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PHOTOACTIVITY MEASUREMENTS OF BACTERIORHODOPSIN IMMOBILIZED IN POLYACRYLAMIDE GEL

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Dedicated to
İSMET ARALP
and
MURAT KURAN
Who are now
in The Dark Side of The Room

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Adnan AYDEMIR

M.S. in Chemical Engineering

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ABSTRACT

Biomimetic photoenergy conversion systems have acquired a spreading interest in relation to solar energy conversion into chemical or electrical energy. Bacteriorhodopsin (BR), which is a retinal protein synthesized by a photosynthetic bacteria called *Halobacterium halobium* is known as the simplest light driven proton pump.

Halobacterium halobium S-9 strain was grown and purple membrane fragments (PM) were isolated.The PM fragments were immobilized into polyacrylamide gel and constructed between a two compartment glass made chamber. This chamber was later improved to eliminate possible environmental effects and a compact jacketed cell was constructed.

The photoactivity of bacteriorhodopsin (BR) immobilized in polyacrylamide gel (PAG) was determined by measuring pH in each compartment of the cell. Upon illumination of the system proton association and dissociation of bacteriorhodopsin (BR) have been observed.This behaviour was same in both compartments.It was concluded that there was no ligth driven [H^+] ion tranfer from one compartment to the other.

Keywords:*Halobacterium Halobium*, Bacteriorhodopsin, Purple Membrane, Photoactivity

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POLİAKRİLİK JELE TUTTURULMUŞ BAKTERİORODOPSİNİN FOTOAKTİVİTESİNİN ÖLCÜMÜ

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ÖZET

Biyomimetik ışık enerjisi çevrim sistemleri, güneş enerjisinin kimyasal veya elektrik enerjisine çevrimiyle ilgili olarak gittikçe artan bir ilgi kazanmaktadır. Fotosentetik bir bakteri olan *Halobacterium halobium*'dan sentezlenerek elde edilen ve retinal bir protein olan Bacteriorhodopsin biyolojik sistemlerde bilinen en basit ışık etkileşimli proton pompasıdır.

Halobacterium halobium S-9 suju büyütüldü ve mor zar fragmanları ayrıştırıldı. Mor zar fragmanları poliakrilik jele tutturuldu ve iki odacıklı camdan yapılmış bölmelerin arasına konuldu. Daha sonra çevresel etkileri en aza indirebilmek için pleksiglasdan iki bölmeli su ceketli bir ölçüm sistemi yapıldı.

Poliakrilikamid jele tutturulan bacteriorhodopsinin fotoaktivitesi her iki bölmede pH ölçümleri ile belirlendi. Sistemin ışıkla uyarılması sonucunda bacteriorhodopsinin proton bağlama ve bırakma özelliği gözlendi.Bu davranış her iki bölmede de aynı idi. Bu gözlemlerden sistemde bir bölmeden diğerine ışık etkisi ile herhangi bir [H^+] iyonu transferi olmadığı sonucuna varıldı.

Anahtar Sözcükler : *Halobacterium halobium*, Bacteriorhodopsin, Mor zar, Fotoaktivite

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LIST OF SYMBOLS

AGTCC : All glass two chamber cell

BR : Bacteriorhodopsin

EPC : Egg phosphatidyl choline

GCGP : Gel constituted glass plate

HH : Halobacterium halobium

H^+ : Hydronium ion concentration

JTCC : Jacketed two chamber cell

PAG : Polyacrylamide gel

PC : Phosphatadyl choline

PCB^- : Phenyldicarbaundecarborane anion

PM : Purple Membrane

T : Temperature

CHAPTER 1

INTRODUCTION

Since it is unfortunately revealed that the energy resources already-existing on the earth will continually have been dwindling in the mid of next century and also to reduce the effect of pollution due to fossil fuels; alternative energy resources are to be investigated. Among them there exist nuclear energy, wave and tidal energy, solid waste energy,biomass, biogas and eventually solar energy. Because of its cheapness, clearness and plentifulness, the solar energy seems to be the most challenging energy source. A great tendency toward the realization of collection and usage of this energy has been achieved by development of solar converters named the photovoltaic cells.

The solar energy can directly be altered either into electrical or chemical energy form by means of solid-state cells or photoelectrochemical cells. Another approach to achieve the photovoltaic solar cells is biomimetic systems.

Yücel and Eroğlu (1987) have reviewed the studies about biomimetic photoenergy conversion systems. Various

biomimetic systems have been constructed as models for the conversion of light energy into electrochemical potential energy. Photochemical potential energy can be further converted to electrical energy with a properly designed photovoltaic cell.

Purple membrane synthesized by *Halobacterium halobium*, contains BR a retinal protein endowed with the ability to pump protons when it is exposed to light. This ability can be used for direct conversion of solar energy into electrical energy.Upon illumination BR undergoes a fast cycling photoreaction passing through a number of phototransients. During each photochemical cycle a proton is released on one side and a proton is bound on the other. Bacteriorhodopsin is, therefore, believed to function as a light driven proton pump. Accordingly, attempts have been made to use this natural ion pump for the direct conversion of light into electrical energy.To accomplish this, mechanically sturdy synthetic membranes incorporating BR are required for the construction of light-energized electrochemical cells. For such a synthetic membrane to be photoresponsive it is essential that the incorporated BR molecules be preferentially oriented so that they all pump protons in the same direction.

The efficiency of energy conversion achieved is

exceedingly low. This is, perhaps due to the fact that none of the techniques used for membrane formation produced perfect or even significant orientation of the purple membranes. Even a reliable quantitative assessment of the extend of orientation in synthetic membrane systems has not yet proved possible. Furthermore, although BR continues functioning without any diminution in electronic activity, the supporting structures develop signs of early deterioration. Satisfactory solutions to the dual problems of orientation and mechanical stability will have to be found before any BR-containing membrane system of practical utility can be devised. (Singh and Caplan, 1980)

In this research, the objective is to construct a two chamber cell for the measurement of photoactivity of BR immobilized in polyacrylamide gel. Photoactivity of BR is investigated by measuring [H^+] ion concentration variation in each cell independently. The aim is to verify the light driven [H^+] ion transfer of BR immobilized in PAG, and to supply data to evaluate the rate of pumping and the stoichiometry.

CHAPTER 2

LITERATURE SURVEY

2.1 *Halobacterium halobium*

Halobacterium halobium is a photosynthetic bacteria which belongs to halobacteria family. It was discovered by Stoeckenius and Oesterhelt in 1971. *Halobacterium halobium* lives in hypersaline ponds and salty lakes of Middle East region.It can not live below 3 M NaCl concentration which causes complete loss of cell wall rigidity.

Halophilic bacteria are aerobes and use oxygen to oxidize fuel molecules. The solubility of oxygen is so low in brine ponds that the halobacteria needs another energy source and provides from sunlight.As a part of their evolutionary adaptation to life, halobacteria have developed a double linkage between light-sensitive membrane proteins and the polar flagella system by which they are mobile.

The plasma membrane of *Halobacterium halobium* contains a light absorbing pigment which is called bacteriorhodopsin. Upon illumination the excited BR

molecules undergo bleaching reactions. As the light excited BR molecules in the membrane revert to their initial ground state, the energy released is harnessed to translocate [H^+] ions from inside to outside of the cells thus produce pH gradient across the cell membrane. Since [H^+] concentration is higher on the outside, [H^+] ion diffuses back into the cell through an ATP-forming enzyme in the membrane, similar to the ATP-synthetase of mitochondria and of chloroplasts. As [H^+] ions pass through the bacterial ATPase, ATP is synthesized from ADP and phosphate. Thus halobacteria can conserve light energy in the form of ATP. However, the halobacteria does not evolve oxygen.

2.2 PLASMA MEMBRANE OF *HALOBACTERIUM HALOBIUM*

The cell membrane of *Halobacterium halobium* has been subdivided into four sections; red, brown, white and purple membranes (Stoeckenius, 1980). The yellow fraction is composed of gas vacuoles. Bacteria can adjust its level in the water by controlling the buoyant density.

The red fraction functions in the respiratory chain and in enzymatic machinery for oxidative phosphorylation. Another domain of halobacterial plasma membrane termed as brown membrane takes place in the biogenesis of purple

membrane. This membrane fraction has a lower buoyant density than PM. It contains BR and a cytochrome-b type protein besides other membrane protein species. (Sumper, 1988)

The white membrane is synthesized by respiratory enzymes. This membrane is not light sensitive. The white membrane is composed of bacterio-opsin in a hexagonal crystalline array almost identical to that of BR in the PM (Mukohata and Sugiyama, 1988)

Bacteriorhodopsin is found as a single protein in the purple membrane.Consideration of the evidences from x-ray diffraction, electron microscopy and chemical analysis, the most satisfactory arrangement of BR is found to be in patches of BR forming a hexagonal pattern of trimers where BR is spanning the membrane as seven α-helical rods (Blaurock,1975). The patches can easily be isolated in relatively large quantities. They may constitute more than 50% of the total cell membrane under anaerobic conditions. They remain at low ionic strength where the rest of the membrane dissociates. Because of their deep color, they are named as purple membrane. PM fragments are composed of 25% lipid and 75% protein. The major lipids are diether of phosphatidyl glycerophosphate (about 50%) triglycosyl diether (about 20%)sulpholipids (about 15%) neutral lipids including squalene and phosphatidylglycerol. Dihydrophytol ether-linked to

glycerol is the major component of these lipids as hydrophobic moiety (Stoeckenius ,1980). The rest of the lipid is carotenoids.

The cell membrane of *Halobacterium halobium* contains two more photochemically reactive pigments in addition to BR (Spudish,1983). One of them is halorhodposin which is an ion pump for [Cl^-]. The other one is S-rhodopsin which functions as a receptor pigment for phototaxis. All three pigments have very similar absorption spectra. At neutral pH, S-rhodopsin has an absorption maximum at 587±2 nm, halorhodopsin at 578±2 nm and BR at 570 nm.

2.3. BACTERIORHODOPSIN

Bacteriorhodopsin consists of a single polypeptide chain and a chromphore retinal. The molecular weight of BR is about 26.500 daltons.Both the primary and secondary structures of the protein are known. Since BR is forming two dimensional ordered hexagonal arrays in the plane of the membrane, it is also possible to characterize its three dimensional structure at low resolution by x-ray diffraction (Blaurock, 1975).

The complete amino acid sequence of BR has been

determined by two research groups (Khorana *et al.*,1979, Ovchinnincov, 1979). Proteolytic digestion experiments have shown that it is a transmembrane protein with a blocked pyroglumatic acid N-terminus (3-7 amino acids residues) exposed at the extracellular surface and the C-terminus (17-24 amino acids) exposed on the Cytoplasmic site. Bacteriorhodopsin has one retinal molecule which is bound stoichiometrically through a schiff base linkage to a lysine ε-amino group (lys 216). The chromophore retinal is not destroyed between pH 1 to 10 and will stand to the temperatures up to 80 °C. The location of the retinal, both in the sequence and the three dimensional structure,has been investigated. The location of the chromphore perpendicular to the membrane have been controversial. By neutron diffraction method, Wallace (1982) has found that the retinal was placed near the center bilayer, whereas fluorescence energy transfer measurements indicated that it was within the cytoplasmic side.

2.4 ELECTROGENECITY OF THE BACTERIORHODOPSIN

2.4.1. PROTONS AND THEIR PHOTOREACTION CYCLE

The BR photoreaction cycle is the basis of its function as a light energy proton pump. The available

spectroscopic data are insufficient to determine unequivocally the number of photoreaction cycle intermediates, their kinetic constants, absorption spectra, and connectivity. Models must be suggested and criteria must be found to test them.

The photocycle originally proposed by Lozeir *et al* (1977) has number of intermediates. They are called Bath-(k_{590}),Lumi,(L_{550}), Metabacteriorhodopsin (M_{412}) and (O_{640}). Photocycle of BR proposed by Liu *et al* (1985) is shown in Fig 2.1. The configuration integrity of 13,14 bond of retinyl chromophore is not maintained at room temperature, giving approximately equal amounts of all-trans(BR_{560} and 13-cis BR_9BR_{550}) which is called dark adapted BR. Light adaptation results in fast 13-cis to all-trans conversion. At <0°C where the dark adaptation process is suppressed,the light conversion to the BR_{570} becomes complete. Light excitation generates a series of intermediates. During transition of L_{550} to M_{412}-I, a proton is released on one side of the membrane. However, during the transition of M_{412} to O_{640P} one proton is bound from the other side of the membrane. The cycle is completed by conversion of O_{640} to BR_{570}. Also, low temperature spectroscopic studies showed that K_{610},L_{550} can all photo-converted back to BR_{570}, and more recently different forms of M have been observed that originate from two different forms of L.

2.4.2. Stoichiometry of Proton Pumping

There are conflicting results in literature on the number of proton released or translocated in one cycle from PM sheets ranging from 1 [H^+] / photocycle (Lozier et al, 1975) to 2 [H^+] / photocycle (Kalisky, 1981 after Ort and Parson,1978). Also at different experimental conditions the investigators obtained different results. For fresh preparations of BR sheets, Govindjee (1980) found that the [H^+]/M_{412} ratio was 1.0 at low salt concentrations and 2.0 at high salt concentrations. Eisenbach *et al* (1977) have proposed that the light-induced proton release from PM sheets were resulted not only from pumping process but also from [H^+] release and rebound on the surface of the membrane (Bohr protons) (Stoeckenius,1982).

