_{H2}/L_{kültür}$.sa olarak gerçekleşti.

Moleküler azot gazının ve sodyum glutamat derişiminin *Rhodobacter sphaeroides* O.U. 001 suşundan hidrojen üreten fotosentetik bakterilerin büyümeleri üzerindeki etkileri 500ml-hacimli şişeler kullanılarak araştırıldı. 15mM asetat ve 10mM sodyum glutamat içeren kontrol besiyerlerinden elde edilen büyüme eğrileri arasında bir farklılık gözlenmedi. Ancak daha az miktarda azot kaynağı içeren diğer şişelerde bakterilerin azot atmosferi altında daha erken büyümeye başladıkları görüldü. Ayrıca 15/2'lik bir asetat/sodyum glutamat oranının ekim yapılacak bakterileri yetiştirmede yeterli olduğu ve fazladan sodyum glutamat harcamayta gerek olmadığı bulundu.

Bu çalışmada geliştirilen yeni tasarımlar fotobiyolojik hidrojen üretimini geliştirmeyi, ve fotobiyolojik sistemlerin büyük ölçekli uygulamalar için dış dünya koşullarına uyumunu kolaylaştıracak yeni yolların sağlanmasını amaçlamaktadır.

Anahtar kelimeler: fotobiyoreaktör, fotosentetik bakteriler, fotofermentatif hidrojen üretimi.

To my family

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CHAPTER 1

INTRODUCTION

Hydrogen is the most plentiful and the lightest element in the universe, making up about three-quarters of all the matter. However the earth contains very little of it, since the gravity of earth cannot hold free-hydrogen molecules from leaving off out to the space.

Hydrogen is considered as the energy for future since it is a clean energy source with high energy content as compared to hydrocarbon fuels. Today global energy requirements are mostly dependent on fossil fuels. This will eventually lead to the consumption of limited fossil energy resources. Moreover, the fossil fuels are causing global climate change mainly due to the emission of combustion products. Combustion of hydrogen produces mainly water; therefore it is a clean fuel.

Hydrogen can be produced from fossil fuels, biomass, and water. However, the biological hydrogen production is the most environment-friendly and the least energyintensive process. There are a few biological hydrogen production techniques. Hydrogen can be produced via (a) biophotolysis of water by algae and cyanobacteria, (b) photodecomposition of organic compounds by photsosynthetic bacteria, (c) fermentative hydrogen production from organic compounds, and (d) hybrid systems using photosynthetic and fermentative bacteria. The vessel systems employed to produce hydrogen from organic compounds with the help photosynthetic microorganisms are called with a name implying all these components; the photobioreactors. Various types of photobioreactors have been devised and tested for bacterial growth and hydrogen production. These are usually either tank reactors or tubular reactors. Closed tubular photobioreactors with working volumes ranging from 5 to 36,000 L for laboratory use and also for pilot and large-scale technical production have been reported in the literature. Some were designed as horizontal serpentine tubular or plate type bioreactors, others as vertical tubular bioreactors (Hai *et al.*, 2000).

An oscillatory tubular photobioreactor was developed in this study. This is the first application of oscillatory flow on photobioreactors. Oscillatory flows are known to increase the transfer process. In oscillatory flow reactors (OFRs), a two-directional flow and a controlled stage-wise mixing is present, which can be achieved using tubes fitted with low constriction orifice plate baffles and superimposing oscillatory motion upon the net flow of the process fluid. The oscillatory motion creates a flow pattern conducive to efficient heat and mass transfer while maintaining tubular flow. Unlike conventional tubular reactors where a minimum Reynolds number must be maintained, the degree of mixing is independent of the net flow, allowing long residence times to be achieved in a reactor of greatly reduced length-to-diameter ratio.

In specialty or fine chemicals manufacturing processes batch reactors are traditionally used, partly due to the fact that they are a tried and tested method, but also because no continuous reactor type is feasible for the particular reaction or process. However, in practice, a number of inherent inefficiencies in batch plants often limit the degree of optimization that can be achieved, and the very large solvent inventories inherent in batch reactors are becoming increasingly difficult problems to resolve in certain applications for safety reasons. In a batch process, external mixing must be provided in order to promote heat or mass transfer, or simply to maintain uniform conditions for a chemical reaction. This is usually achieved using rotary agitation. An oscillatory flow offers a highly effective, alternative means of external agitation for batch as well as continuous processes, and provides a range of specific process enhancements (Ni *et al.*, 2003).

An OFR can therefore be much more compact than conventional tubular flow reactor designs, allowing reactor designs for longer residence times to be of practical dimensions. It has been shown that OFRs can be used for totally new applications, such as fermentation, bio-chemical and bio-medical processes, where the conventional devices are mainly limited to liquid–liquid extraction.

The mixing in OFRs is very uniform. This has been shown to be an advantage when handling shear-sensitive materials, such as certain pharmaceutical crystals and in flocculators. The scale-up and design of OFRs are well understood. Indeed, the understanding of the OFR's scale-up is believed to be one of its advantages. A full review of the history and further details of the theory and applications of OFR technology are available (Akay, 2006).

The objectives of this thesis are;

- To develop and operate an oscillatory helical tubular reactor for growth of hydrogen producing photosynthetic bacteria, and
- To construct and operate a recycling reactor to produce hydrogen by photosynthetic bacteria.

The second chapter of this thesis, literature on photobioreactor design has been summarized with an emphasis for tubular and oscillatory reactor.

CHAPTER 2

PHOTOBIOREACTOR DESIGN

2.1 Photobioreactor types

Phototrophic microorganisms need light to convert the substrates into hydrogen. For this reason, all photobioreactors must provide optimum ways to collect and distribute the light throughout their contents. Various types of photobioreactors have been devised and tested for hydrogen production with photosynthetic bacteria. The most widely used photobioreactor types are described below.

2.1.1. Flat Panel Reactors

Flat panel reactors consist of a rectangular transparent box with a depth of only 1–5 cm. The height and width can be varied to some extent, but in practice only panels with a height and width both smaller than 1 m have been studied (Akerman *et al.*, 2002). The photobioreactors are either not mixed or mixed with a carrier gas introduced via a perforated tube at the bottom of the reactor. A typical photobioreactor with pneumatic agitation is shown in Figure 2.1 below.

The panels are usually illuminated from one side by direct sunlight and the panels are placed vertically, or inclined versus the sun. Hu *et al.* (1996) stated that when the front surface of the flat panel reactor was inclined towards to the sun, it receives the major thrust of solar irradiance and the back side of the reactor surface was also illuminated

by diffuse and reflected light which may be very effective for photosynthesis. In this research, the individual reactors were facing south with inclinations of 30° and 60° for summer and winter, respectively. Similarly, Rechenberg (1998) noticed at his group's experiments in Sahara Desert that purple bacteria are not able to make use of the high concentrated energy of the sun. They discovered the reflected radiation from the dunes were more effective. Therefore, they positioned their flat panel reactors in a position facing the dunes.

Tabanoğlu (2002), designed and constructed a flat plate solar photobioreactor with 8L of volume in order to produce hydrogen by *Rhodobacter sphaeroides* O.U.001. She found out that under limited sunlight conditions, i.e., light density < 10 klux, no hydrogen production occurred. Rather, fermentation end product formate together with carbon dioxide was produced when malate was used as the carbon source.

She also observed that malate was the best carbon source among the ones tried, yielding a hydrogen production rate of 0.0104 $L_{H2}/L_{culture}$.h. Acetate resulted in less hydrogen production but high poly- β -hydroxybutyrate accumulation. Lactate consumption by the non sulfur purple bacteria was little, and use of different basal medium resulted in highest carotenoid production and bacterial growth.

Hoekema *et al.* (2002) developed a lab scale flat-panel photobioreactor with pneumatic agitation for the anaerobic cultivation of purple non-sulfur bacteria (*Rhodopseudomonas* sp. HCC 2037) and concomitant hydrogen production from the organic acid salts, namely succinate and acetate. They used yeast extract as the nitrogen source in the medium. They showed that the photobioreactor with argon gas-recirculation was successful in attaining a steady chemostat culture of photoheterotrophic bacterium under anaerobic conditions.

A disadvantage of the flat-panel reactors is that the power consumption of aeration (or mixing with another gas) is high, although mixing is always necessary in any reactor. Light/dark cycles are short in these reactors, and this is probably the key factor leading to the high photochemical efficiency. Akkerman et al. (2002) believed that separating light collection from biological cultivation in flat-panel bioreactors was an effective method. They proposed a system based on collection of solar beam radiation in 'clear-sky' areas and its concentration into optical fibers with lenses or parabolic mirrors, through which the light can be guided into a large-scale photobioreactor.



Figure 2.1. Schematical drawing of the flat-panel photobioreactor set up: (1) membrane gas pump; (2) gasbag for collection of produced gas; (3) two 1-l pressure vessels; (4) pressure valve; (5) mass flow controller; (6) condenser; and (7) pH/redox electrode. (Hoekema *et al.*, 2002)

2.1.2. Tubular Reactors

Tubular photobioreactors consist of long transparent tubes with diameters ranging from 0.5 to 6 cm, and lengths ranging from 10 to 100 m. The culture liquid is pumped through these tubes by means of mechanical or air-lift pumps. The tubes can be positioned on many different ways: in a horizontal plane as straight tubes with a small or large number of U-bends; vertical, coiled as a cylinder or a cone; in a vertical plane, positioned in a fence-like structure using U-bends or connected by manifolds; horizontal or inclined, parallel tubes connected by manifolds; in addition, horizontal tubes can be placed on different reflective surfaces with a certain distance between the tubes.