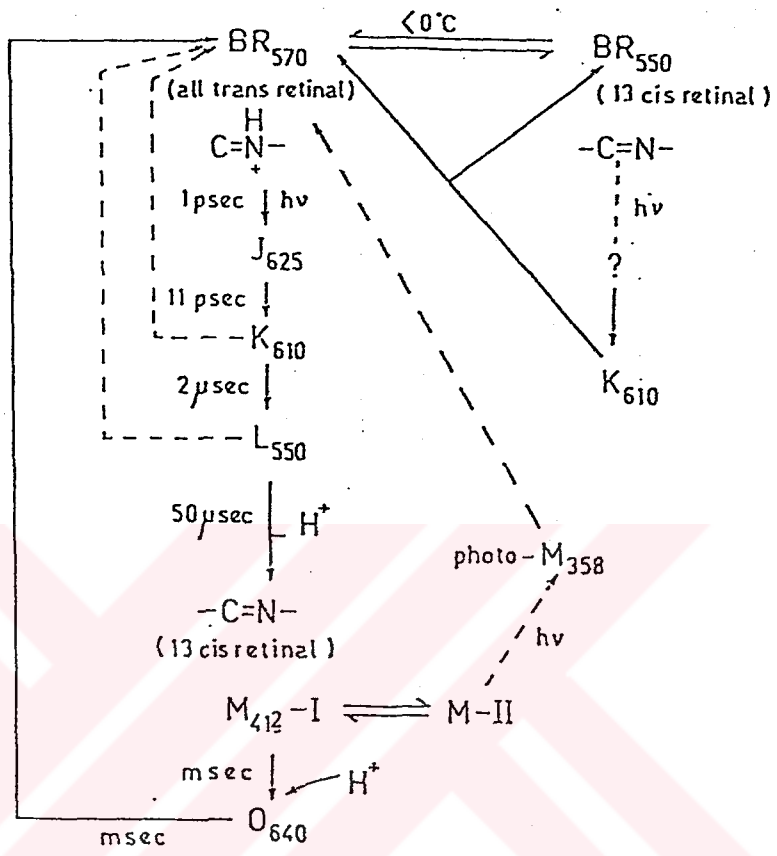

Figure 2.1. Photocycle of Bacteriorhodopsin

2.5 PHOTOACTIVITY MEASUREMENTS OF BACTERIORHODOPSIN

According to the chemiosmotic theory of Mitchell (1966), some membraneous proteins should carry electrons or protons through the membrane against an electrochemical gradient. Somewhat ambiguous results were obtained from attempts to test this hypothesis by means of microelectrode techniques. Development of methods for membrane potentials detection, other than microelectrode techniques, resulted in the obtaining of various pieces of evidence of electric generation in coupling membranes such as in mitochondria, chloroplasts, bacteria and their membranous fragments. Unfortunately, all these systems are too complex to study molecular mechanisms of electric generation.

The studies on bacteriorhodopsin suggest this component to be simplest biological electric generator found in coupling membranes. Some observations indicated that bacteriorhodopsin carries out light energy conversion into a utilizable form have been made. Illumination of the bacteria gives rise to an increase in the intracellular ATP level and a decrease in respiration rate, the effect being sensitive to uncouplers (Oesterhelt and Stoeckenius, 1973). Reconstitution of phospholipid-protein vesicles containing BR and

mitochondrial oligomicin-sensitive ATPase result in the system capable of light-supported ADP phosphorylation by inorganic phosphate. (Racker and Stoeckenius, 1974).

Considering these data in terms of chemiosmotic theory of energy coupling one may suggest that bacteriorhodopsin mediates light dependent formation of a transmembrane electrochemical potential of $[H^+]$ ions. Indeed illumination of bacterial cells as well as of proteoliposomes was shown to induce some changes in pH of the incubation medium.

Kayushin and Skulachev (1974) report data demonstrating the generation of electric potential difference ($\triangle\theta$) and a pH gradient ($\triangle$pH) by BR. Proteoliposomes reconstituted from BR and mitochondrial phosholipids were studied. The penetrating anion (PCB^-) and atebrin were used as probes for $\triangle\theta$ and $\triangle$pH, respectively. It was found that illumination of BR or proteoliposomes gives rise to (PCB^-) uptake which indicates that a transmembrane is generated (positive inside the proteoliposomes). Turning-off the light caused extrusion of the (PCB^-) taken up under illumination of proteoliposomes, evidence of the formation of a transmembrane $\triangle$pH (acid inside the proteoliposomes). Parallel measurements of pH of the incubation medium showed an alkalinization. In the dark,

both the atebrin fluorescence and the external pH returned to initial levels.

Gerber *et al*,(1977) studied the orientation of BR in the purple membrane of *Halobacterium halobium* by proteolitic degradation of PM sheets, reconstituted vesicles and whole cells. As a result they reported that the carboxyl terminus as well as the additional cleave site near the amino terminus observed in apomembrane are on the cyctoplasmic site of the purple membrane.

Govindjee *et al*,(1980) measured the quantum yield of $[H^+]$ released in PM sheets and $[H^+]$ uptake in phospholipids of egg phosphatidylcholine vesicles. They reported that the proton relaxation time was much longer than the photochemical cycling time. Therefore they have suggested that protons were pumped across the membrane but there was no contribution as a result of reversible binding and the release of protons on one side of membrane.

Zabut *et al*, (1987) have measured the photoactivity of BR reconstituted in egg phosphatidylcholine liposomes. They have observed that the orientation of BR was the same in liposomes as in the native bacterial membrane at low light intensity. Light induced proton pumping characteristic of the system is changed at high light intensity. The protons were first released and then were

taken inside of the vesicles during illumination. They have suggested that might indicate the light induced confirmational change of BR molecules in vesicles. The proton pumping activity were also effected by pH of the external medium and lipid to protein ratio.

Eroğlu *et al* (1990) have suggested models for expressing the net rate of proton pumping of BR reconstituted in (EPC) liposomes. According to the phenomenological model the net rate of proton pumping was the combination of the rates of photoreaction and simple diffusion of protons across the lipid membrane to compensate the concentration difference between two sides of the membrane. A mechanistic model was also proposed for the photoreaction which contains activation, proton dissociation, translocation and association reactions. Activation and translocation steps were considered to be fast, however proton dissociation and association steps were considered to be slow. Models were in accordance with data for initial pH values around seven.

Several other studies were conducted with macroelectrodes and using conventional electrometer techniques to measure light-induced photocurrent developed by BR incorporated into synthetic membranes.

2.5.1 BR Reconstituted On Membranes

2.5.1.1. BR Reconstituted on Lipid Film

Drachev et al, (1973) measured the electric potential difference across a planar membrane by Ag/AgCl electrodes. The planar membrane was prepared by mixing BR with a decane solution of soya bean phospholipids. They eventually concluded that illumination of the membrane resulted in the appearance of transmembrane electric potential, the maximum value of this difference being about 50 mV.

Drachev *et al* (1974) have shown that when BR or protoliposomes were integrated to only one side of the planar membrane,it could generate a higher transmembrane electric potential difference at the expense of light energy, and reported that the magnitudes of the maximal potential values were 150 mV.

Packer *et al*,(1977) studied the factors involved in orientation of BR in planar membranes and their effect on photo-induced [H^+] gradients. They examined the electrical potentials and chemical modification studies of BR with cross-linking reagents. They incorporated PM

fragments into a black lipid planar membrane system composed of oxidized cholesterol-decane.They claimed that the increase in the amount of externally added $Ca^{2}+$ caused eightfold increase in proton pumping activity.

Ormos, *et al*, (1978) have investigated the photoelectric properties of BR incorporated into a bimolecular lipid membrane. They have suggested two alternative schemes for photochemical cycle of BR.

Hong and Montal,(1979) applied a quasi-short circuit measurement technique using BR in the surface film of the water-hexane interface. They have postulated that their novel interfacial technique is very sensitive for studying of fast capacitative photoresponses in model membranes.They have claimed that they have detected the charge displacements in BR associated with distinct stages of the photochemical transformation.

2.5.1.2 BR Reconstituted on Millipore Filter

Block *et al*, (1977) have reconstituted BR vesicles on one side of millipore filter. They have concluded that the association of BR vesicles with lipid impregnated millipore filter was similar to that of planar membrane. Eventually, the photoactivity they have reported was 215

mV.

Block and Van Dam, (1978) studied the factors effecting the association of BR containing vesicles with phospholipid-impregnated millipore filter.

Abdulaev *et al*, (1978) elucidated whether or not the water exposed sites of polypeptide chain of BR were involved in the generation of transmembrane electric potential difference. They studied the products of the partial proteolysis of BR by papain and thermolysis. As a result they reported the kinetic parameters of BR as a photoelectric generator do not deteriorate in spite of removal of 17-amino acids from the C-end, 3-amino acids from the N-end and 5-amino acids from the middle part of polypeptide domain.

2.5.1.3 BR Reconstituted on Hard Surfaces

Karoly, (1978) obtained dried layers of PM oriented in an electric field on glass plate. He has measured the photopotential and absorption spectra after exciting PM vectorially. The absorption maximum was around 560 nM.

Hwang*et al* (1978) have studied the photovoltaic properties of BR molecules and their photochemical intermediates in a cell containing multilayered films of

highly oriented dry fragments of PM and lipid sandwiched between two Pd electrodes. As a result, they reported that the bright illumination of these cells containing 5 to 30 layers generates transient photovoltages. By use of photovoltaic signals, they have suggested that M_{412} and M_{520} are to be kinetically distinct photointermediates of BR.

Korenstein and Hess (1978) have studied the flash photoselection of rotational mobility of BR dried on a glass plate. They have concluded that either protein was completely immobilized or its rotational relaxation rate was much slower than rotational rate which was attributed to the mobility of retinal chromophore.

2.5.2. BR Reconstituted in Polymers

Eisenbach *et al* (1977) have prepared a membrane by mixing PM with acrylic acid and acrylamide polymers and the mixture was polymerized on a cotton cloth. BR was oriented under the action of an electrical field. The cloth was added to increase the mechanical strength of the gel. They illuminated the membrane from both sides and measured the light induced short-circuit electric current in oriented and non-oriented BR containing gels.With non-oriented BR they observed no response while they obtained 30 μA maximum response in oriented BR with

a light intensity of 750 W/m². They have applied the electrical field of orientation (100 V/cm for 1 to 2 min) after initiation of the polymerization when the suspension appeared viscous enough to prevent the migration of acrylic acid molecules and PM fragments in the field but fluid enough to permit rotation of fragments. They have reported that the mechanical stability of the gel was unsatisfactory, and its shape became distorted with time.

Hristova *et al* (1986) ; Liu and Ebrey (1988) ;Liu (1990) ; Liu *et al* (1990) have studied the fast kinetics of the photocycle and the charge transfer of BR. They have immobilized BR in PAG. They have initiated the photoexcitation using flash light (time of flash was less than 1 msec), and measured the electrical signals as mV or nA generated between two platinum electrodes placed on each side of the gel slabs.

Packer *et al* (1987) studied on proton translocation by chemically modified BR. Purple membrane containing modified BR were oriented by electrical field and immobilized in a PAG as described in (Kasthelzy and Ormos ,1983). They have given flash light and measured mV change with time. They have concluded that modification of carboxyl residues influences the time course of the photocycle but does not change the number of protons per photocycle.

Kunugi *et al* (1987) studied oriented immobilization of BR in synthetic polymer membranes by use of electrostatic field. Purple membrane containing pre-polymer solution was placed in a cell and sandwiched between indium oxide evaporated polyester film with the conductive surface facing the solution. An electro-static field was applied between the film below the solution and point type counter electrode placed over the top of the film by a high voltage power supply. They have measured the photocurrent of the cell using an electrometer connected to the two IOIO film (250 µm in thick and 2x3 cm in size). Sandwiching the membrane while illuminated by two halogen lamps from both sides of the membrane. They have observed that the photocurrent was enhanced by application of electrical field but it was constant only for 1 min ,and become practically zero after 40-50 min.

2.5.3 Studies on BR Fragments

Ort and Parson (1979) have measured the quantum yield of proton release from the volume changes after excitation of PM fragments by short flashes. They claimed that relaxation half time was 25 min at 35°C and during relaxation BR absorption spectra did not change significantly. They have concluded that the quantum yield

of proton release increases gradually with increasing ionic strength and that two protons can be released and rebound in each photochemical cycle at high ionic strength. They also shown that the quantum yield decreases with increasing the intensity of flash light.

Marinetty and Mauzerall (1982) measured the transient ion movements of BR suspensions after a flash light. They have measured the conductance of the medium. Eventually, they elucidated that $[H^+]$ uptake proceeded release at pH=4 and the transient conductance was virtually independent of buffer composition, showing that the ions other than $[H^+]$ were first released and then taken up by PM. For monovalent cations such as $[Na^+]$, the quantum yield was found to be 2 or more at high salt concentrations.

Slifkin *et al* (1986,1988) measured the relaxation half times of both light and dark-adapted BR suspensions exposed to be modulated by monitoring conductivity changes. They have determined apparent activation energies, pK values and equilibrium species for the light adapted BR.

Zabut (1990) measured the effect of temperature, pH and ionic strength on the photoactivity of PM fragments . He has suggested a mechanism for the photoreactions taking place under continuous illumination.

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

The reagents commonly used during the experiments satisfy the limiting criteria of being above a certain quality.

The components Sodium Chloride, Potassium Chloride, Magnesium Sulfate, Trisodium Citrate were from Merck (Germany). The Bacteriological Peptone (L-37) was from Oxoid (England). N,N,Metylene Bis Acrylamide, Acrylamide, Ammonium Persulphate, TEMED, DNA cleaving enzyme DNAase were from Sigma Co. (USA). For calibration of the electrode, the buffers of pH 4,7,9 were bought from Ingold (Germany).

Halobacterium halobium S-9 strain was provided by Dr.Baker Zabut in Department of Biology, METU, TURKEY.

3.2. METHODS

3.2.1. Growth of *Halobacterium halobium*

Halobacterium halobium cells were grown referring the method suggested by Osterhelt and Stoeckenius (1974). The cells were cultivated to breed in a sterilized medium which is composed of NaCl, 250.0 g; $MgSO_4.7H_2O$, 20.0 g; KCl, 3.8 g; Trisodium Citrate $5.5H_2O$ ($C_6H_5Na_3O_7.5.5H_2O$), 3.64 g. These components are diluted to 1000 ml with distilled water and the solution is called Basal Salt. After adjusting the pH of the Basal Salt to 7, the bacterial Peptone (L-37) is mixed to form the bacteria growth medium.

Halobacterium halobium strains are maintained on the growth medium (pH 6.8-7.8) containing 1.5 % agar and transferred every 3 months.

Before starting the growth of the *Halobacterium halobium* for further usage, the broth was prepared, by inoculating the bacteria taken from the stock slants, into two flasks of 25 ml medium and they were incubated at 38°C, having shaked in Julabo SW-20C Shaker for 3 days.

The *Halobacterium halobium* culture taken out of this broth was transferred into two 50 ml flasks containing sterile growth medium and proliferated by shaking at 130 rpm at 38°C for 3 days. 100 ml of two flasks were shared to 5 separate flasks for total volume of 750 ml growth medium, then *Halobacterium halobium* was incubated at 40°C, 140 rpm for 4 days.

Growth was followed by measuring the optical density in an Absorption Spectrophotometer at 660 nm.The steadily increasing formation of Purple Membrane was observed by following the increase in absorbance at 560 nm.The formation of Purple Membrane was maximum at the end of exponential growth curve (Oesterhelt and Stoeckenius, 1974). The proliferated culture was harvested after 7 days elapsed for isolation.

3.2.2. Isolation of the Purple Membrane

The isolation of the Purple Membrane was carried out according to Oesterhelt and Stoeckenius (1974) with certain modifications. All steps were accomplished in cold room at 4°C. Cells from 5 flasks of total 750 ml culture were harvested by centrifugation for 60 min. at 6620 g. using Sorwall Centrifuge (SS-34 rotor). The cell pellets were washed by basal salt, centrifuged again by

the same procedure and suspended in 20 ml basal salt solution overnight at -20°C (in deep freezer). 0.5 ml of DNAase solution (2000 unit/ml) was added to the pellet to reduce the viscosity and dialyzed 3-days at 4°C against 2.0 lt of 0.1 M NaCl. After dialysis, the lysate volume was 40 ml, centrifuged at 43000 g (Beckman L265 B ultracentrifuge) for 60 min. After resuspending the pellet in 12 ml of 0.1 M NaCl, it was repeated once more. The resulting pellet which was purple in color is suspended in 5 ml distilled water, and kept in the deep freezer (-20°C) until use.

3.2.3. Characterization of the Purple Membrane

3.2.3.1. Protein Determination

The protein content was determined spectrophotometrically at 570 nm. In this case absorptivity (extinction coefficient) of 63000 M^{-1} cm^{-1} was used. (Oesterhelt and Hess, 1973)

3.2.3.2. Molecular Weight Determination

The molecular weight of BR was determined by using SDS polyacrylamide gel electrophoresis. The electrophoresis was performed on 3% stacking and 7.5% separating gels according to Laemmli (1970), using Bovine Serum Albumin, Bovine τ-Globumin. Catalase and Lysosyme

as standards. Electrophoresis was carried out at room temperature with a current 2.5 mA per gel until bromophenol marker reached the bottom of the gels (2-3 hours).

After electrophoresis, dye fronts were marked with a wire, gels were fixed and the proteins were stained in a solution containing 0.25% comassive blue in 50% (v/v) methanol and 7% (v/v) glacial acetic acid for 60 min. The gels were destained by diffusion of unbound dye from gels by extensive washing with a solution containing 7% acetic acid and 30% methanol for 2-days at room temperature.

3.2.3.3. Absorption Spectrum of Bacteriorhodopsin

The absorption spectrum of BR is measured in distilled water or in 0.15 M KCl solution by using Hitachi U-3200 double beam spectrophotometer.

3.2.4. Activity Measurement of BR

The photoactivity of PM fragments has to be tested before they are immobilized in PAG to achieve this test, the pH of the medium was recorded by Nel (mod 821) pHmeter after this instrument was calibrated with buffers. The temperature was set to 25°C and 1 M KCl stock solution was mixed with 1 ml of PM fragments.

The activity of BR was measured by light induced pH changes by combined pH electrode. The measurement system was described by Zabut(1990).

3.3. CONSTRUCTION OF PHOTOACTIVITY MEASURING CELLS

3.3.1. All Glass Two Compartment Photoactivity Measuring Cell

In order to construct all glass two compartment photoactivity measuring cell, eight glass plates of 3.5x3.5x4 cm with a thickness of 4 mm in size were engraved. The sides of the plates were perfectly cleaned with alcohol and congruously constructed by sticking the walls with silicon as it is shown in Figure 3.3.1 Gel Constituted Glass Plate (GCGP) which was prepared as it is described in Section 3.4. was centrally placed between two chambers and it was stucked to the chamber with silicone. This confirmed cell was placed in a glass water bath. The volume of one compartment was about 50 ml. This cell was placed in water bath which was made of all glass. The dimensions of the water bath was 30x12x12 cm with a water capacity of 1.5 lt with cell

3.3.2. Jacketed Two Chambers Photoactivity Measuring Cell

The preliminary all glass two compartment photoactivity cell has been modified, and a new cell replacing the water bath by a jacket surrounding the chambers has been designed. The details of the jacketed chambers are given in Figure 3.3.2. The jacketed chambers were made up of plexiglass plates of 0.5-1 cm thick. Inner chamber has an empty volume of about 30 ml and the dimensions are 2x2x8 cm. The jacket is in U shape surrounding the chambers only from two facing sides and from the bottom. The tap of the jacket is closed and there are two openings of 1 cm in diameter which will let to the circulation of water through the jacket. The front and back faces of the chambers have a hole of 1 cm in diameter on the same axis. The holes on the facing sides of the chambers are left open. However, the holes on the front face of front chamber and the back face of back chamber are closed by a glass plate, so that the incident beam of light directed from the light source is allowed to pass. There are two flanges and a leach on the connection of the two chambers have been achieved by means of these flanges. On the outside face of the facing sides of the chambers there is a 2x2 cm silicon pillow

surrounding the open hole. Gel constituted glass plate is placed between these silicon pillows, and then two chambers are clenched.

On the upper lid of cell two holes of 0.75 and 1.15 cm in diameter were drilled for immersion of temperature probe and pH electrode. These holes can be well-closed with screws to get rid of diffusion of CO_2 into the solutions if probe or electrode was not immersed.

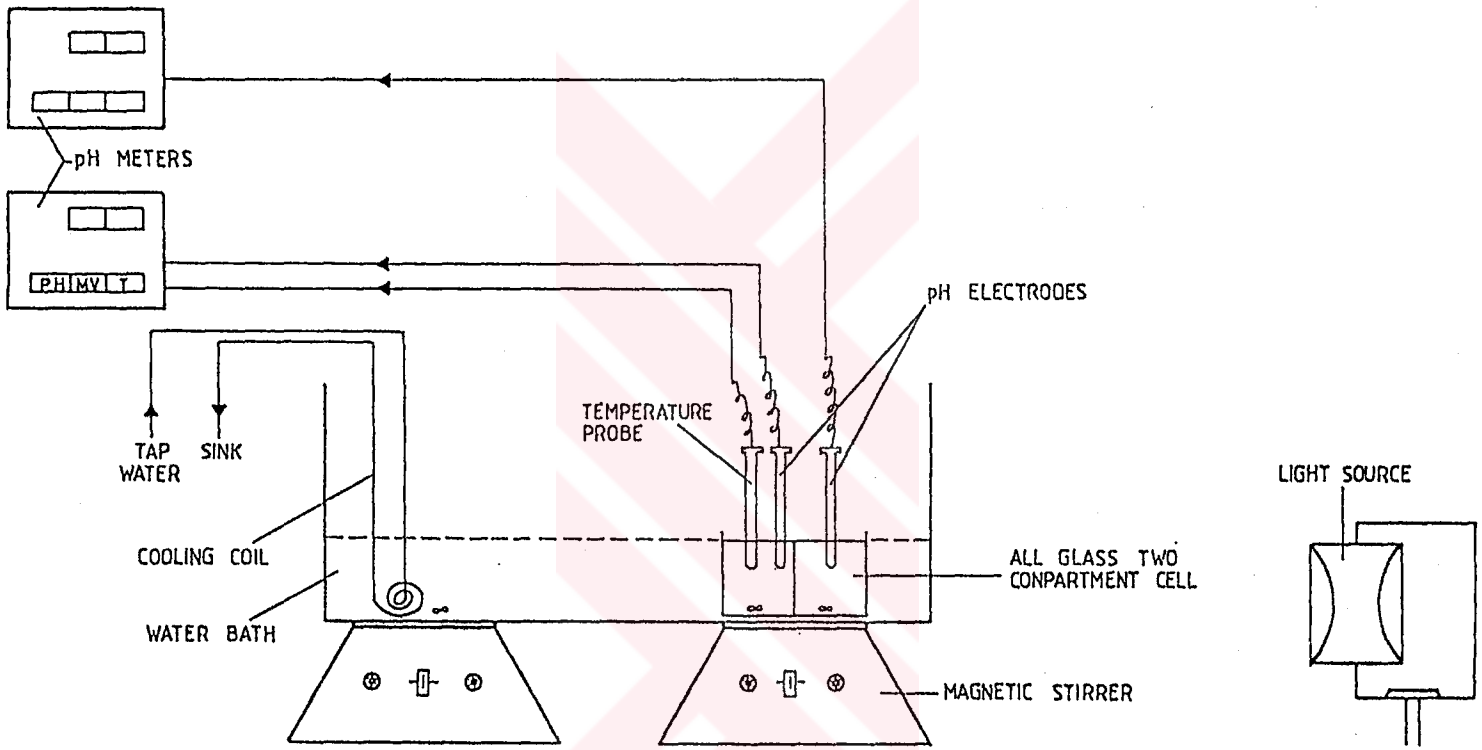

Figure 3.3.1. Experimental Setup with All Glass Two Compartment Cell.

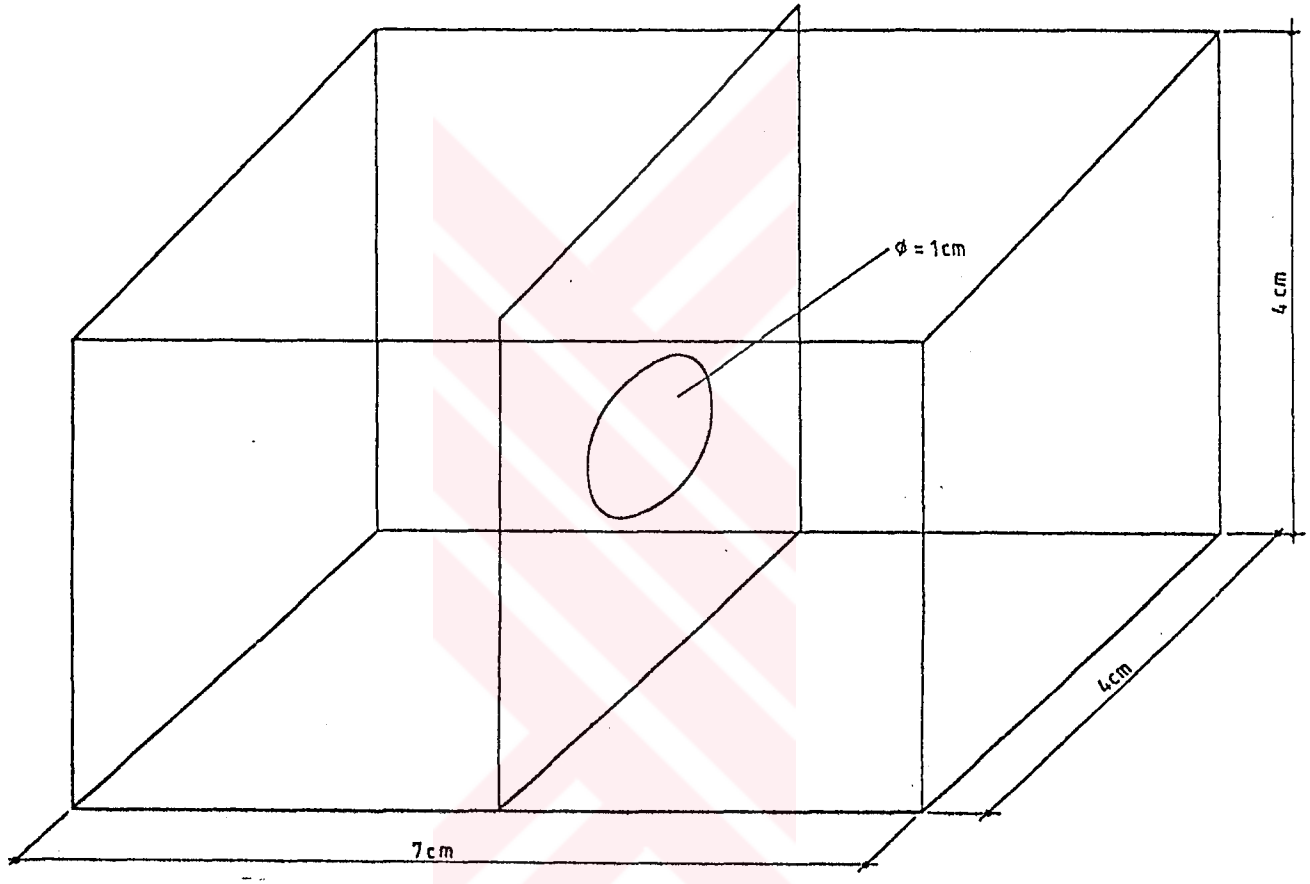

Figure 3.3.2. All Glass Two Compartment Photoactivity Measuring Cell.