Although tubular reactor design is very diverse, the predominant effect of the specific designs on the light regime is a difference in the photon flux density incident on the reactor surface. The shape of the light gradient in the tubes is similar in most designs. Also with respect to liquid mixing, i.e. light/day cycling, the circumstances in most designs are similar.

The length of the tubes is limited because of accumulation of gas, though this might not be so important for nitrogenase-based processes, since they may be less inhibited by H_2 (Akerman *et al.*, 2002). The way to scale-up is to connect a number of tubes via manifolds.

Hai *et al.* (2000) constructed a closed tubular glass photobioreactor allowing axenic cultivation of phototrophic microorganisms. They arranged standard glass tubes in a helical array providing a working volume of 80 L, and connected the tubular part with a degassing chamber. A pump module allowed agitation of the medium in the bioreactor at a laminar flow rate of 1.5 m/s. Their reactor set-up is displayed in Figure

2.2. They reported successful cultivations of the anoxygenic phototrophic bacteria and microalga in this photobioreactor.



Figure 2.2. The 80-L photobioreactor of Hai et al. (2000).

2.1.3. Column Reactors

These types of reactors are simply the closed vertical vessels with a length-to-diameter ratio usually higher than 5. There are lots of column reactor types. They can be packed or hollow, and they accommodate countercurrent or co-current flows. Since the penetration depth of light is of utmost importance in photobiological systems, the column reactors used for hydrogen production usually cannot have diameters larger than 10 cm.

Arık (1995) designed and constructed a water-jacketed glass photobioreactor for semicontinuous hydrogen production by *Rhodobacter sphaeroides* O.U.001. In the photobioreactor, the growth phase and the hydrogen production phase were differentiated. They found that the photoproduction of hydrogen was better at 31°C.

Yetiş (1999) employed a 400 ml-volume glass column photobioreactor in order to produce hydrogen from the sugar refinery wastewater by *Rhodobacter sphaeroides* O.U.001. He achieved continuous hydrogen production in 20% sugar refinery wastewater for approximately 100 days.

Palazzi *et al.* (2002) used a laboratory-scale continuous flow column type bioreactor for his experiments to produce hydrogen from organic wastes. The reactor was assembled by packing a sterile water-jacketed glass column (54 cm long \times 4 cm inner diameter) with glass beads. The fresh medium was introduced from the bottom of the reactor using peristaltic pumps. The researchers developed a kinetic model for hydrogen production in fixed-bed column tubes and tried to optimize the parameters. A schematic diagram of their experimental equipment is reproduced in Figure 2.3.



Figure 2.3. The fixed-bed column photobioreactor (Palazzi et al., 2002).

2.1.4. Oscillatory Reactors

In oscillatory flow reactors (OFRs), the tubes are fitted with low constriction orifice plate baffles and then an oscillatory motion is imposed upon the net flow of the process fluid, thus a controlled stage-wise mixing is obtained. This pattern of flow yields to better heat and mass transfer at even low Reynolds numbers, and allows longs residence times.

Ni and Pereira (2000) have studied the parameters affecting fluid dispersion in a continuous oscillatory baffled tube. It consisted of 14 glass tubes, operated vertically and connected to each other by U-bends, having an outlet for either venting or draining, as shown in Figure 2.4.



Figure 2.4. Oscillatory baffled tubes of Ni and Pereira's reactor (2000).

They have reported that the reproducible and controlled residence time distributions were obtained in a continuous oscillatory baffled tube with very low axial dispersion. The axial dispersion was calculated using both the plug-flow-with-axial dispersion model and the CST-with-feedback model. They found that the truly plug-flow behaviors dominated in the continuous oscillatory baffled tube for a range of laminar-flow net Reynolds numbers.

2.1.5. Immobilized Reactors

Most of the reactor types mentioned above can be operated as an immobilized reactor. In this type of reactors, the bacterial cells taking part in the photobiological hydrogen production are attached on transparent materials. The technique is usually applied in fermentative hydrogen production and it is scarcely tried in photobiological hydrogen systems because of the problem that the attaching materials may prevent the full penetration of the light into the reactor and its availability to the cells may be diminished.

Tsygankov *et al.* (1994) developed a rectangular glass chamber with a porous glass sheet for bacteria immobilization. They managed to produce hydrogen from organic materials by photosynthetic bacteria.

2.1.5. Other Bioreactors

There are some other photobioreactors devised to enhance the hydrogen production. They usually combine the good aspects of existing reactors in the literature.

Modigell and Holle (1998) investigated hydrogen production with an outdoor reactor in the form of hollow channel plates made of acrylic glass that are connected at the top and at the bottom to form a loop and erected vertically and placed at east-west position. The total area was $8m^2$ and they used *R. rubrum* cells and used lactate for outdoor hydrogen production experiments. Constant hydrogen production was observed with exchange of the half of the medium every fifth day for almost two months, indicating that there was no significant contamination of the reactor although no sterile methods or substrates were applied. They were able to obtain 40 $L_{hydrogen}/m^2$ ·day. The schematic view of the reactor is shown in Figure 2.5.



Figure 2.5. Scheme of the modular outdoor reactor (Modigell and Holle, 1998).

Teplyakov *et al.* (2002) integrated an active membrane system for hydrogen production. The sun-powered bioreactor was being operated in the closed mode with the active membrane system allowing to remove the gas produced and to monitor the level of components at the optimum level of concentration.

Chen *et al.* (2006) utilized a novel optical fiber-based photobioreactor to produce hydrogen by indigenous purple non-sulfur bacterium *Rhodopseudomonas palustris* WP3–5 using acetate as the sole carbon source. They inserted side-light optical fibers into photobioreactors as the internal light source. The hydrogen production performance and light conversion efficiency of the photobioreactor were assessed when various illumination systems were used. They obtained a highest H_2 yield of 17.06 ml/h l.

Kondo *et al.* (2006) developed a multi-layered photobioreactor (MLPR) for efficient hydrogen production, where the light paths were formed by the localization of bacterial cells. They employed *Rhodobacter sphaeroides* RV as the bacterial strain and a sodium-lactate, sodium-glutamate, and sodium hydrogen carbonate mixture as the hydrogen producing medium. They investigated the performance in order to clarify the effect of this reactor on hydrogen production. The analysis of the hydrogen production profile showed that the MPLR utilizes both the light that directly illuminates its surface and the light induced and diffused from its light paths for hydrogen production. It was also found that the hydrogen productivity in the MLPR was more than twice that in a plate-type reactor. When a photosynthetic bacterium mutant with reduced pigment, MTP4, was used, the maximum hydrogen production rate reached 2.0 l/m^2 h, which was 38% higher than that of a conventional plate-type reactor. The schematic representation of their reactor is provided in Figure 2.6.



Figure 2.6. Structure of the multi-layered photobioreactor (Kondo et al., 2006).

2.2. Criteria for Photobioreactor Design

A reactor for photobiological hydrogen production has to meet several conditions, and there are a certain number criteria that can be used in assessment and comparison of photobioreactors.

2.2.1. Cost of the Reactor

All the energy on earth is originated from the sun, and the situation becomes more critical in the case of photobioreactors, which need sun rays to operate. However, the diffuse nature of solar energy and the consequent low energy density places severe economic restrictions on potential light-driven processes for biological conversion of solar energy to hydrogen.

Benemann (1994) illustrated the issue by calculating the value of hydrogen produced as low as only \$10 worth of $H_2/m^2/year$. They assumed that the yearly average solar irradiation at very favorable locations could be as high as 5 kWh/m²/day; the conversion efficiency of the process was 10%, which is the usual value accepted; and the USDOE benchmark price guiding the hydrogen R&D program was H_2 of \$15/GJ. This places severe constraints on expenditures for construction and operations, even without including operational costs or considering energy inputs for mixing, gas exchange and compression, and cooling, etc. Obviously, lower efficiencies translate to increased bioreactor surface areas and consequently cost for the production per unit output of hydrogen fuel.

Tredici *et al.* (1998) investigated the photobioreactors for biological hydrogen production and conducted a preliminary cost analysis. The researches calculated all

the capital and operating costs and they concluded that single-stage processes could only be considered if total photobioreactor costs were at most $50/m^2$.

2.2.2. Light Energy Efficiency

The productivity of photobioreactor is light limited, and a high surface- to-volume ratio is a prerequisite for a photobioreactor. The photochemical efficiencies in biological hydrogen production systems are low. In theory, photosynthesis in general could achieve as much as a 10% total light energy conversion into a primary product, such as CO₂ fixed into biomass or even H₂. As pointed out by Kok (1973), under full sunlight the limiting factor is the rate of the dark reactions, which is roughly ten-times lower than the rate of light capture by photosynthetic pigments (e.g. chlorophyll). This results in up to 90% of the photons captured by the photosynthetic apparatus (e.g. absorbed by chlorophyll and other pigments) under full sunlight not being used in photosynthesis but rather decaying as heat. The pigment antenna systems of photosynthetic bacteria are capable of a much higher light absorption rate at high-light intensities than can be productively used due to slow dark reactions. This is a big waste which is only somewhat ameliorated in dense cultures where not all cells are near the culture surface and thus subject to maximal solar intensities. This so-called "light-saturation effect" is a major reason that photobiological productivities are not nearly as high as those projected from extrapolations of laboratory data at low light intensities (Hallenbeck and Benemann, 2002).