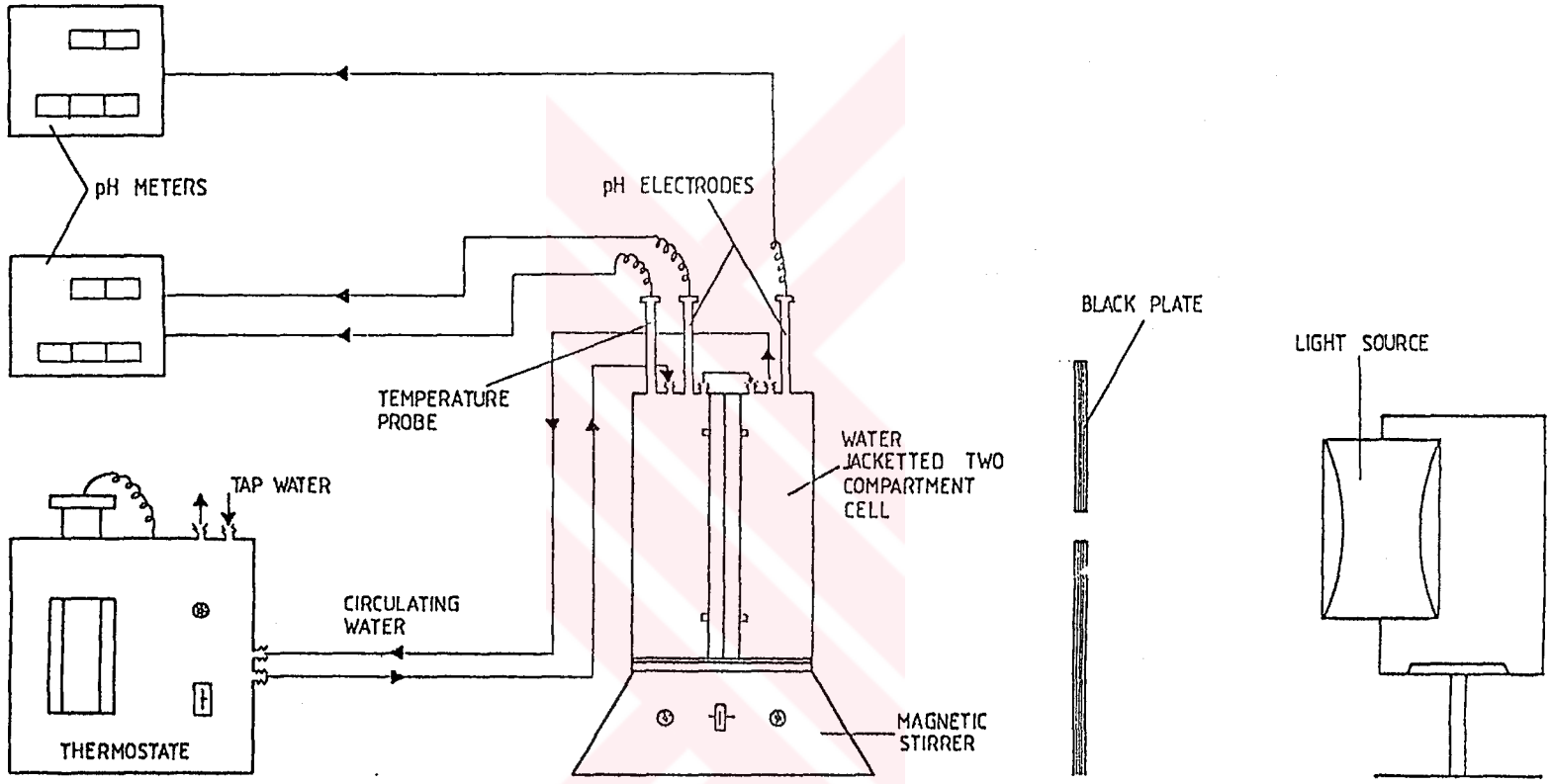

Figure 3.3.3. Experimental Setup with Jacketed Two Compartment Cell

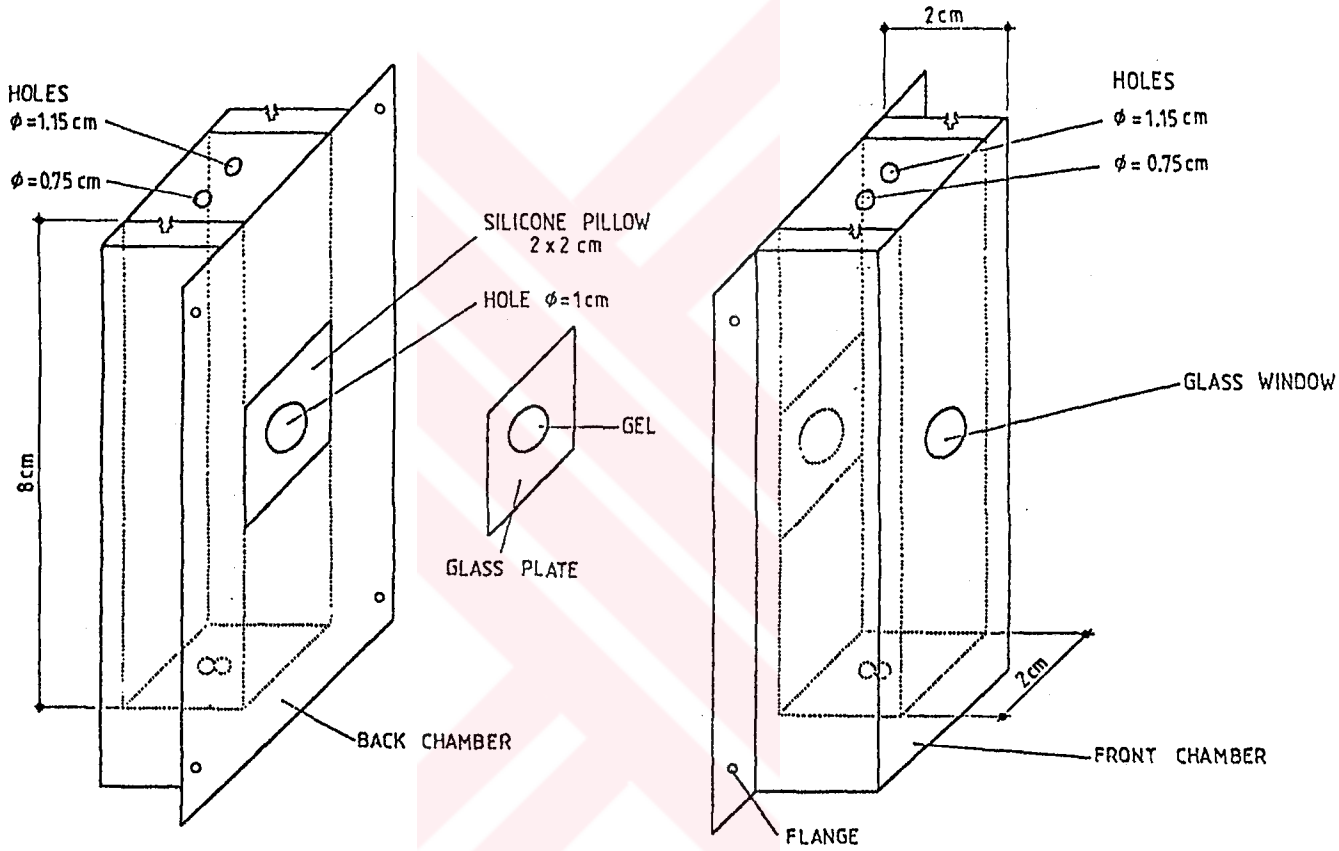

Figure 3.3.4 Jacketed Two Compartment Photoactivity Measuring Cell

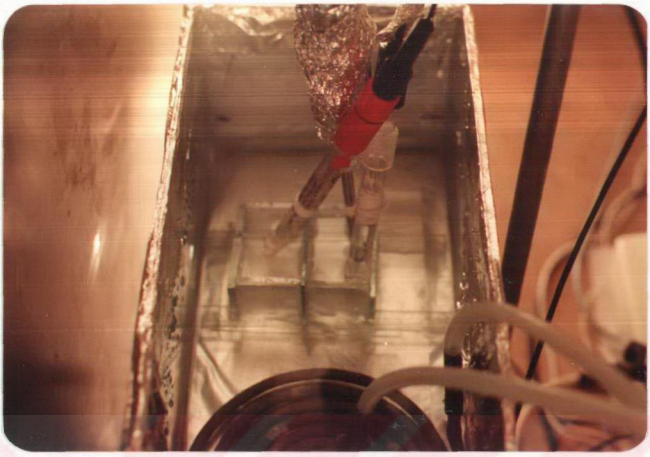

Figure 3.3.5. Photograph of Experimental Setup with All Glass Two Compartment Cell.

Figure 3.3.6. Photograph of Experimental Setup

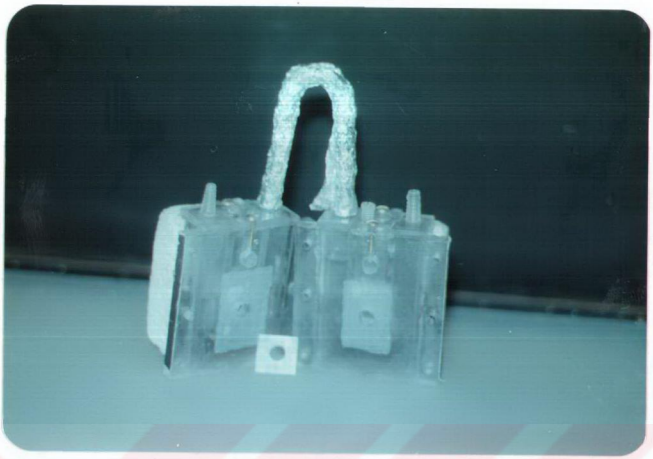

Figure 3.3.7. Photograph of Experimental Setup with Jacketed Two Compartment Cell.

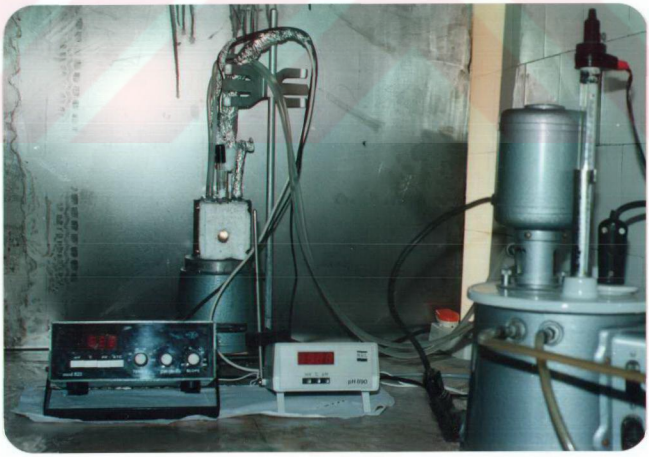

Figure 3.3.8. Photograph of Focused Illumination through the GCGP

3.4. PREPARATION OF GEL CONSTITUTED GLASS PLATES

The Polyacrylamide Gel (PAG) is formed by polymerization of a reagent which is composed of 14 g acrylamide and 0.37 g bis acrylamide which is diluted with distilled water to 50 ml. In order to prepare 2 ml of 7.5% PAG, 0.5 ml of 7.5% gel solution reagent is mixed with 0.725 ml of 1% w/v of ammonium persulfate, 0.775 ml distilled water, and 3 ml TEMED. The pore size of PAG can be adjusted by means of different composition ratios of these components.

Bacteriorhodopsin containing polyacrylamide gel is prepared by the same procedure as described above. The only difference is that instead of 0.775 ml distilled water, 0.775 ml of purple membrane fragments isolated as described in Section 3.2.2. are added to gel solution. The concentration of BR could be varied by the amount of purple fragments added. However, the care should be taken to the fact that the sum of PM and distilled water should not be more than 0.775 ml.

Glass Plates of 1 mm in thickness are cut as squares of 2x2 cm in size. In the center of the glass plate a hole of 0.4 or 0.8 or 1.0 cm in diameter has been drilled. The glass plates are well rinsed with alcohol

and wetted with distilled water for easy dismantlement. The hole is placed on another clean glass plate, and after it is closed by an upper glass plate the gel solution is poured into the hole. The upper and lower glass plates were clenched.

Gel solution polymerizes within the hole and nestles down in this niche by sticking to periphery of the hole. Polymerization will be completed in about 30 minutes. After that first upper glass support and then the lower glass support are dismantled very carefully. Gel constituted glass plates thus prepared are sank into 0.15 M KCl solution to attain uppermost swelling of gel and kept in this solution in a refrigerator if it is not used.

It should be noted that a perfect coverage of the hole by the gel without any leakage has been achieved by experience and a novel technique has been developed. The pinch point of this technique is to control the time of waiting just before the polymer solution is poured into the hole.

For the orientation of BR, electrical field is applied by a direct current generator to the electrodes placed above and below of the upper and lower glass plates, during gelation. The voltage applied to the

electrodes is 15 V/cm or 40 V/cm.

3.5. EXPERIMENTAL SETUP

In Figure 3.3.1. experimental setup with all glass two compartment cell is schematically shown. The compact glass cell is placed in the water bath. There is a cooling coil inside the water bath which is connected to the tap water. There are two magnetic stirrers placed under the water bath one for mixing the bath and the other is for the cell. The bath could be heated to the desired temperature with the use of heater of the magnetic stirrers. There are two combined glass pH electrodes (Russel, Model DIN Connection and Ingold, Model) and a temperature probe (Ingold) which are connected to two pH meters, (NEL Model 821 and NEL Model 980) respectively. Two light sources are used; a 150 W light projector and a projector (Reflecta Lamp) which have 500, 1000, 1500 and 2000 W light sources. The light source is 50 cm apart from the cell.

In Figure 3.3.3. experimental setup with jacketed two compartment cell is schematically shown. A circulation water bath has been set to a preset temperature by means of a thermostat and water is circulated through the jacket of the chambers by a pump. Outside of the chambers are encrusted with a black

hardcover, except the glass window in front face of the front chamber. In order to eliminate light scattering to environment a black shelter having 1 cm hole was interposed just in front of the cell. Light is focused on GCGP through this black body. The center of all holes along incident light beam are on the same line.

3.6. EXPERIMENTAL PROCEDURE

Experiments are carried out in a dark room. Gel constituted glass plate is saturated in 0.15 M KCl for at least 24 hours, after it is prepared. A leakage test is carried out for each newly prepared gel. For this test 0.15 M KCl solution is poured into two chambers by the same volume. pH electrodes and the temperature probes are immersed into both chambers. The system is incubated in dark for 30 min at 25 C and a steady state has been reached in both chambers. Then 5 µl of concentrated HCl has been injected as a pulse into back chamber and the pH change in both chambers have been recorded. If there is a sudden change in pH of front chamber this indicates leakage,otherwise a slow change in pH is expected.

Photoactivity of GCGP have been tested by the following procedure.

i) The system is incubated in dark at 25 °C until

constant temperature and pH values in both chambers are reached

ii) The light is turned on

iii) Temperature and pH values in both chambers are recorded.

iv) Wait in light until steady pH values are attained in both chambers

v) Turn off the light

vi) Continue recording the temperature and pH changes until steady values are attained.

Temperature was tried to be kept constant at 20-25 °C in all the experiments.

Three different types of GCGP were tested. Those were

i) Polyacrylamide gel without BR

ii) BR immobilized in Polyacrylamide gel

iii) Oriented BR immobilized in Polyacrylamide gel

Three different light sources were used, such as 150, 500 and 1000 W.

Initial pH of the chambers have been varied. Experiments were carried out as having the same initial pH in both chambers and different initial pH values in two chambers.

CHAPTER 4

EXPERIMENTAL RESULTS

4.1. GROWTH OF *HALOBACTERIUM HALOBIUM*

The growth curve of *Halobacterium halobium* S-9 strain is given in Figure 4.1. The growth of bacteria was followed by direct measurement of absorptivity at 660 nm and there was no turbidity problem during these measurements. According to this figure maximum growth was achieved after 54 hours at 40 °C.

Bacteiorhodopsin was isolated after 64 hours of growth which corresponds to the maximum accumulation of BR as given in Stoeckhenius and Oesterhelt (1971).

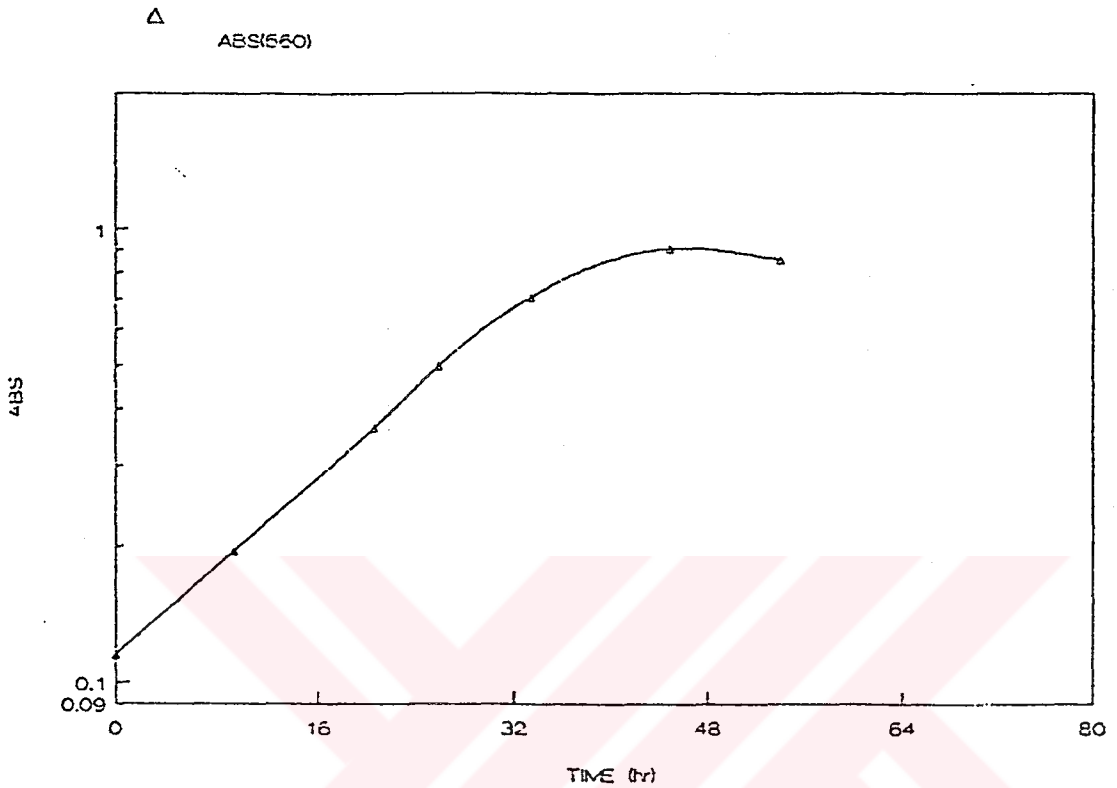

Figure 4.1. Growth of *Halobacterium Halobium* S-9 Strain. Absorbance Value is given for samples from 125 ml culture medium.

4.2. ISOLATION AND CHARACTERIZATION OF PURPLE MEMBRANE

The concentration of BR in isolated PM was found to be in the range of 30-85 µM which corresponds to a protein concentration of 0.78-2.17 mg/ml.

Molecular weight of BR was determined by SDS gel electrophoresis and a single polypeptide band was observed at 26,000 d .

The absorption spectrum of PM was given in Figure 4.2.The absorption was measured in distilled water and it showed maximum peak at 564 nm. This result is consistent with literature Stoeckhenius and Oesterhelt (1971).

Results of activity tests are shown in Figure 4.2.2. and Figure 4.2.3. Maximum $\triangle$pH was measured as 0.12 after 10 minutes of illumination by 1000 W, 45 cm apart light source. This result has indicated that BR is active.

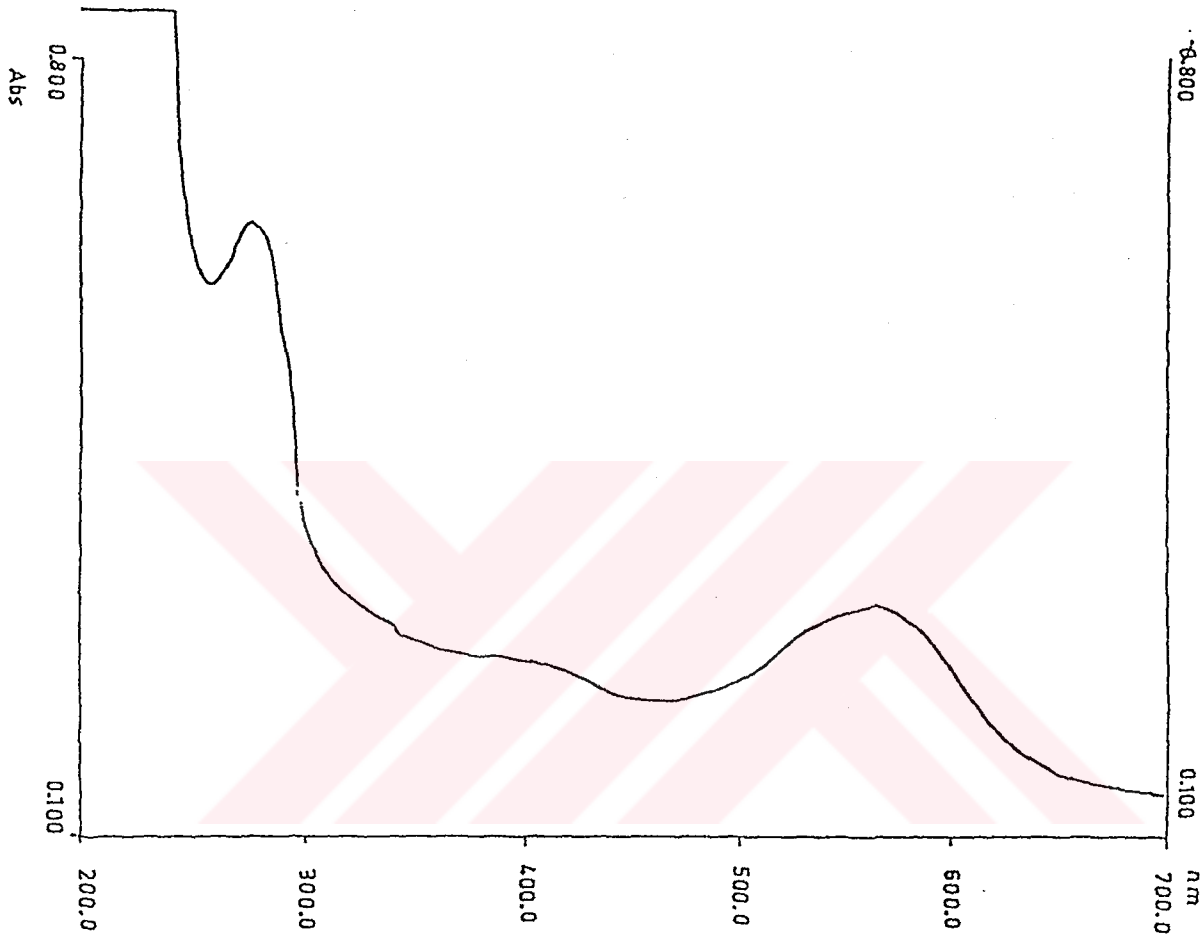

Figure 4.2.1. Absorption Spectra of Purple Membrane Suspended in Distilled Water

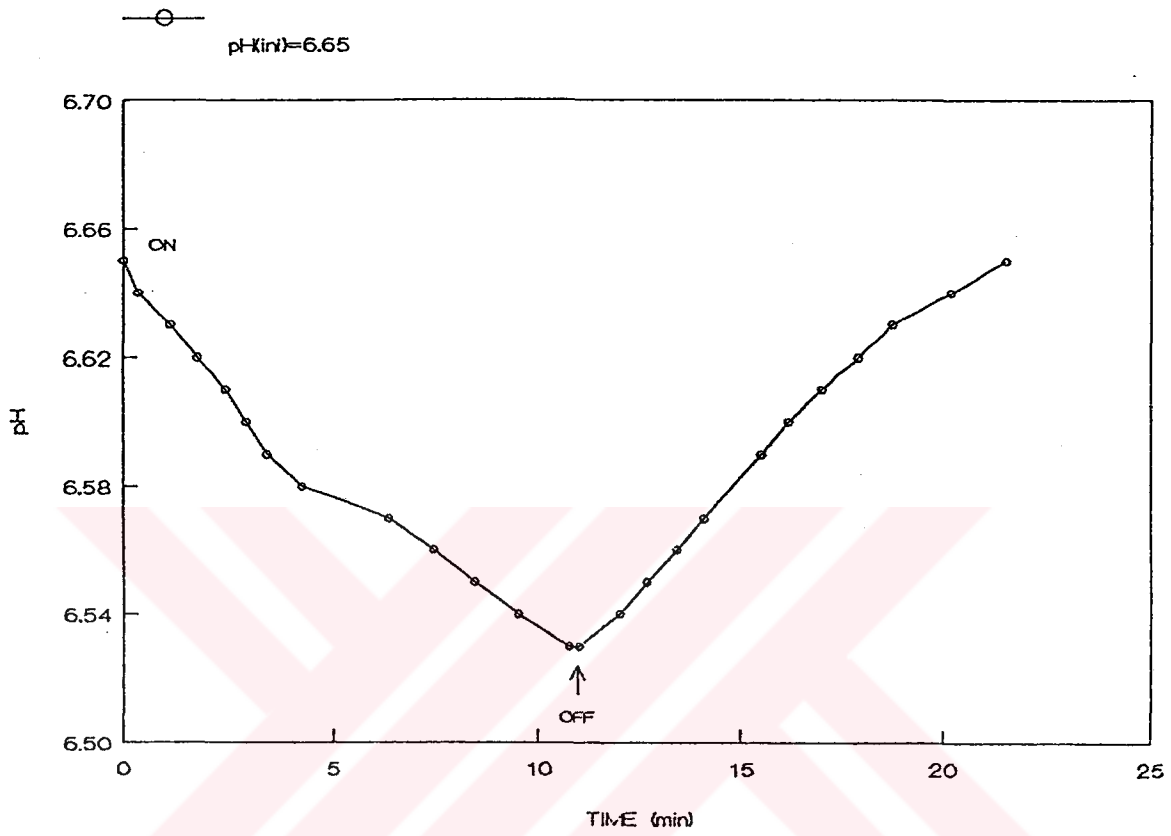

Figure 4.2.2. pH versus time curve for PM fragments upon 1000 W of illumination in activity test.

4.3 RESULTS OF CONTROL EXPERIMENTS

In Figure 4.3.1 result of a leakage experiment carried out in AGTCC is shown. The membrane was PAG without BR. Initial pH of both chambers was the same at pH 6. 94. In this experiment, 5 µl of diluted HCl was added to chamber B at zero time. A sudden decrease from pH 6.94 to 6.20 was observed in chamber B. pH in chamber A did not show any variation with respect to time. This result indicated that there was no leakage or simple diffusion of $[H^+]$ during the experiment. A gradual increase in $[H^+]$ was observed in chamber B which could be attributed to surface protonation of the gel. After about 40 minutes pH reached a steady value at 6.61 in chamber B. That may indicate the saturation of gel surface at the experimental conditions.

In Figure 4.3.2 the result of another leakage test carried out at the same initial conditions of previously described experiment is shown. In this experiment 5 µl of concentrated HCl was introduced to chamber B at zero time. A sudden decrease of pH from initial value of 6.94 to 3.4 has been observed in chamber B. As it can be seen from Figure 4.3.2, pH in chamber A remained constant for about 40 min. This result indicated that there was no $[H^+]$ transfer between chamber A and chamber B. However after 40 minutes a gradual decrease of pH in chamber A

was detected. This result indicated that there was a $[H^+]$ transfer between two compartments which might be due to rupture of the membrane at relatively low pH at longer times. It was calculated that $9E^{-10}$ moles of $[H^+]$ was transferred to chamber A after 200 minutes. This value was negligible as compared to the $[H^+]$ concentration in chamber B. Therefore, no pH change was observed in chamber B which can be seen from the data in Appendix B.

Similar results were obtained with JTCC. Further control experiments were carried out to test the effect of light on polyacrylamide gel without BR. In those experiments no change of initial pH was observed with illumination.