A few solutions were proposed for this problem. For example, a rapid mixing in algal cultures helped to overcome this light saturation effect. Besides, Wakayama and Miyake (2002) examined the effect of light shade bands set on the surface of the reactor to overcome this problem, and obtained remarkable results on conversion efficiency under the excessive light condition. Therefore, it is important to either dilute the light and distribute it as much as possible over the reactor volume, and/or

mix the culture at a high rate, so that cells are light exposed only for a short period in order to create an efficient biological process (Akkerman et al., 2002).

2.3.3 Optical Considerations and Arrangements

Artificial illumination systems are usually considered inappropriate in the biohydrogen production processes since the only potentially viable photonic source is the sunlight. Therefore, good optical designs are needed fore the better performance of the reactors.

For that reason, the most widely used photobioreactor type is the flat-panel reactor, which has the largest projection area for sunlight collection. However, due to the light saturation effect mentioned in the previous section, flat-panels have some problems with direct excessive sunlight, and they usually kept at inclined positions.

Rechenberg (1998) and his work group also noticed at their experiments in the Sahara Desert that purple bacteria are not able to make use of the high concentrated energy of the sun. The discovery that the reflected radiation from the dunes is so effective gave rise to the idea of building heliomites for use under excessive sunlight. They designed a 1500-L volume with a theoretical power production of 1 kW. The heliomites are shown in Figure 2.7.



Figure 2.7. Heliomites (300 L) in an art exhibition (Rechenberg, 1998)

Although the sunlight as the energy source is the cheapest solution, it may not be available at each day of the year, or outdoor conditions may not be appropriate for biological hydrogen production. Besides, the reactor thickness is a limiting factor under natural illumination. Therefore, the high-performance optical systems can be applied and adapted to artificial illumination photobioreactors.

Gordon (2002) claimed that one need not compromise the design of photobioreactors because of the limitations of conventional optical designs, in particular, with regard to desired power densities, flux distributions and radiative efficiencies. Using the principles of non-imaging optics, he tailored inventive solar optical designs to many aspects of the light input that optimize reactor bio-performance. These include higher flux levels and more uniform flux distributions than achieved in solar photobioreactors to date, while respecting high collection and distribution efficiencies. Gordon (2002) developed two contrasting categories of solar concentrators for photobioreactors; one was tailored to completely stationary systems, and hence was restricted to optical concentration ratios in the range of 1.0–2.6. (In contrast, a standard tubular solar bioreactor has an optical concentration ratio of 0.32.) The unit-concentration collectors can insure near-perfect flux homogeneity on the reactor surface. When flux uniformity is not crucial, concentration can be boosted by about a factor of two. Part of the attractiveness of these designs is trading off expensive reactor against relatively inexpensive static concentrators that are rooted in off-the-shelf materials and technologies.

One method suggested by Akkerman *et al.* (2002) was the collection of sunlight with a photon collector and distribution of this light into the photobioreactor by optical illuminators. Chen *et al.* (2006) utilized a similar optical fiber-based photobioreactor to produce hydrogen by inserting side-light optical fibers into the reactors as the internal light source. The schematic drawing of their reactor is shown in Figure 2.8.



Figure 2.8. Schematic diagram of the photobioreactor system with internal (optical fiber) light sources. (Chen *et al.*, 2006)
Wakayama and Miyake (2002) developed a reactor with light shade bands set on the surface of it to test the effect of irradiation area/shading area ratio. They found that the hydrogen production rate was almost constant and not related to the ratio of irradiation area/shading area of the reactor, so they applied a ratio of 1/1 in the experiments. The light shade bands of various widths at equal space were placed in a horizontal and vertical stripe pattern on the irradiation area of reactor surface. With narrow down of the widths of light shade bands, the hydrogen production rate increased. The maximum hydrogen production rate of 24 1 m⁻²day⁻¹ was observed using 1.0-cm wide horizontal light shade bands. In this width, the hydrogen production rate was 1.4 times larger than the reactor without light shade bands. As the width of light shade bands increased, the hydrogen production rate decreased to the same level as the reactor without light shade bands. These results showed that the hydrogen production rate when using the same irradiation area could be different depending on the width of light shade bands.

2.2.4. Mixing

Mixing is one of the most crucial criteria of photobioreactors as it is in other reactors. The application of mixing is beneficial for hydrogen production because it facilitates the removal of hydrogen gas, and distributes the cells within the culture, aiding the homogeneous exposure of the microorganisms to light and substrate. However, the relative delicate nature of biological systems does not allow the use of powerful mixing techniques. Besides, the air-tightness requirement of most photobioreactors eliminates the possibility of conventional mechanical stirring, since it is extremely difficult to seal these systems.

Therefore, some milder techniques devised for providing mixing in photobioreactors. The most common method is sparging a gas from the bottom of the reactor and agitating the liquid by means of turbulence created by gas flow. In these systems, usually an inert gas or a reactant gas is used in the sparger.

One other method is the recirculation of reaction fluid within the system and providing continuous or sporadic mixing by the liquid flow. Magnetic stirrers can also be used especially in vessels with a circular cross-sectional area.

Zabut, *et al.* (2006), found out that stirring increased both the total gas produced and enhanced the rate of hydrogen production in their studies employing the combined systems of *H. salinarum* packed cells with *R. sphaeroides* O.U.001 cells in 400ml water jacketed-glass column stirred photobioreactors.

Oscillation might also be employed as a means of mixing. Ni and Pereira (2000) studied the mixing capabilities of oscillatory flow an oscillatory baffled tube. They have reported that the reproducible and controlled residence time distributions were obtained in a continuous oscillatory baffled tube with very low axial dispersion. They found that the truly plug-flow behaviors dominated in the continuous oscillatory baffled tube for a range of laminar-flow net Reynolds numbers.

An oscillatory baffled photochemical reactor was developed for three phase heterogeneous photocatalytic reactions (Fabiyi and Skelton, 2000). In this reactor, the catalyst-coated particles are suspended in the liquid phase by oscillation of the fluid, whilst being contacted with gas bubbles through the column, the hold-up of which is increased by the fluid oscillation. The catalysis is activated by ultraviolet light supplied by an axially positioned cylindrical lamp. The solid particles are separated from the liquid at the top of the column by means of a baffle-free disengagement zone. The reactor has been applied to the oxidation of contaminants in wastewater, using titanium dioxide as the catalyst, where it has been shown to have higher photon utilization efficiencies than conventional photoreactors and to be less prone to fouling.

2.2.5. Heating and Cooling

In biological systems, the temperature of the reactor is also very important, since the enzymes of microorganisms within the vessels have an optimum temperature to sustain their activities. The purple non-sulfur bacteria are reported to live most happily in the temperature range of 25 - 35 °C (Sasikala *et al.*, 1993).

Therefore, the temperature within the photobioreactors must be kept within a certain interval. The temperatures may go up to 60°C under direct sunlight (Rechenberg, 1998), or it may drop below zero degrees in winter days. A few possibilities exist for heating or cooling the photobioreactors. When the reactors work under sunlight, the heating usually does not pose a problem. A heater can be employed to sustain a certain temperature in indoor experiments in winter.

The photobioreactors can be cooled by passing cold water through the coils to be placed within the reactors or through the water jackets wrapped around the vessels. The algae producing companies usually spray water onto the surfaces of reactors, and that alone provides sufficient cooling. Some researchers reported the usage of mechanic fans in indoor experiments. Rechenberg *et al.* (1998), managed to maintain the temperature at desired levels even in Sahara Desert by the help of a solar driven Peltier-equipment.

2.2.6. Operation of the Reactor

The photobioreactor should be as easy as possible to operate. Since the light-aided hydrogen producing systems are very fragile to impact of other microorganisms, the bacterial culture within the reactor must be kept uncontaminated. The sterilization is

usually carried out by chemical agents. However, the smaller reactors with heatresistant components can also be sterilized by being kept at 121°C for 15 minutes.

The reactor should allow easy loading of the reactants and unloading after the operation. Some geometrical configurations and reactor materials might be resulting in better light absorption or mixing. However, these should not make it difficult to clean the system after use if possible.

Since the produced hydrogen gas has to be collected, a prerequisite of the bioreactors is for it to be an enclosed system. The configuration should allow for the gas to leave the system easily. However, sometimes the produced gas is stripped by an inert gas or it is mixed by the sparging gas. In these cases, the further separation of hydrogen from the outlet gas mixture brings an additional cost for the operation.

2.3.7. Scale-up

The ultimate goal of biohydrogen research with the photosynthetic bacteria is the operation of large-scale bioreactors under natural sunlight (Koku *et al.*, 2002).

The modular reactors are easier to scale up. For example, the process was scaled-up 100 times in the heliomites of Rechenberg (1998) by simply putting a hundred of conical tubular reactors in series on a field. They called this hypothetical system as an energy farm.

Scale-up of oscillatory flow reactors is achieved simply by linear geometric scaling and by maintaining dynamic similarity via the oscillatory and net flow Reynolds numbers. This has been shown to be a successful approach, where the axial dispersion was shown to be the same irrespective of scale, provided geometric and dynamic similarity were maintained. Hence, this method is used as the basis for two industrialscale OFR design case studies presented in Harvey and Stonestreet (2002).

The predictability of OFR scale-up is an advantage, as it is better understood than the scale-up of stirred tanks, where discrepancies in the flow and mixing characteristics predicted by the standard correlations for laboratory and full-scale increase significantly with size of reactor.

Ni and Gao (1996) studied the effect of scale-up on mass transfer. It was shown that mass transfer coefficients increased as a function of increasing scale, for a given power density, i.e. the scale up is linear.

Scale-up and design of oscillatory flow reactors are now well-understood and fullscale industrial reactors are close to being developed (Ni *et al.*, 2003).

2.3. Applications

Although developed in great numbers and variety, the photobioreactors for hydrogen producing photosynthetic bacteria has not been applied to the industrial scale, yet. The most widely used industrial photobioreactors are the tubular or column type bioreactors that are used for algae cultivation.

Ni *et al.* (2003) illustrated a pilot-scale study that 100-fold (or greater) reactor volume reduction was possible for a particular reaction, by converting to the continuous OFR, as shown in Figure 2.9. The reduction in size resulted from a large reduction in required residence time, as the reaction was no longer mixing-limited (as it would be for any batch reactor of this scale), and from a full occupancy time (the reaction time in the batch had been only one-twelfth of the batch cycle time).



Figure 2.9. Process intensification using OFR (Ni et al., 2003)

In their review article also, Ni *et al.* (2003) gave the chronological summary of the main applications of oscillatory flows (Table 2.1).

First development	Process	Equipment	
1950s	Solvent extraction	Pulsed packed columns Pulsed plate columns Reciprocating plate columns	
1970s	Gas-liquid mass transfer	Pulsed plate columns Furrowed channel oxygenator	
1980s	Mixing and dispersion	Baffled tubes	
	Heat transfer enhancement	Oscillatory baffled columns	
	Gas liquid mass transfer	Oscillatory baffled columns	
1990s	Solid-liquid contacting Chemical reaction Bio-processing Membrane separation Filtration Bulk mixing and mass transfer	Pulsed packed columns Baffled tube reactors Baffled tube column Pulsed baffled cell Pulsed filtration cell Vortex generation in large tanks	
2000	Flocculation Saponification Suspension polymerization Fermentation	Oscillatory baffled column Baffled tubular reactor Oscillatory baffled column Oscillatory baffled column	

Table 2.1. Chronological summary of the development of pulsed and oscillatory flow technology (Ni *et al.*, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1. Reactor Construction

3.1.1. Oscillatory Flow Helical Reactor

The reactor mainly consisted of three parts; that is, two reservoirs and a conical part. The conical part was assembled by first constructing a pyramid out of metal bars with 1-cm diameter. The pyramid was 50 centimeters in height and 40 centimeters in width. Then, 30 meters of transparent PVC tubes with an internal diameter of 8 mm were coiled around the pyramid, giving it a conical appearance. The total volume of this helical part was 1.5 L.

Two 5-L ordinary glass jars were used as the reservoirs. Circular polyamide (Kestamit®) plates were prepared for each reservoir, and a number of ports necessary for liquid inflow, outflow, sampling, inoculation, temperature control, and gas outlet were fitted on these plates, as can be seen in the Figure 3.1 below.

The conical part was placed between the two reservoirs, and the tubes were connected. A solenoid valve system operated by an electronic switch and timer was constructed in order to provide the oscillation between the two reservoirs. A pressured nitrogen gas (%99.99) tube was used as the carrier gas. The gas coming from the tube was first passing through a rotameter, and then through the solenoid valves, which determine the direction of flow. The gas line was connected through blue gas-tight plastic tubes

with 6-mm internal diameters. The carrier gas and the hydrogen produced within the reactor during this time leave the system through the same valve system, but from a different port. The schematic of the connected system is depicted in Figure 3.2.



Figure 3.1. The 5-L reservoir and the fittings on the polyamide cover.

After the system is fully constructed, the medium and the bacteria were inserted into the reactor, and the required illumination configuration was provided by putting the 100 W lamps in front of the reactor. Operation was started by first opening the gas valve, and then pressing the "on" button of the solenoid valve switch. The opening and closing times of the valves were arranged so that after a certain time the flow totally was reversed. The switching operation is shown schematically in Figures 3.3A and 3.3B. The solid lines correspond to the gas flow and the double lines correspond to the liquid flow. Whereas, the blue arrows show the direction of gas flow and the brown ones show direction of the liquid flow. Operation of the reactor was achieved by opening valves 1 and 3 while 2 and 4 are closed, and in the reverse direction, opening valves 2 and 4 while 1 and 3 are closed.



Figure 3.2. The representation of fully connected oscillatory flow helical reactor.

The system was sterilized by a 3% hydrogen peroxide solution, and it was later cleaned by passing distilled water from the reactor. After the sterile the growth medium was placed into the reactor under the laminar hood, the covers and the fittings were closed.

The inoculations were carried out through one of three ports placed in this system. These ports were placed either left or right reservoirs' top cover or the port placed on the middle of tubular part. Inoculation volume was always 10% of the total liquid volume, and they were made by 50-ml syringes. Total liquid capacity of the system was 11.5 L, however 4.5 L of liquid volume was occupied.



Figure 3.3A. With only valves 1 and 3 are open, the flow direction is from left to right.



Figure 3.3B. With only valves 2 and 4 are open, the flow direction is from left to right.

3.1.2. Recycling Flow Helical Reactor

In this configuration, there is only one reservoir and a conical part. The liquid is circulated between the reservoir and the tubes on the helical part by means of a peristaltic pump (Watson-Marlow 505 S).

The gas produced by the bacteria within the tubes is carried by the liquid flow from down to up, and it is freed on the liquid-gas interphase when the liquid is poured onto the reservoir from the top. Since there is no carrier gas in this system, only one port is enough to collect the produced hydrogen. A water displacement technique was employed for this purpose and it was connected to the reactor. The schematic of this system is shown in Figure 3.4 below.



Figure 3.4. Schematic representation of the recycling helical reactor

Once the connections and the sterilization processes are completed, the medium was inserted into the reactor, and it was made anaerobic by flushing argon gas. Then it was

inoculated with a 10% volume ratio by *R. sphaeroides* O.U. 001 species. This time, the inoculation was made only from the reservoir.

After the lamps were placed, the peristaltic pump was adjusted to clock-wise direction, so that it sucks the fluid from the bottom of the reservoir and sent it to the bottom entrance of the conical part. The liquid containing bacteria and evolved hydrogen gas bubbles left the cone from the upper end and poured to the gas phase left on the top of the reservoir. The gas was separated and was collected in the burette by water displacement technique. The flow rate was adjusted to 150 ml/min (15 rpm). Figure 3.5 below demonstrates the system at start-up. The volume of the reservoir was 5-L, and the total volume of the system was 6.5 L with the tubular part combined. On top of the reservoir, a 50 ml of space left for the gas separation.



Figure 3.5. The recycling helical photobioreactor at start-up.

3.2. Photofermentation Process

A non sulfur purple bacterial strain, *Rhodobacter sphaeroides* O.U.001 (DSM 5864) was used as the hydrogen-producing microorganism in this study.

3.2.1. Anaerobic growth of *Rhodobacter sphaeroides*

In the anaerobic photoheterotrophic growth mode, malate as carbon source and sodium glutamate as nitrogen source were shown to be effective for the growth of bacteria (Sasikala *et al.*, 1995). Besides, the influent of the photobiorectors in the HYVOLUTION project will contain large percentages of acetate. Therefore, both malate and acetate were used as the carbon source; and nitrogen source was sodium glutamate throughout this study. A 10% inoculum of the pre-activated bacteria was transferred into the hydrogen producing liquid media containing either acetate- or malate-based media.

The minimal medium of Biebl and Pfennig (1981), supplied with L-malate (7.5 mM) and sodium glutamate (10 mM) without ammonium chloride and yeast extract, was used. In the acetate based-medium preparation, two-fold of 7.5 mM was used as malate molecules contain twice as many carbon atoms as acetate molecules do. This way, the Biebl and Pfenning (1981) medium was conserved with a little modification in all medium preparations. Vitamins, trace elements and iron-citrate were also added to this solution; their compositions are given in Appendix A.

The initial pH of the medium was adjusted to 6.7 by adding small amounts of NaOH. Then, 50 mL medium was injected into a 100 mL (or 550 mL) glass bottle with a rubber cap. This bottle was sterilized in an autoclave (Prior Clave or Nuve) for 15 minutes at 121 °C. After cooling to room temperature, either argon gas or nitrogen gas (99.995 % purity) was sparged at a flow rate of 100-150 mL/min for about 5 minutes, in order to create an anaerobic atmosphere inside the bottle. Anaerobic culture preparation bottle is shown in Figure 3.6.

Finally, a 10% inoculum of *Rhodobacter sphaeroides* O.U.001 was injected using sterile syringe needles into the prepared anaerobic liquid media, which were then incubated at 30 °C either in an incubator (Gallenkamp PLC) or in hot room where the temperature is kept around 30 °C. The bottles were illuminated by a 100 W tungsten lamp, placed at a distance of 25-30 cm.



Figure 3.6 Anaerobic culture preparation bottle (100 or 500 ml bottles).

For the storage purpose of bacteria, 30 mL growth medium contained in penicillin bottles was inoculated with 3 mL active culture of *Rhodobacter sphaeroides* O.U.001.

After 48 hours of growth, approximately 3.5 mL of sterile glycerol (10% of total volume) was injected into the bottle with a sterile syringe, in order to protect the cells from freezing. Then, that bottle was shaken by hand and stored in a freezer (Uğur) at - 20 °C.

When inoculating a new culture, the bacteria were transferred at least two times into a fresh medium to get rid of the effects of glycerol. Renewals of the stocks were carried out every 2 or 3 months (Eroğlu, 2006).