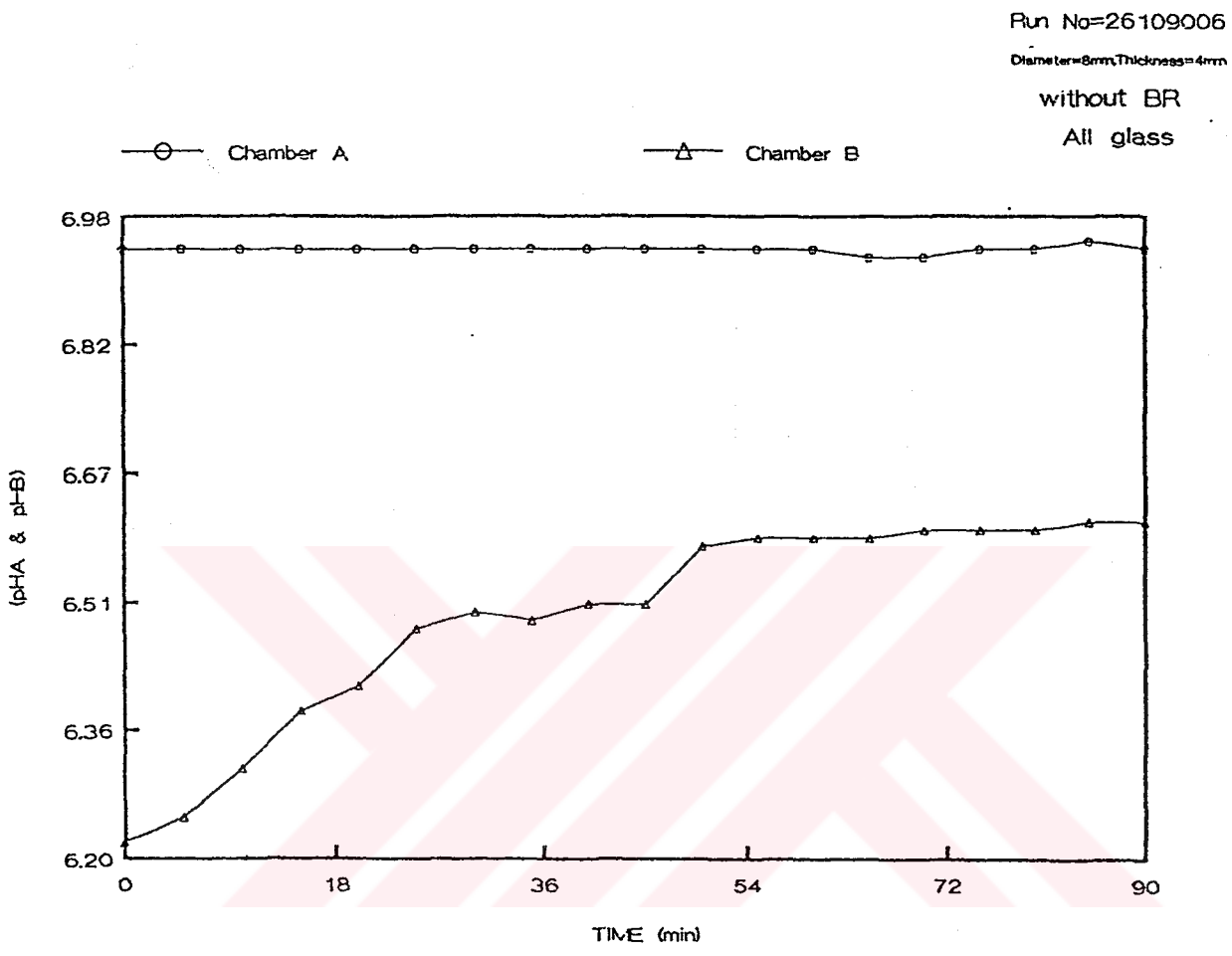

Figure 4.3.1. Variation of pH in both Compartments with Time After Injection of a Pulse of 5 µl Dilute HCl at Zero Time.

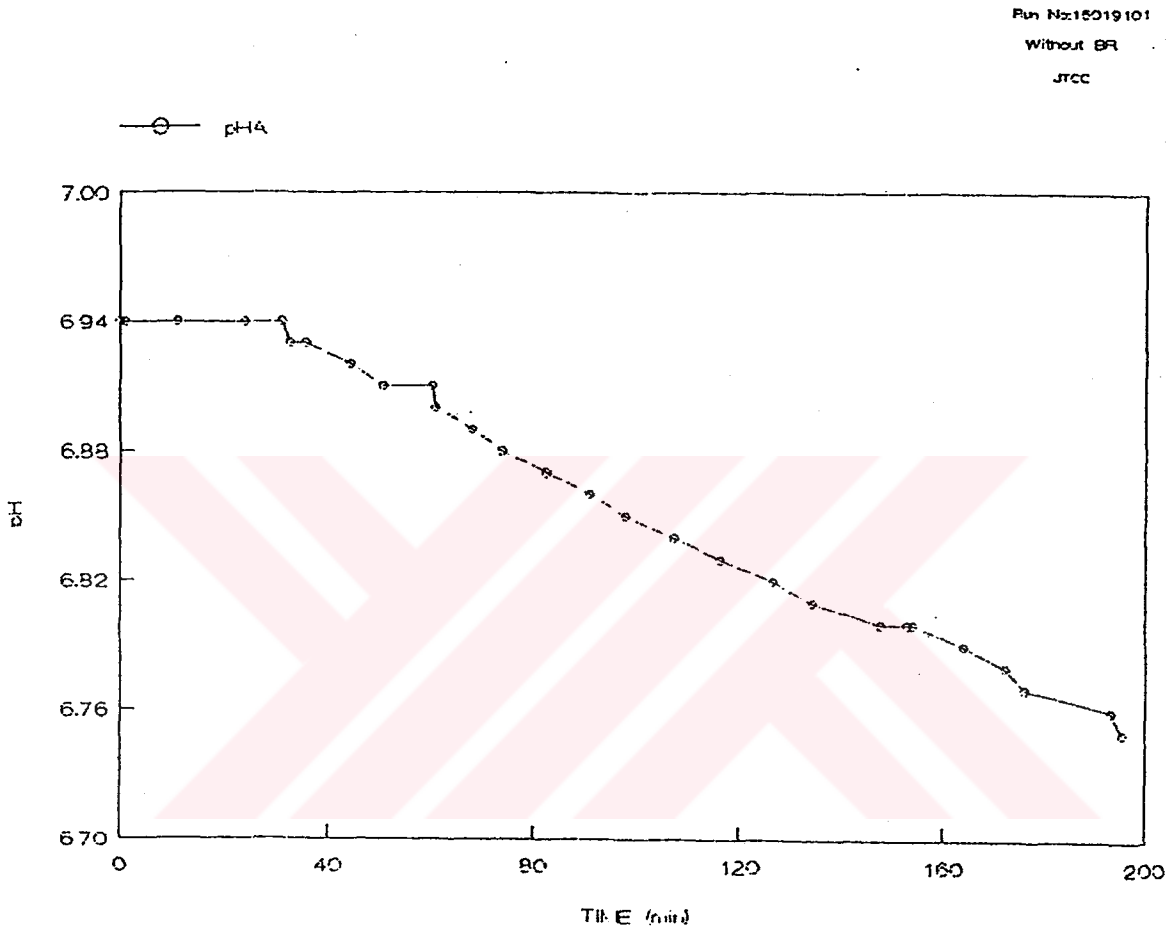

Figure 4.3.2 Variation of pH in Chamber A with Time After Injection of a Pulse of 5 µl Concentrated HCl at Zero Time.

4.4 PHOTOACTIVITY MEASUREMENTS OF BACTERIORHODOPSIN IMMOBILIZED IN POLYACRYLAMIDE GEL

4.4.1 Non-oriented BR Immobilized in PAG

In Figure 4.4.1 results of an experiment carried out in AGTCC are shown. The membrane was non-oriented BR immobilized in PAG with size of 6 mm diameter and 4 mm thickness. The membrane was incubated in 0.15 M KCl and initial pH in both chambers was 6.96. At zero time the membrane was illuminated with 150 W light source. Variation of pH in both chambers were recorded and the data was plotted. As it can be seen from Figure 4.4.1 pH variations was quite similar in both chambers. When the light was on pH in both chambers decreased up to a minimum value, remained constant for a certain time and then increased. The value of pH in both chambers eventually reached to 6.88 and remained constant at this value. When light was turned off no further change was observed.

In Figures 4.4.2 the results of another experiment having the same conditions with the previously experiment are shown. However in this experiment the light was turned off after 16 minutes. pH decreased up to 6.89 and then remained constant. When the light was turned on

again, pH in both chambers remained constant for about 20 minutes but after that a gradual increase was observed in both chambers almost up to initial value. However final pH in chamber B was higher than its initial value while it was less for chamber A.

In Figure 4.4.3 the results of experiments carried out in AGTCC with non-oriented BR immobilized in PAG are shown The dimensions of the membrane were the same as it is described previously. The membrane has been incubated in dark in 0.15 M KCl with initial pH 7.19. However during the incubation pH of chamber B reached to 7.16 and chamber A to 7.05. When the cell was illuminated with 1000 W light source pH in both chambers slowly decreased upto a steady value. When light was turned off and later on again, no significant change in pH was observed.

In Figure 4.4.4 the results of an experiment carried out with non-oriented BR immobilized in JTCC were shown.The membrane had the dimensions of 4 mm in diameter and 1 mm thickness. After incubation, initial pH values in chamber A and B were 7.04 and 6.86 respectively. When the cell was illuminated with 500 W light source no significant change in pH was observed in both chambers.

4.4.2 Oriented BR Immobilized in PAG

In Figure 4.4.5, the results of experiments carried out with oriented BR immobilized in PAG in AGTCC are shown.Bacteriorhodopsin was oriented by applying electric field of 10 mA and 140 mV. The dimensions of the membrane were 6 mm in diameter and 4 mm in thickness. Initially pH was 6.48 in chamber A. when the cell was illuminated with 1000 W light source an increase in pH was observed. When the light was turned off at pH 6.76, the increase in pH continued however much more slowly and it reached a maximum at 6.82 after that ph decreased to 6.76. When it is illuminated again no significant change was observed. In this experiment pH variation of chamber B was not recorded,unfortunately.

In Figures 4.4.6 the results of the experiment carried out with same conditions as described above except initial pH are shown.Initial pH of both compartments were 6.63. When light was on a gradual increase in pH in both chambers was observed. When light was turned off,the increase in pH has continued but less steeply. When light was turned on again the behavior did not change. In Figure 4.4.7, the difference between pH values of chamber A and B versus time are plotted. It is

interesting to note that pH difference increases with time upto 25 minutes and then it remains constant. Maximum pH difference observed was 0.12.

In Figure 4.4.8, the results of the experiment carried out in JTCC with oriented BR immobilized in PAG are shown. Bacteriorhodopsin was oriented by applying 20 mV static electricity. The size of the membrane was 10 mm in diameter and 1 mm in thickness. Initially 0.15 M KCl at pH 8.0 was poured into both compartments. After 14 hr of incubation period, pH values in chamber A and B were reached to 7.94 and 8.07, respectively. When the cell was illuminated with 1000 W light source pH values in both chambers decreased. The light was turned off after 150 minutes and decrease in pH continued. After 380 minutes, the experiment was stopped.

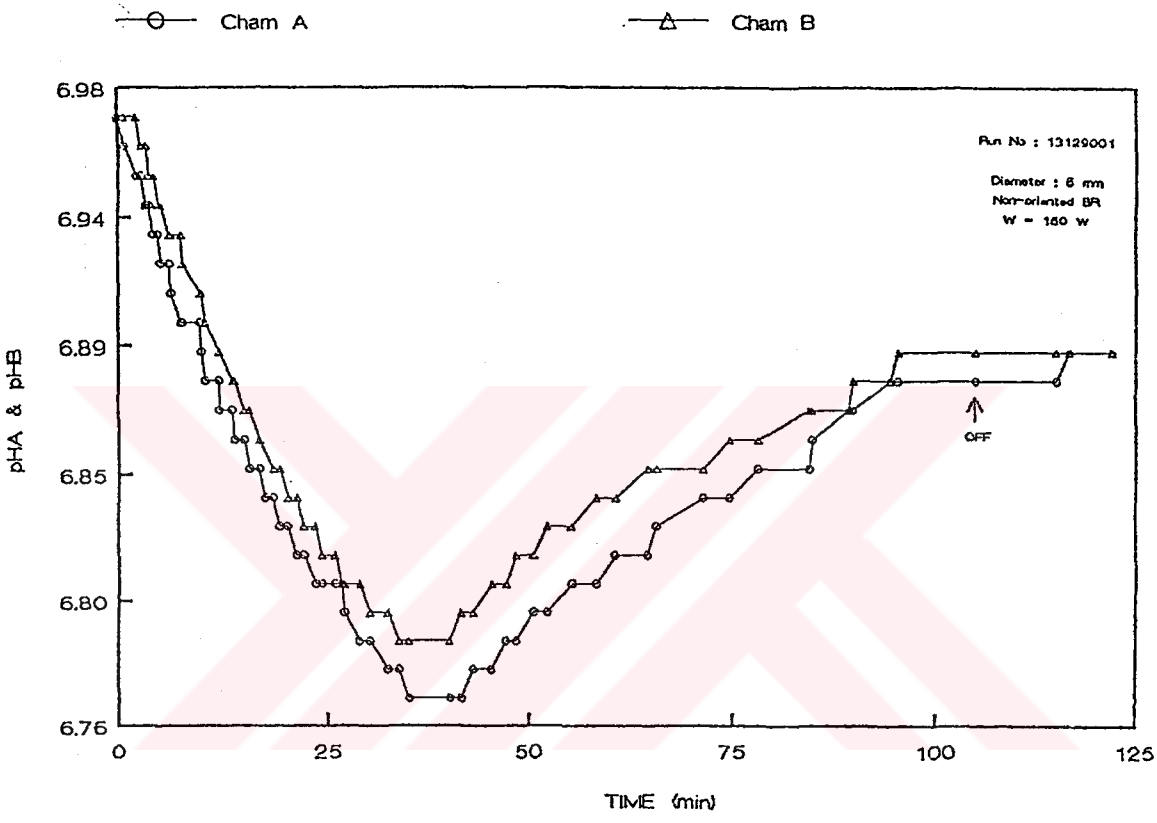

Figure 4.4.1. pH versus time plot for non-oriented BR immobilized in PAG upon illumination of 150 W.