3.3. Liquid Sampling and Analysis

Liquid samples were collected from the sampling ports. The pH of the culture was measured with a standard combination of a pH electrode (Mettler Toledo 3311) and an electronic transmitter (Nel pHR-1000 Transmitter). Cell concentrations were detected by measuring the optical density of the culture at 660 nm (OD₆₆₀). The calibration curve obtained for the cell concentration versus OD₆₆₀ is given in Appendix B.,

The cell concentration was obtained by measuring the optical density of culture and also performing the dry cell weight analysis. Measuring the optical density of the culture is one of the simplest and a direct way of the bacterial cell concentration determination. For this purpose, absorbance of the culture at 660 nm was detected by a visible spectrophotometer (Shimadzu UV-1201). Fresh medium was used as a blank solution.

The calibration curve for *Rhodobacter sphaeroides* O.U. 001 of dry cell weight versus OD_{660} are given in Appendix B. Calibration shows that OD_{660} value of 1.0 corresponds to 0.58 g_{dry weight} / L_{culture} (Eroğlu, 2006).

3.4 Tracer Analysis

The system was operated with distilled water, and at a time zero, a pulse input of 20% (w/w) tracer solution (65 ml aqueous NaCl solution) was inserted to the system as a tracer.

The peristaltic pump was operated at the lowest allowable speed (15 rpm), and at the highest speed it is capable of (50 rpm). The tracer concentrations were measured in terms of conductivity readings by means an ion-meter (Mettler Toledo MA130), and then converted into concentration values. A calibration curve relating the conductivity readings to the tracer concentrations is given in the Appendix E.

The samples were taken at port 1 at the outlet of the reservoir, port 2 in the middle of the tubular part, and port 3 at the inlet of the reservoir.

3.5. Scope of the Experiments

Experiments were carried out in a hot room. The temperature of the liquid in the photobioreactors was kept around 30 °C.

3.5.1. Adjustment of Liquid Flowrate in Tubular Photobioreactors.

Liquid flow rate was adjusted by changing the carrier gas flow rate in oscillatory helical tubular photobioreactor. Calibration is given in Appendix C. No liquid flow was observed when the rotameter reading was below 3.

Liquid flow rate was adjusted by changing the peristaltic pump revolution per minute in recycling helical photobioreactor. The calibration of pump speed to the liquid flow rate is given in appendix D. The reactors were checked for leakage by placing it in a bucket full of water. If no gas bubbles was observed it was leakage-proof.

Oscillatory helical tubular reactor was kept under nitrogen atmosphere, while the recycling helical tubular reactor was flushed with argon gas.

3.5.2. Factors Affecting the Bacterial Growth on Acetate

Factors affecting the bacterial growth of *Rhodobacter sphaeroides* O.U.001 on acetate (initial concentration was 15 mM) were tested in 550 ml glass bottles. These factors were selected as,

- a. the carrier gas used either argon or nitrogen
- b. initial concentration of sodium glutamate

Two sets of experiments with different concentrations of sodium glutamate as nitrogen source were prepared. 15mM of acetate was used as the carbon source, and it kept fixed for all sets. However, six different sodium glutamate concentrations (0.2, 2, 4, 6, 8, and 10 mM) were used in order to better differentiate the effects in two sets of media. The 550ml-volume bottles were filled with 250 ml of medium and the remaining part was spared for the atmosphere gas. One set was filled with nitrogen gas, while the other was flushed with Argon gas. After the usual 10%-volume inoculation, the lights were opened and the experiment was started. A photograph of the experiment was put as Figure 3.7 below.



Figure 3.7. Two sets of experiment; one set carrying argon, and the other set carrying nitrogen gas within the bottles.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Hydrodynamics of the Helical Tubular Reactors

4.1.1. Hydraulic Retention Times for the Oscillatory Helical Reactor

The flow rate of the liquid within the system was controlled by the gas-flow rate. Therefore, the speed was adjusted by the gas valve and was monitored through a rotameter. The flow properties at different rotameter readings are shown in Table 4.1. A calibration curve relating the rotameter readings to the liquid flow rate is provided in Appendix C.

			Hydraulic	
Rotameter	Liquid Flow rate	Liquid velocity	Retention Time	Reynolds
readings	(111/5)	(CITI/S)	(11111)	number
3	4.7	9.28	5.36	825
4	6.7	13.3	3.75	1180
5	8.7	17.2	2.88	1530
6	10.7	21.2	2.34	1890
7	11.3	22.6	2.21	2000
8	13.3	26.5	1.88	2360
9	15.3	30.5	1.63	2710
10	16.7	33.2	1.50	2950

The flow is laminar for the rotameter readings below 7, and then it gets turbulent at the last reading. Turbulent flow can be achieved at a relatively high liquid velocity at about 34 cm/s. Laminar flow occurs at low Reynolds numbers (Re<2100), where viscous forces are dominant, and is characterized by smooth, constant fluid motion, while Turbulent flow, on the other hand, occurs at high Reynolds numbers (Re>3000) and is dominated by inertial forces, producing random eddies, vortices and other flow fluctuations (Bird, et al, 2002).

A logical method could be to circulate the system at the lowest velocity during bacterial growth and then to speed up the system at appropriate intervals to ensure good mixing and to strip any gas formation trapped within the tubes.

4.1.2. Hydraulic Retention Times for the Recycling Helical Reactor

The pump system could be operated at speeds ranging from 0 to 55 rpms. The flow regime within the tubes is a function liquid velocity, and it can be monitored through the Reynolds number. Table 4.2 shows the Reynolds numbers and hydraulic retention times for a few pumps speeds that could be selected. A calibration curve relating the pump speed to liquid flow rate is provided in Appendix D. Sample calculations were also provided in the Appendix D.

The flow regime is laminar at all pump speeds. However, this does not pose a big problem in terms of insufficient mixing, since our system is not a continuous tubular reactor. Instead, the liquid flowing laminantly through the tubes returns to the 5-L volume reservoir at the end of each turn and is mixed in the bulk fluid there. The following discussion provides empirical answers on this issue.

The mixing properties of the reactor were of utmost importance in order to be able to determine if there is a good distribution of contents and whether additional mixing is necessary.

Pump speed (rpm)	Liquid Flow rate (ml/s)	Liquid velocity (cm/s)	Hydraulic Retention Time (min)	Reynolds Number
5	0.83	1.66	30.2	133
10	1.67	3.32	15.1	265
15	2.50	4.97	10.1	398
25	4.17	8.29	6.0	663
30	5.00	9.95	5.0	796
35	5.83	11.61	4.3	928
40	6.67	13.26	3.8	1060
45	7.50	14.92	3.4	1190
50	8.33	16.58	3.0	1330
55	9.17	18.24	2.7	1460

Table 4.2. The flow properties of the recycling reactor at various pump speeds.

The concentration variation of the tracer with respect to time in the reactor at 150 ml/min (15 rpm) and 500 ml/min (50 rpm) flow rates are depicted in the Figures 4.1 and 4.2 below. The experimental procedure is described in Section 3.4.

As can be seen from Figure 4.1, the first detectable molecules of NaCl ions reach the mid point of the reactor at about the 9^{th} minute, while the hydraulic retention time in that pump speed is calculated to be 10 minutes. In other words, the ions could only traveled halfway through the reactor in the time they would finish the circle if the flow was a perfectly plug one. This is an expected picture, since the flow regime within the tubes is highly laminar at this speed (Re=400).

A CSTR-like mixing pattern was observed at the reservoir, as evidenced by the fact that the maximum concentration was reached within the first minute. The concentration did not drop below a certain level since the flow was recycled back to the reservoir. The complete mixing throughout the reactor took place at about 40th minute for the slowest flow rate and at only 4th minute for the fastest flow rate.

Considering the delicate characteristic of the biological process, the slowest flow rate was chosen for the operation. It is quite safe since the hydrogen production usually begins after 12th hour, and our system will reach a homogenous state in one hour at the latest. The characteristic of the flow within the tubes are also checked by means of a tracer experiment, and the axial distribution terms are found for the tubular part of the reactor, which are shared for both type of reactors.



Figure 4.1. The concentration variation of tracer with respect to time in the recycling reactor at 150 ml/min (15 rpm).



Figure 4.2. The concentration variation of tracer with respect to time in the recycling reactor at 500 ml/min (50 rpm).

4.1.3 Axial Dispersion within the Recycling Helical Reactor

The tracer experiment was carried out to determine to flow properties within the tubular part of the reactor. The experiment was carried out at a flow rate of 2.4 ml/s (i.e., 14 rpm), since this was the chosen flow rate for the bacterial operations.

A pulse input of salt solution at a concentration of 0.2 g/ml was injected from the beginning of the tubular part, and the concentrations were measured at the end of coils. The graphical demonstration of NaCl concentrations is provided below in Figure 4.3.



Figure 4.3. The plot of concentration readings at the end of the tubular part of the reactor.

The mean residence time was calculated as 14.6 minutes from the mean of the C_{pulse} curve. The variance was 0.0464 in dimensionless form, and by using van der Laan (1958) equation for closed vessels, the distribution term, (*D/uL*), was computed as 0.029. The dimensionless group characterizing the spread in the whole tube, i.e., *D/uL*, is greater than 0.01, therefore we can conclude that the flow deviates from the plug flow. Peclet number was 34.5. The calculations are provided in Appendix F.

The hydraulics experiments were carried out by using distilled water to get an idea on the behavior of liquid bacterial culture within the reactor. However, the physical properties of the bacterial culture, such as density, viscosity, or surface tension, would be different than pure water. Therefore, further studies should also investigate the possible effects of these parameters on the bioreactor.

4.2. Bacterial Growth in the Oscillatory Reactor

4.2.1. Bacterial Growth in Malate-Based Medium

4.5 liters of growth medium containing 7.5 mM Malate/10 mM Sodium glutamate was put into the oscillatory flow helical reactor, and was inoculated by the species R. *sphaeroides* O.U. 001, at a volumetric ratio of 10:1.

The inoculation was performed through three ports; namely through two reservoir ports and one helical port. Nitrogen was used as the carrier gas in the system. The flow velocity of the liquid was adjusted to 10 cm/s, which was decided to be appropriate after previous calibrations. The system was illuminated by three lamps located in front of the reservoirs and the conical part.

The bacteria grew very well under the conditions described above. The photograph of the system can be shown in Figure 4.4.



Figure 4.4. Bacteria grown in malate-based medium under nitrogen atmosphere.

The bacteria sustained its growth for quite a long period, more than expected, in fact. The growth curve is displayed in the Figure 4.5 below. In this plot, the average values of samples collected from three ports were used.

The bacteria showed a consistent behavior with the logarithmic growth model of Koku *et. al.* (2002). They reached a quite a high concentration first, and then started to die. However, after a few hours, they recollected themselves and started to grow again, in gradually diminishing waves, just as projected by the logarithmic growth model.



Figure 4.5. The growth curve of the *Rhodobacter sphaeroides* O.U.001 in malate-based medium in the whole oscillatory reactor under nitrogen atmosphere.

However, the samples were collected from each end of the reactor, namely from two reservoirs and form the tubular part. Therefore, the cell concentrations differ at each point. In order to be able to see these fluctuations, the cell concentration read from each port were plotted separately in Figure 4.6 below.



Figure 4.6. Cell concentrations obtained at three ports of the oscillatory helical reactor with malate-based medium under nitrogen atmosphere.

The bacteria survived about 24 days before the system was shut down. This is a significant finding, because the previous work on the same bacteria usually reports a life-span around 10 days under anaerobic conditions (Arık, 1995; Yetiş, 1999). Therefore, we can now conclude that we can keep our inoculum alive with this system for a quite long time while working with large-scale outdoor reactors.

Another important finding could be related to the effect of nitrogen-gas present in the system atmosphere on the elongation of bacterial life. An experimental set-up was prepared, and the question of whether the nitrogen atmosphere has any effect on the growth of bacteria was tested. The findings related to this issue are put in Section 4.3 of this thesis.

4.2.2. Bacterial Growth in Acetate-Based Medium

The sixth frame European Union project, HYVOLUTION, aims to optimize the hydrogen production by combining the fermentative and photosynthetic techniques. The main component of the fermentation outlet, which will be the feed of photosynthetic reactor, is the acetate. Therefore, the performance of the system and the bacteria was tested with also acetate-based medium. 4.5 liters of growth medium containing 15 mM Malate/10 mM Sodium glutamate was poured into the oscillatory flow helical reactor, and was inoculated by the species *R. sphaeroides* O.U. 001, at a ratio of 10:1, through three different ports again, as described in the previous section.

Nitrogen was used as the carrier gas in the system, and the flow rate of nitrogen was adjusted so that the liquid velocity within the tubes was 10 cm/s. Again, the system was illuminated by three lamps located in front of the reservoirs and the conical part.

The bacterial growth turned out to be very vigorous, even stronger than the growth observed within Malate-based medium. The system looked like as displayed in Figure 4.7 on the fourth day.



Figure 4.7. The appearance of the acetate-based medium on the 4^{th} day.

The experiment was terminated at the 11th day, and its growth curve is displayed in the Figure 4.8 below. The hydrogen ion concentrations were also plotted in the same Figure. It is obvious from these data that pH increases with growth. However, this time pH increases too much, compared to the one in malate-based medium, which usually never exceed a pH of 8. As the Figure shows, the pH of acetate system breaks the 10.0 barrier, which is highly detrimental to the bacteria and their hydrogen producing enzymes.



Figure 4.8. The growth curve of *Rhodobacter sphaeroides* O.U.001 and the hydrogen ion concentration of the medium in acetate-based medium in whole of the oscillatory flow reactor under nitrogen atmosphere.

The pH increase seen in the acetate-based medium is interesting. The buffer in the solution is not enough to hold it under a certain limit, and if we raise the buffer concentration, the increased ionic strength within the solution will cause an extra stress on the bacteria. However, this seems to be the only solution right now, and a four-fold of buffer compound (KH_2PO_4) was added to the medium.

Addition of an acid could solve the problem. However, if this acid is chosen as the substrate, i.e., acetate, the experimental reliability will suffer since we would be adding fresh medium to the system. HCl addition can drop the pH also, but then the adjustment of the new pH would be extremely difficult, given the complexity of the mixing profile of the reactor.

According to a theory, the cells release hydroxide ions (OH⁻) as they take acetate molecules in, in order to preserve the ionic balance of their environment. This process, thus, increases the pH of the system as acetate is being metabolized.

The cell concentrations of samples collected from each port is given in Figure 4.9, and the pH values of the same samples in Figure 4.10. As can be seen from the plots, the values differ very little. Thus, we can safely assume a homogenous system.



Figure 4.9. Cell concentrations obtained at three ports of the oscillatory helical reactor with acetate-based medium under nitrogen atmosphere.



Figure 4.10. Hydrogen ion concentrations obtained at three ports of the oscillatory helical reactor with acetate-based medium under nitrogen atmosphere.

The best temperature for *R. sphaeroides* species lies in the range of $30-34^{\circ}$ C (Koku *et al.*, 2003; Eroğlu *et al.*, 1999). The temperature of the hot room and that of the reactant medium was continuously monitored. As can be seen in Figure 4.11, the temperature of the culture was maintained within the optimum range.



Figure 4.11. The temperature of the culture during the bacterial growth experiment in acetate-based medium.

4.3. Factors Affecting the Bacterial Growth

In the growth experiments carried out in the oscillatory reactor, nitrogen gas used as the carrier gas. The longer survival periods of bacteria noticed in this system brings about the possibility of the nitrogen gas present in the system atmosphere being utilized as an additional nitrogen-source supplementary to sodium glutamate.

The growth curves of bacteria under argon atmosphere and the ones under nitrogen atmospheres were shown in the Figures 4.12 and 4.13, respectively.



Figure 4.12. The growth curves of bacteria put into different sodium glutamate concentrations under argon atmosphere. The initial acetate concentrations were 15 mM for all cultures.



Figure 4.13. The growth curves of bacteria put into different sodium glutamate concentrations under nitrogen atmosphere. The initial acetate concentrations were 15 mM for all cultures.
These curves present an interesting pattern. As expected, the best growth occurs with the control concentration of 15mM Acetate/10 mM of sodium glutamate. However, almost all other bottles containing a lesser amount of N-source started to grow earlier. The longer lag time observed at the 10mM sodium glutamate concentration might be resulting from the substrate inhibition. This could be an important finding because the lag time required for bacteria to initiate its growth will be very important when the large-scale outdoor reactors are used. Besides, it can be seen that even a 15/2 acetate/sodium glutamate ratio is sufficient to grow the bacteria, and maybe there is no need to spend extra sodium glutamate in this case.

The growth profiles of both atmospheres look quite alike when the figures drawn for argon and nitrogen atmospheres are examined. In both cases, the bacteria grew very well, but when examined carefully, it would be seen that the second plot, i.e. the bacteria under the nitrogen atmosphere, did get mature earlier. Bacteria almost in all bottles reached their peak concentrations and started to die, while the other set that uses argon gas as the atmosphere is still on the rise and did not come to the stationary phase yet.

This finding suggests that the nitrogen atmosphere enhances the bacterial growth. The most plausible explanation for this is the dissolution of molecular nitrogen in the liquid medium and its subsequent usage by the bacteria as an additional nitrogen source. The amount of dissolved nitrogen at room temperature (~25°C) can be calculated by Henry's Law, and it turns out to be 1.2 mM dissolved nitrogen at double atmospheric pressure (where $k = 1639.34 L_{soln}.atm/mol_{gas}$ for N₂; www.wikipedia.org), which is a significant number considering the maximum nitrogen concentration of 10mM coming from the sodium glutamate.



Figure 4.14. Comparison of bacterial growths in two different atmospheres media with the same 15/10 acetate/sodium glutamate ratio.

The 15mM acetate to 10mM sodium glutamate ratio is the routine concentration used in growth media of *R. sphaeroides* O.U. 001 species. The growth curves of bacteria put into these control media are shown in Figure 4.14. It is clear that there is not much difference between the two atmospheres, but the nitrogen atmosphere starts to decline earlier. The nitrogen gas present within the bottle does not seem to have any significant effect on the growth of bacteria. This observation might be resulting from the fact that the medium already contains sufficient amount of nitrogen-source for bacteria to grow joyfully, and the additional nitogen present in the second set does not contribute to the bacterial growth. However, when looked at the lower sodium-glutamate concentrations, such as 15/8 molar ratio as shown in Figure 4.15, the faster growth of bacteria under nitrogen atmosphere can easily be noticed. The same is valid for 15mM acetate/2mM sodium glutamate medium also as illustrated in Figure 4.16.



Figure 4.15. Comparison of bacterial growths in two different atmospheres media with the same 15/8 acetate/sodium glutamate ratio.



Figure 4.16. Comparison of bacterial growths in two different atmospheres media with the same 15/2 acetate/sodium glutamate ratio.