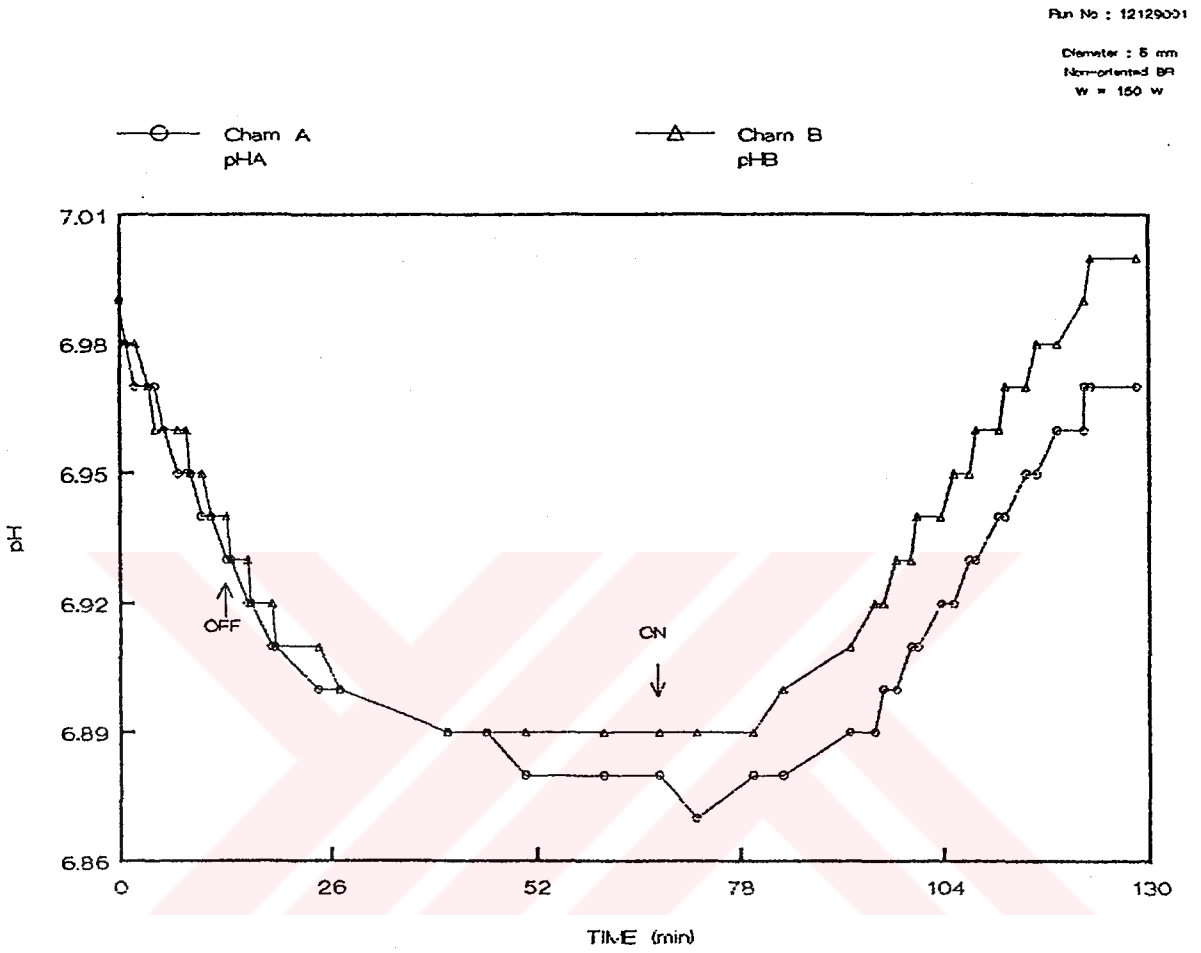

Figure 4.4.2. pH versus time plot for non-oriented BR immobilized in PAG upon illumination of 1000 W.

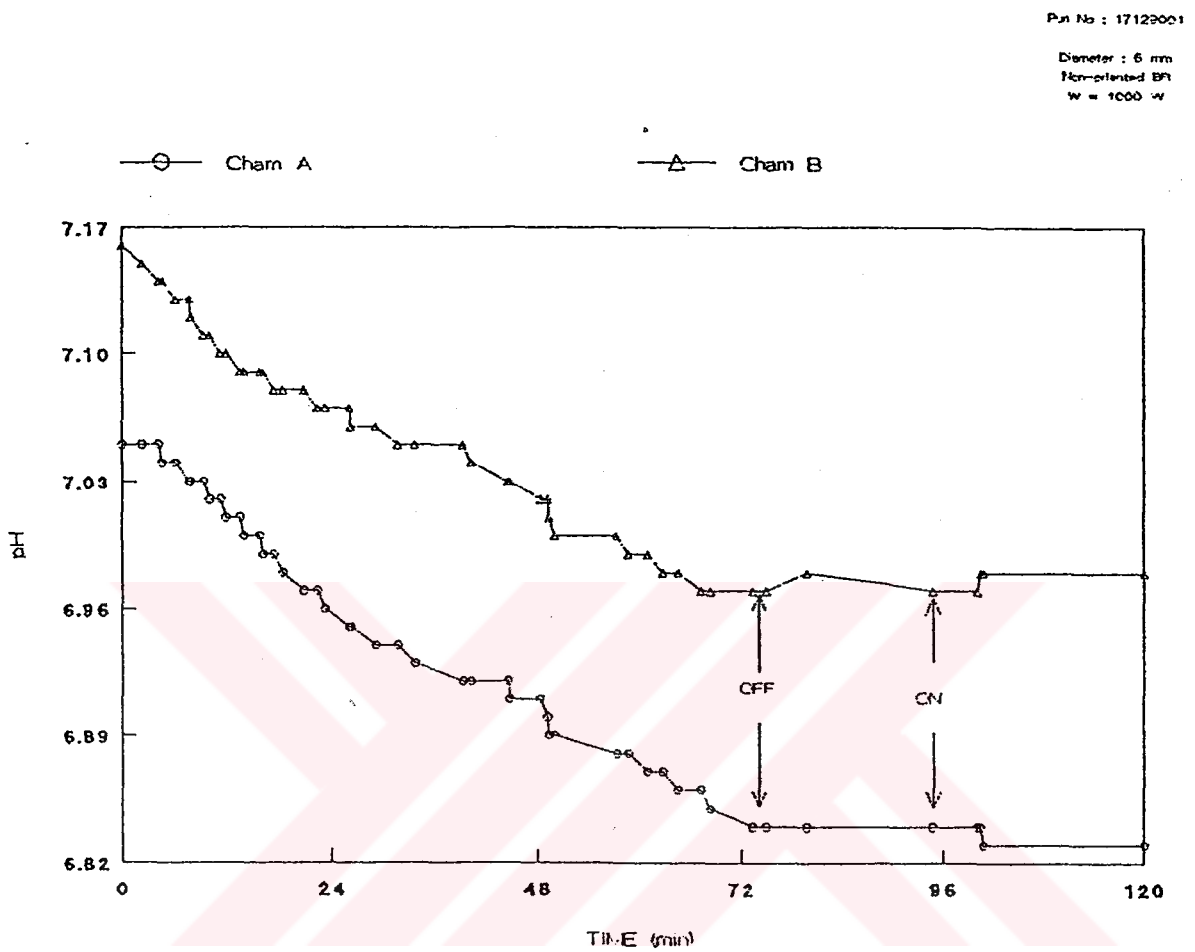

Figure 4.4.3. pH versus time plot for non-oriented BR Immobilized in PAG upon illumination of 150 W.

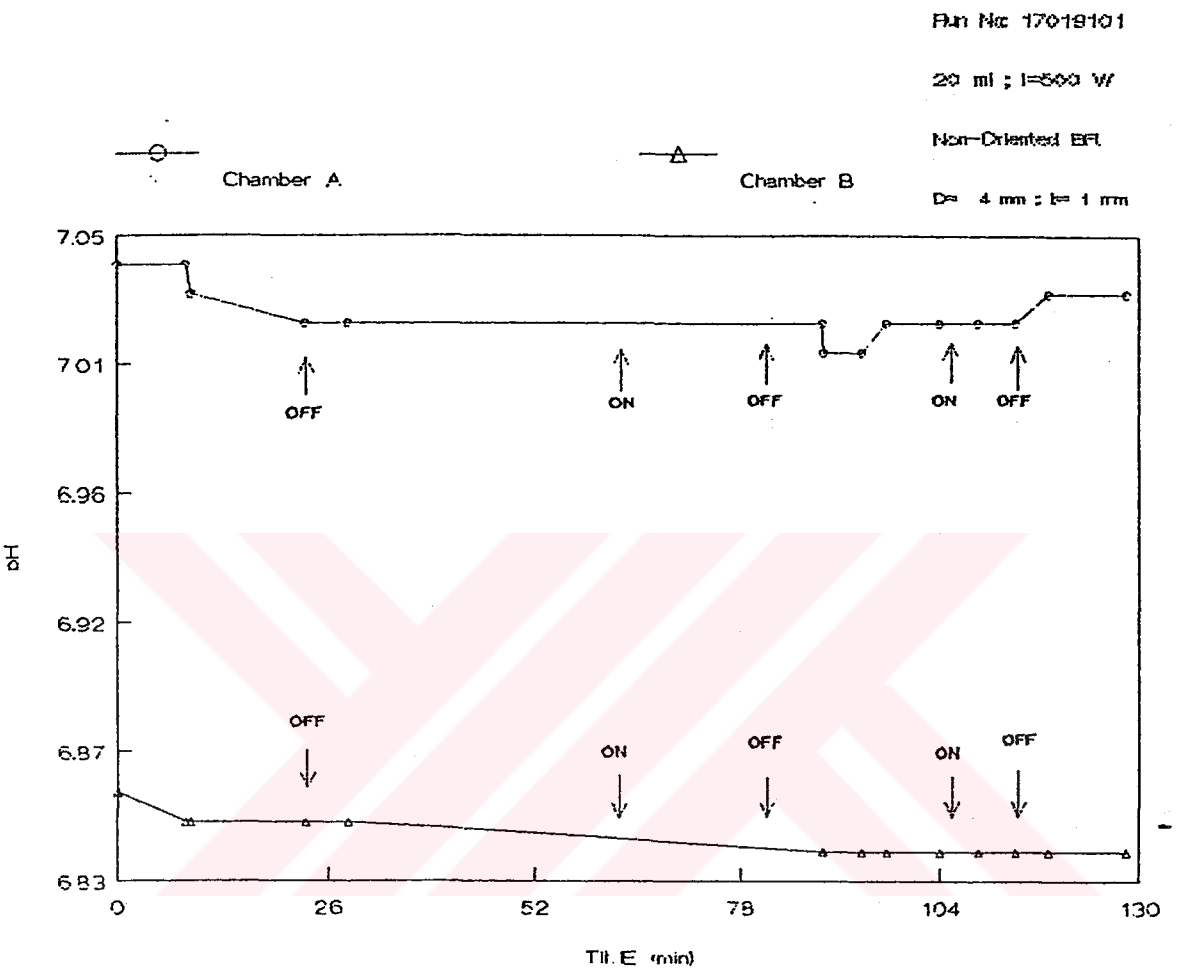

Figure 4.4.4. pH versus time plot for non-oriented BR immobilized in PAG upon illumination of 500 W.

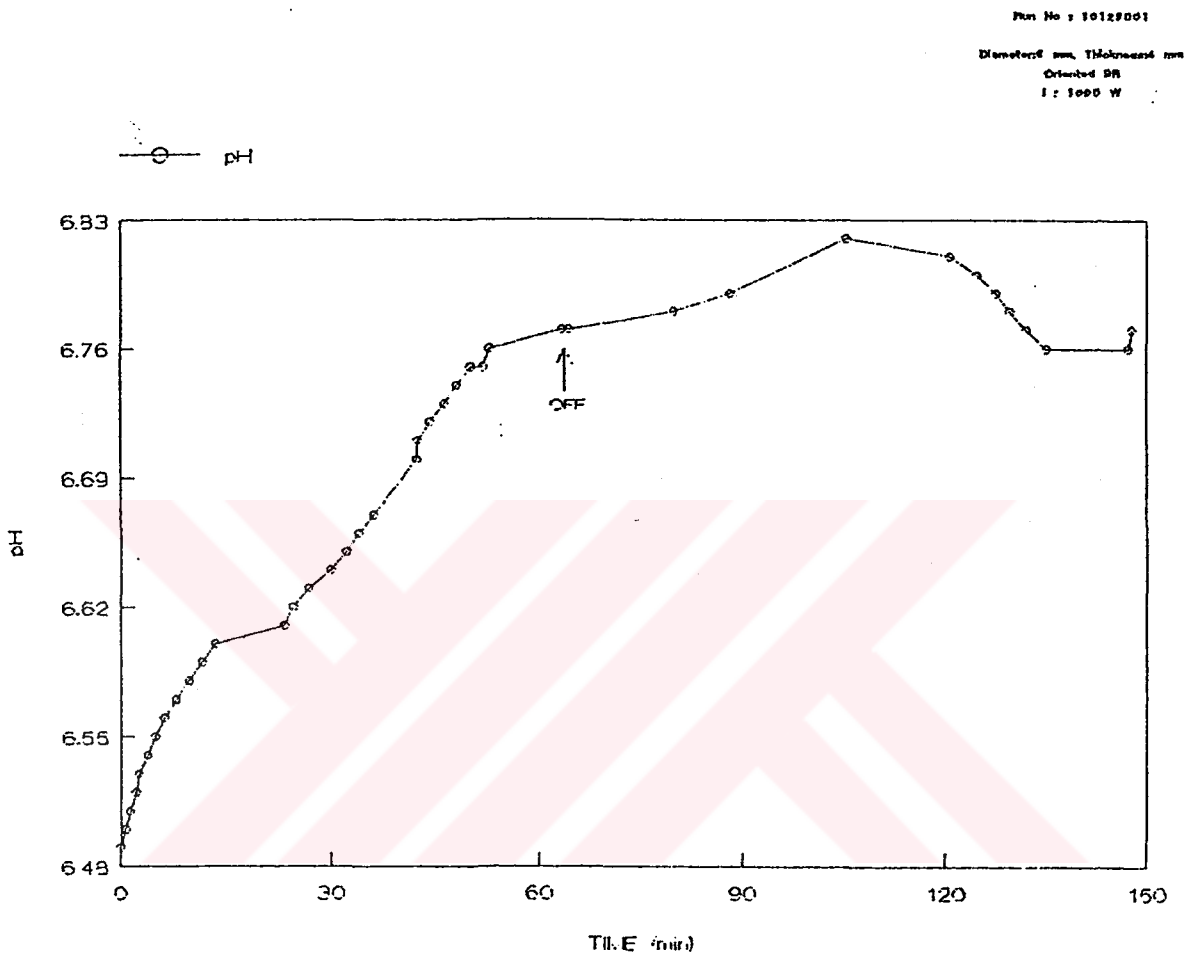

Figure 4.4.5. pH versus Time Plot for Oriented BR Immobilized in PAG upon Illumination of 1000 W .