The hydrogen ion concentrations of growth media change as the bacteria grow. The pH values of both sets are displayed below in Figures 4.17 and 4.18. It can be noticed that the pH of the medium was following the same pattern as the growth of the bacteria. For example, the pH of 0.2 mM sodium glutamate-containing virtually did not change as no bacterial growth was observed. This is an expected finding, because as the bacteria consume the organic acids within the medium, they release hydroxide ions out of their cell membranes in order to maintain their internal ionic balance. Therefore, as the number of bacterial cells increases within the medium, the hydroxide ions are expected to accumulate more, increasing the pH value. No significant

difference in the medium was observed between the argon and nitrogen atmosphere in terms of their pH trends.



Figure 4.17. The pH curves of bacteria in different sodium glutamate concentrations under argon atmosphere. The initial acetate concentrations were 15 mM for all cultures.



Figure 4.18. The pH curves of bacteria in different sodium glutamate concentrations under N_2 atmosphere. The initial acetate concentrations were 15 mM for all cultures.

4.4. Hydrogen Gas Production

The recycling reactor was originally designed for the hydrogen production since it allows the gas separation on top of the reservoir.

The system was filled with the hydrogen-producing medium that contains a 15 mM of malate and 2 mM of sodium glutamate. The reactor was inoculated and started just as described above. The appearance of the system was photographed on the third day, as can bee in Figure 4.19.



Figure 4.19. The recycling helical reactor in operation.

The amount of hydrogen produced and the growth of bacteria was measured and put into one graph as displayed in Figure 4.20 below. The hydrogen production started at about the 20th hour, and it went very well till the fourth day (70th hour), when the experiment had to be stopped since bacterial culture assumed a bright red color, revealing an oxygen leakage into the reactor. The bacteria produced approximately 1.9L of hydrogen in four days on malate-based media. The hydrogen production rate was $0.009L_{H2}/L_{culture}$.h, and substrate conversion efficiency remained at 22% since the experiment had to be stopped before all the nutrients were consumed.

For the hydrogen production experiment, a well-proven medium composition containing malate as the carbon source and sodium glutamate as the nitrogen source was selected since the hydrogen production with acetate-based media and with other nitrogen sources, such as ammonium chloride or urea, has not been fully proven to be effective, yet.

The results, however, are very promising. Because, if the system can exceed the common flat-panel reactors in performance, then its other advantages, such as process intensification, low production cost, and acceptance of sun light from any angle and at any hour of the day, become more evident. Therefore, this type of reactor can be a real candidate to be used in large scales, and even in industrial operations, since the scaling-up is easy and the costs of materials are very inexpensive.



Figure 4.20. The total hydrogen production and growth curve of the bacteria in the recycling reactor.

The temperature and the pH of the medium during the operation was also recorded and demonstrated in Figure 4.21. The hydrogen ion concentration of the system remained in very reasonable ranges, and the temperature was almost constant at about 33 °C.



Figure 4.21. The temperature and the pH of the medium during the operation of recycling helical reactor.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

There is an urgent need for development of renewable energy systems due to the depletion fossil fuels and environmental problems. Hydrogen is one of the most favorable alternatives in the clean energy studies. Its only combustion product is water; therefore, it is a clean fuel.

An oscillatory tubular photobioreactor was developed in this study. This is the first application of oscillatory flow on photobioreactors. Oscillatory flows are known to increase the transfer process, and they allow long residence times to be achieved in a reactor of greatly reduced length-to-diameter ratio, thus decreasing the required volume by providing process intensification.

A pneumatically driven, low-cost photo-bioreactor was devised in this study to cultivate phototropic microorganisms. The geometry of the reactor was inspired from a study of a German group who performed hydrogen production experiments in the desert. The excessive direct sunlight was found to be ineffective in phototrophic hydrogen evolution, therefore a conical photobioreactor was proposed as a concept to work in outdoor conditions.

Two reservoirs were added to the system and the fluid was forced to move by gas pressure between the reservoirs in a pendulum-like pattern. The movement of the system provided additional advantages. By this way, the bacteria could reach the photons required for hydrogen production independent of sun's position. Besides, a good mixing was achieved and no equipment was required for agitation.

The liquid flow within the tubes did not exhibit a plug pattern, and axial dispersion was significant. The mean residence time was calculated as 14.6 minutes, and the distribution term, (D/uL), was computed as 0.029. Peclet number was 34.5. The experiment was carried out at only one pump speed that was appropriate for bacterial growth. However, further studies should test the mixing and dispersion pattern of the reactor at a higher number of speeds, and considering other parameters such as the injection position, tube diameter, etc.

The bacteria growth experiments within the oscillatory flow reactor were very successful both with acetic acid-based and malic acid-based media under nitrogen atmosphere. The bacteria sustained their growth for a longer period than expected. The bacteria survived about 24 days before the system was shut down. This was a significant finding, because it showed that the inoculum could be kept alive with this system for a quite long time while working with large-scale outdoor reactors.

The elongation of bacterial life-span was attributed to the effect of molecular nitrogen present in the system atmosphere. An experimental set-up was prepared, and the question of whether the nitrogen atmosphere has any effect on the growth of bacteria was tested. The bacterial growth curves did not show any difference at the control medium containing 15mM of Acetic acid and 10 mM of sodium glutamate. However, other bottles containing a lesser amount of N-source started to grow earlier under the nitrogen atmosphere.

This is an important finding because the lag time required for bacteria to initiate its growth will be very important when the large-scale outdoor reactors are used. Besides,

even a 15/2 acetate/sodium glutamate ratio is sufficient to grow the bacteria, and there is no need to spend extra sodium glutamate in this case.

The effect of reactor atmosphere on the bacterial growth, however, should be further explored. Other possible alternatives, such as carbon dioxide, may also have an impact on the growth. Carbon dioxide is important because the bacteria can use the carbon atoms present in the dissolved gas and may enhance their growth especially in acetic acid-based media. Acetic acid is a two-carbon containing organic acid, and it was reported that the bacteria have difficulty in gathering enough energy to produce molecular hydrogen from this substrate.

A recycling helical tubular photobioreactor was developed for hydrogen production since it allowed gas separation. The bacteria produced approximately 1.9L of hydrogen in four days on malate-based media. The hydrogen production rate was $0.009L_{H2}/L_{culture}$.h.

Considering the additional benefits that this reactor brings such as process intensification, low production cost, and collection of sun light at any angle and at any hour of the day, a well-studied recycling tubular reactor can be a real candidate to be used in large scale applications. Further studies should focus on the optimization of parameters affecting the hydrogen production in this reactor. The flow rate of the liquid, the illumination density and illumination geometry, diameter of the tube and volume of the reservoir are some of the parameters that should be investigated further.

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APPENDIX A

PREPARATION OF PRE-ACTIVATION MEDIUM

Preparation and composition of standard Pre-activation Medium, Trace Element Solution, Vitamin Solution and Fe-citrate Solutions

Table A1. The compositions of standard pre-activation media for malic acid and acetic acid-based experiments.

7.5/10 Mala	te Medi	um	15/10 Acetate Medium		
KH ₂ PO ₄	1	g/L	KH ₂ PO ₄	1	g/L
MgSO ₄ .7H ₂ O	0.2	g/L	MgSO ₄ .7H ₂ O	0.2	g/L
NaCl	0.4	g/L	NaCl	0.4	g/L
Na-Glutamate	1.8	g/L	Na-Glutamate	1.8	g/L
CaCl ₂ .2H ₂ O	0.05	g/L	CaCl ₂ .2H ₂ O	0.05	g/L
Malic Acid	1.0	g/L	Acetic Acid	0.9	ml/L
Trace elements	1.0	ml/L	Trace elements	1.0	ml/L
Fe-citrate	1.0	ml/L	Fe-citrate	1.0	ml/L
Niacyn-Thiamine	1.0	ml/L	Niacyn - Thiamine	1.0	ml/L
Biotin	1.0	ml/L	Biotin	1.0	ml/L

* Medium to be neutralized by addition of NaOH (~pH 6.7)

* Vitamins to be added after autoclave, by filtering

Table A2.	The com	position	of trace	element	solution
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Composition	Amount
HCl (25% v/v)	1 mL/L
ZnCl ₂	70 mg/L
$MnCl_2 \cdot 4H_2O$	100 mg/L
H ₃ BO ₃	60 mg/L
$CoCl_2 \cdot 6H_2O$	200 mg/L
$CuCl_2 \cdot 2H_2O$	20 mg/L
$NiCl_2 \cdot 6H_2O$	20 mg/L
$NaMoO_4 \cdot 2H_2O$	40 mg/L

The components of the trace element solution were dissolved in 1000 mL distilled water and sterilized in an autoclave.

Table A3. The composition of vitamin solution

Composition	Amount
Thiamine	500 mg/L
Niacin (Nicotinate)	500 mg/L
Biotin	15 mg/L

Fe-citrate Solution: Within 100 ml distilled water, 0.5 g Fe(III)-citrate was dissolved and sterilized by autoclaving.

APPENDIX B

OD-DRY CELL WEIGHT CALIBRATION CURVE



Figure B1. Calibration curve and the regression trend line for dry weight versus OD_{660} (Eroğlu, 2006).

APPENDIX C

ROTAMETER READING – FLOW RATE CALIBRATION CURVE



Figure C.1. Calibarion curve of rotameter readings and liquid flow rates in the oscillatory flow helical tubular photobioreactor.

Sample Calculations:

Hydraulic Retention Time = (Reactor volume)/(liquid flow rate)

$$HRT = (1500 \text{ ml})/(4.7 \text{ml/s}) = 320 \text{ s} = 5.33 \text{ min}$$

Reynolds number: Re = $\frac{\rho \partial D}{\mu} = \frac{(1g / cm^3)(9.28cm / s)(0.8cm)}{0.009g / cm.s} = 825$

where,

 ρ : density of water (1g/cm³)

v: flow velocity (cm/s)

D: characteristic lenth of the tube, i.e., diameter.

μ: viscosity of water (0.009g/cm.s at 25°C, 1 atm)

APPENDIX D

PUMP SPEED – FLOW RATE CALIBRATION CURVE



Figure D.1. Calibration curve of the pump speed to the liquid flow rate.

Sample Calculations:

Hydraulic Retention Time = (Reactor volume)/(liquid flow rate)

HRT = (1500 ml)/(0.83 ml/s)= 1807 s = 30.2 min

Reynolds number: Re =
$$\frac{\rho \vartheta L}{\mu} = \frac{(1g / cm^3)(1.66cm / s)(0.8cm)}{0.009g / cm.s} = 147$$

APPENDIX E

CONDUCTIVITY – CONCENTRATION CALIBRATION CURVE



Figure E.1. The calibration curve of NaCl concentration to the conductivity.

APPENDIX F

TRACER EXPERIMENT CALCULATIONS

Data:

t (min)	0	11	12	13	14	15	16	17	18
C (g/ml)	0	0	0.010	0.015	0.060	0.083	0.020	0.010	0

Area under the C_{pulse} curve;

$$A = \int_0^\infty C dt \cong \sum_i C_i \Delta t_i$$

Mean of the C_{pulse} curve;

$$\bar{t} = \frac{\int_0^\infty tCdt}{\int_0^\infty Cdt} \cong \frac{\sum_i t_i C_i \Delta t_i}{\sum_i C_i \Delta t_i}$$

E curve,

$$E_i = \frac{C_i}{A}$$

The variance (in discrete form);

$$\sigma^2 \cong \frac{\sum (t_i - \bar{t})^2 C_i \Delta t_i}{\sum C_i \Delta t_i} = \frac{\sum t_i^2 C_i}{\sum C_i} - \left[\frac{\sum t_i C_i}{\sum C_i}\right]^2$$

The variance in dimensionless form;

$$\sigma_{\theta}^2 = \frac{\sigma^2}{\bar{t}^2}$$

Van der Laan (1958) equation (for closed vessel boundary condition);

$$\sigma_{\theta}^{2} = \frac{\sigma^{2}}{\bar{t}^{2}} = 2\left(\frac{D}{uL}\right) - 2\left(\frac{D}{uL}\right)^{2} \left[1 - e^{-uL/D}\right]$$

The calculations are shown in the table A.4 below.

t _i	Ci	t_iC_i	$t_i^2 C_i$	Е
0	0.000	0.000	0.00	0.0000
11	0.000	0.000	0.00	0.0000
12	0.010	0.120	1.44	0.0505
13	0.015	0.195	2.54	0.0758
14	0.060	0.840	11.76	0.3030
15	0.083	1.245	18.68	0.4192
16	0.020	0.320	5.12	0.1010
17	0.010	0.170	2.89	0.0505
18	0.000	0.000	0.00	0.0000
116	0.198	2.890	42.42	Totals

Table A.4. The processing of data

Therefore,

$$\bar{t} = \frac{2.890}{0.198} = 14.6 \text{ min}$$

$$\sigma^2 = \frac{42.42}{0.198} - \left[\frac{2.890}{0.198}\right]^2 = 1.20 \text{ min}^2$$

$$\sigma_\theta^2 = \frac{1.20}{(14.6)^2} = 0.0564$$

And,

$$0.0564 = 2\left(\frac{D}{uL}\right) - 2\left(\frac{D}{uL}\right)^2 \left[1 - e^{-uL/D}\right]$$
$$\left(\frac{D}{uL}\right) = 0.029$$

Peclet number = 1/0.29 = 34.5 D= 0.029*u*L= 0.029*4.97cm/s*30m = 432.4 cm²/s

where, D is the axial dispersion coefficent, u is the liquid velocity, and L is the lenth of the tube.

APPENDIX G

EXPERIMENTAL DATA

Bacterial Growth in Oscillatory Tubular Reactor

Table A.5. Optical density values of bacteria at 660 nm wavelength, grown on malatebased medium under nitrogen atmosphere:

Time	Optical Density Measurements						
(h)	Port 1	Port 2	Port 3	Average			
0	0.101	0.101	0.101	0.101			
24	0.160	0.160	0.160	0.160			
48	0.405	0.579	0.500	0.495			
72	0.804	1.095	0.810	0.903			
96	1.071	1.719	1.404	1.398			
100	1.251	1.422	1.446	1.373			
120	1.420	1.500	1.490	1.470			
144	1.431	1.752	1.767	1.650			
168	1.690	1.710	1.690	1.697			
192	1.640	1.680	1.640	1.653			
216	1.887	1.824	1.860	1.857			
240	2.052	1.893	1.830	1.925			
264	1.578	1.692	1.731	1.667			
288	1.731	2.064	1.848	1.881			
312	1.620	1.800	1.680	1.700			
336	1.720	1.900	1.780	1.800			
360	1.620	1.845	1.806	1.757			
384	1.683	1.890	1.731	1.768			
408	1.866	1.698	1.437	1.667			
432	2.007	1.767	1.578	1.784			
504	1.881	1.644	1.500	1.675			

Table A.6. Optical density values of bacteria at 660 nm wavelength, grown on acetatebased medium under nitrogen atmosphere:

time		Optical Density	Measurements	
(h)	Port 1	Port 2	Port 3	Average
0	0.081	0.084	0.084	0.083
24	0.282	0.153	0.225	0.220
48	0.759	0.915	0.804	0.826
72	1.554	1.875	1.665	1.698
96	2.193	2.154	2.154	2.167
120	2.437	2.275	2.251	2.321
144	2.392	2.368	2.275	2.345
168	2.085	2.127	2.106	2.106
192	2.016	1.956	2.016	1.996
216	1.921	1.940	2.019	1.960
240	2.046	1.966	2.006	2.006
264	1.952	1.972	1.992	1.972

Table A.7. pH values of bacteria grown on acetate-based medium under nitrogen atmosphere, and the temperature of the culture.

Time		pH measurements							
(h)	Port 1	Port 2	Port 3	Average	Temp. (⁰C)				
0	6.716	6.716	6.716	6.716	19.6				
24	6.930	6.902	7.060	6.964	34.1				
48	7.865	8.006	8.014	7.962	33.2				
72	9.610	9.890	9.655	9.718	36.4				
96	10.062	10.064	10.045	10.057	29.6				
120	10.525	9.824	9.723	10.024	32.2				
144	10.231	10.130	9.729	10.030	30.9				
168	9.940	10.140	10.040	10.040	33.6				
192	10.128	9.827	10.128	10.028	31.8				
216	9.864	9.964	10.367	10.065	34				
240	10.273	9.871	10.072	10.072	32.6				
264	9.965	10.066	10.167	10.066	30.6				

Bacterial growth under argon and nitrogen atmosphere:

Table A.8. OD_{660} and pH values of bacteria grown on acetate-based medium under argon and nitrogen atmosphere. The sodium glutamate conceptartions were different in each bottle.

		Argon atmosphere								
Time	15/0.2	2 A/G	15/2	2 A/G	15/(5 A/G	15/8	3 A/G	15/10) A/G
(h)	OD	pН	OD	pН	OD	pН	OD	pН	OD	pН
0	0.201	6.858	0.219	7.036	0.189	7.008	0.186	7.055	0.204	6.586
24	0.321	6.886	0.786	7.758	0.567	7.547	1.203	8.502	0.405	6.854
48	0.384	7.062	1.467	8.753	1.035	8.942	1.671	9.114	1.377	7.892
72	0.297	7.358	1.762	9.137	1.371	9.452	1.986	9.943	2.301	9.118
96	0.249	7.616	1.899	9.632	1.572	10.174	2.130	10.139	2.472	9.360
					Nitrogen	atmosphere				
Time	15/0.2	2 A/G	15/2	2 A/G	15/6	5 A/G	15/8	3 A/G	15/10) A/G
(h)	OD	pН	OD	pН	OD	pН	OD	pН	OD	pН
0	0.210	6.793	0.192	7.006	0.183	6.810	0.186	7.036	0.210	6.473
24	0.284	6.888	1.152	9.652	0.996	8.433	1.671	9.530	0.396	6.946
48	0.303	6.916	1.827	9.931	1.434	9.684	2.223	9.979	1.452	8.225
72	0.218	6.967	1.641	10.046	1.665	9.882	2.316	10.023	2.322	9.067
96	0.147	6.996	1.560	10.122	1.293	10.006	2.020	10.243	2.124	9.196

Hydogen Production in Recycling Helical Tubular Reactor

Table A.9. OD₆₆₀, pH, temperature, and produced hydrogen values for bacteria grown on malate-based medium in the recycling helical tubular reactor.

Time (h)	OD	рН	(<u>°</u> C) T	H ₂ (ml)
0	0.100	6.654	28.6	0
12	0.351	6.822	34.1	20
24	1.122	7.121	32.5	670
36	1.572	7.206	33.2	1230
48	1.692	7.217	33.2	1640
72	1.821	7.632	34.2	1870