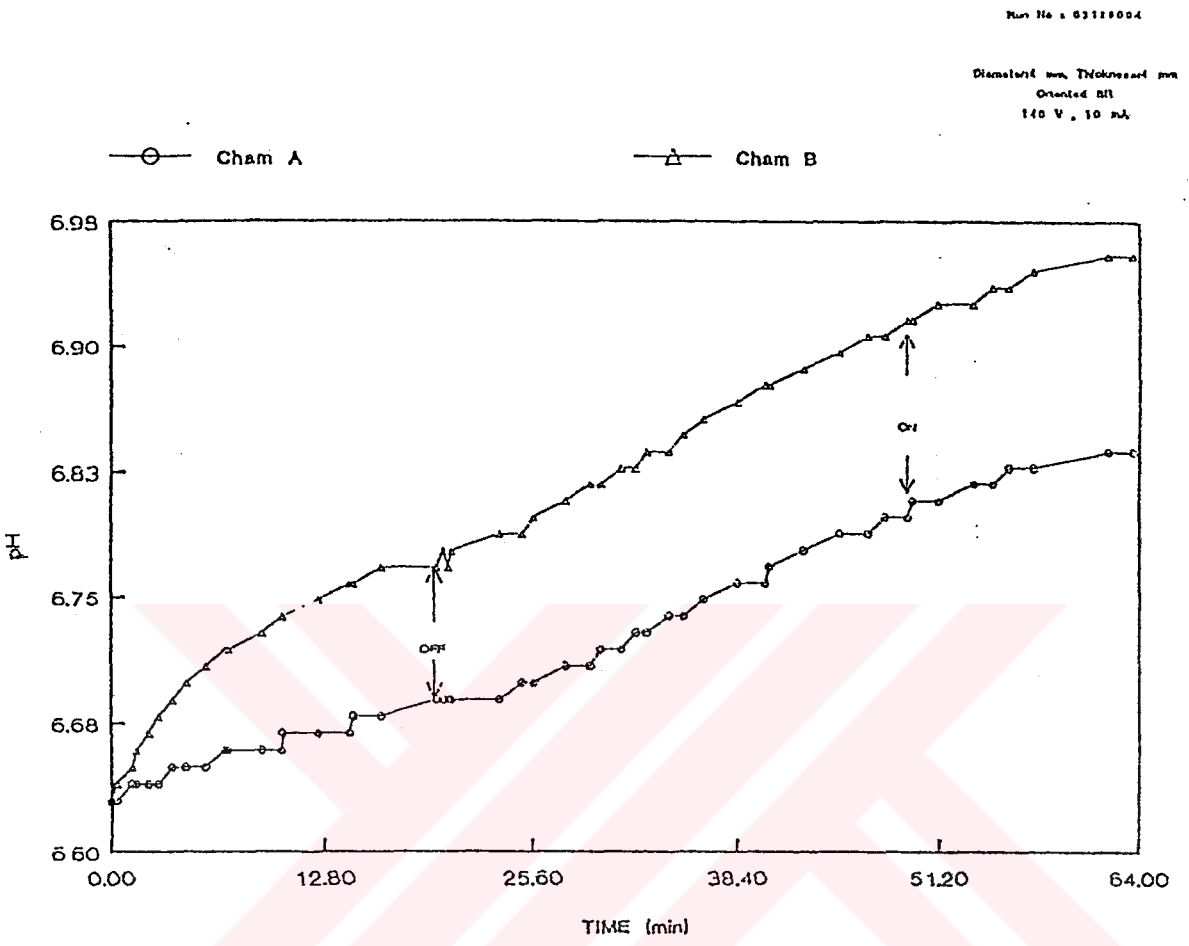

Figure 4.4.6. pH versus Time Plot for Oriented BR Immobilized in PAG upon Illumination of 1000 W .

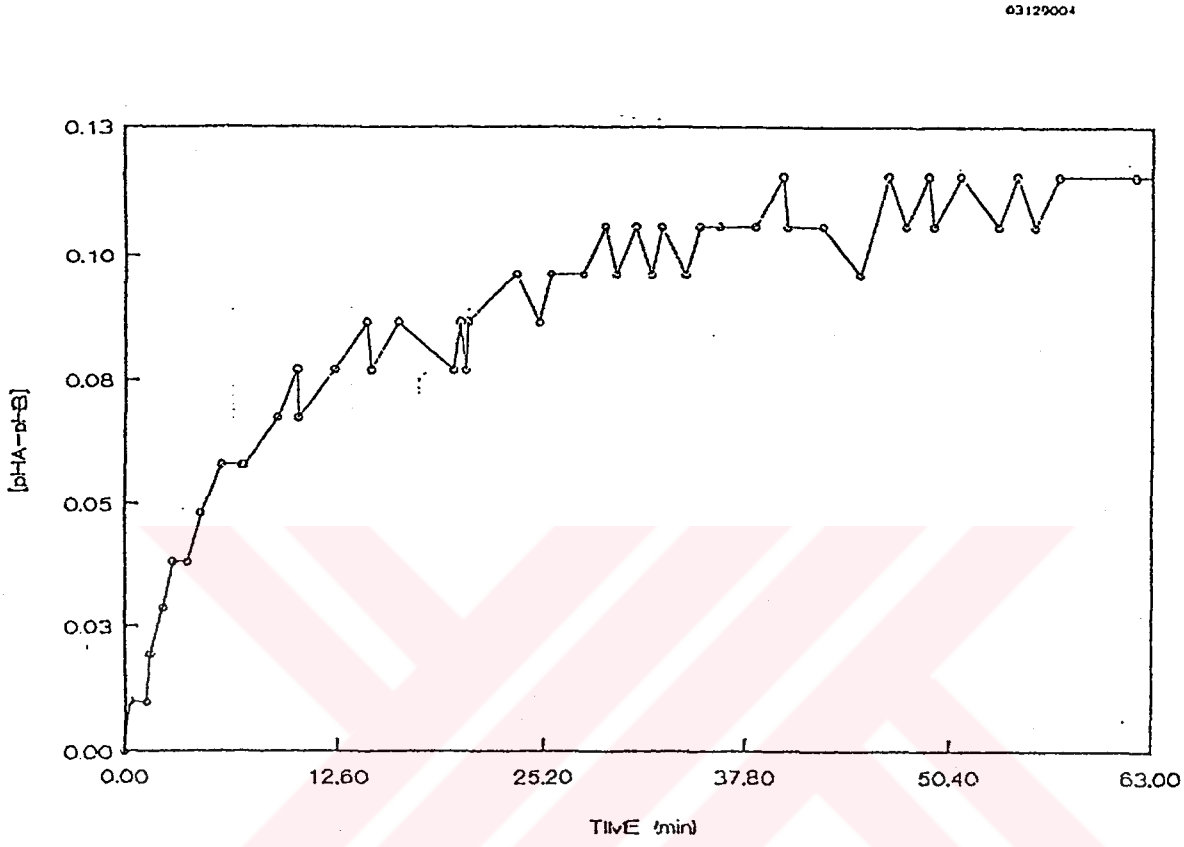

Figure 4.4.7. pH Difference versus Time Plot for Oriented BR Immobilized in PAG upon Illumination of 1000 W .

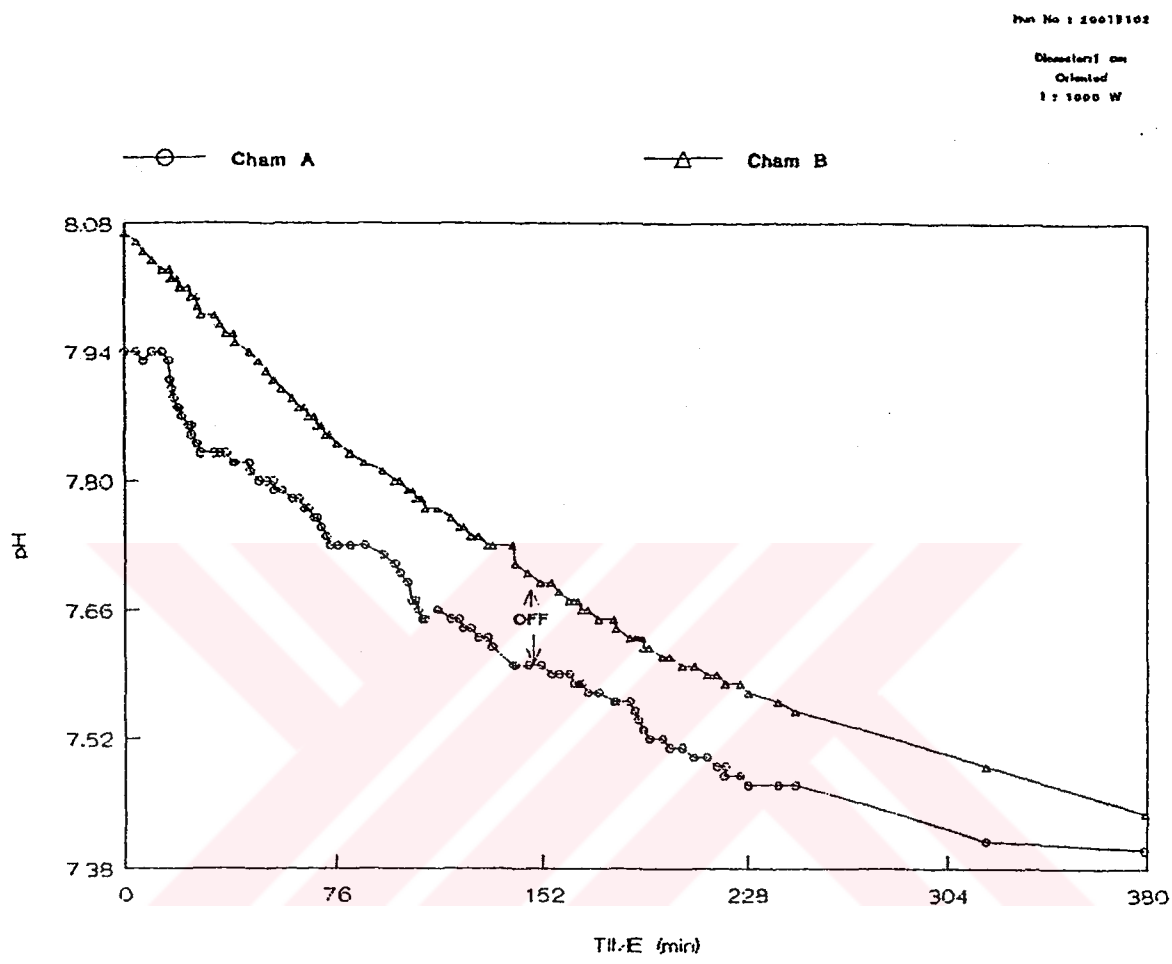

Figure 4.4.8. pH versus Time Plot for Oriented BR Immobilized in PAG upon Illumination 1000 W (pH$_o$=8.0).

CHAPTER 5

DISCUSSION

The studies on BR suggest this component to be the simplest biological electric generator coupling membranes. Accordingly attempts have been made to use this protein for the direct conversion of light into electrical energy. The most of the studies which were given in Section 2.5 were related with the generation of the photopotential with reconstituted BR. However, as Singh and Caplan (1980) have stated the efficiency of energy conversion that has been expressed as electric power generated divided by intensity of illumination is exceedingly low in these devices. The efficiency was reported between 10 $E^{-}7$ to 10 $E^{-}5$ percent. An approximate indication of maximal photoactivity that a BR containing membrane system has been proposed by Singh and Caplan (1980). According to their estimation the theoretical overall light conversion efficiency was about 1 %. This has shown that the synthetic devices are far away from ideality.

This is perhaps due to the fact that none of the techniques used for the membrane formation produced

perfect or even significant orientation of the purple membranes. Even a reliable quantitative assessment of the extent of orientation in synthetic membrane systems has not been yet proved possible. Furthermore supporting structures may interfere electrogenic activity.

The third important cause might be that the mechanism of producing photopotential measured in these devices was different from [H^+] ion pumping mechanism in native bacteria. As Skulachev (1976) has suggested the optimum thickness of BR containing artificial membrane should be around 70 °A, since the natural function of BR is to carry [H^-] ions through the bacterial membrane of this thickness. However, a difficulty arising always in experiments on planar artificial membranes was that they were rather unstable. To stabilize the membranes the thickness of the membranes was increased. Subsequently this might cause a different behavior of BR reconstituted on synthetic membrane.

Those investigators who have measured the photopotential response of BR presumed that [H^+] was transferred from one chamber to the other. In these systems they have not measured the rate of [H^+] transfer between chambers and even they have not proved that [H^+] is transferred through the membrane. In these studies the relative change in photopotential between the electrodes

placed in two compartments have been measured. It is believed that it has to be proved whether or not there exists $[H^+]$ transfer from one compartment to the other.

The experimental setup constructed in this study and the experimental procedure followed have made it possible to comment on some of the points stated above. Examining Figures 4.4.1 to 4.4.8, one may observe that pH change is in the same direction in both compartments. In other words as pH was increasing in chamber B it was also increasing in chamber A, or vice verse. If there had been a $[H^+]$ ion transfer from one compartment to the other, it was expected that pH in one compartment would have increased as the pH of the other compartment decreased. However, this was not the situation. The explanation of this behavior is quite complicated and what is going to be stated here would be a speculation.

It seems like that a surface phenomena is observed instead of $[H^+]$ transfer. Bacteriorodopsin molecules on each surface of the membrane acted independently and showed the same behavior for light phase and dark phase.

In the experiments with oriented BR, the difference in pH in both compartments increased with time, even pH changes were in the same direction in both compartments. During the orientation, BR molecules were either oriented rotationally or translationally. The rotational

orientation means that BR rotates itself so that it has charge position in the electrical field without moving. Translational orientation means that BR molecules migrate during gelation and positions itself towards its isoelectric point. Both types of orientation seem to be possible, since this is a technique called Gel Electrolysis Isoelectric Focusing of proteins to determine their pI values. In this technique proteins are replaced on PAG and an electric field is applied , causing the migration of proteins due to their charges through the PAG. It seems much likely that during the orientation BR molecules accumulate on one side of the PAG. This might have caused the pH difference between the two chambers even though pH changes are in the same direction. The results of Eisenbach *et al* (1977) have supported this explanation. Even though they have measured the potential difference between the two compartments, the results were in accordance with the present results. They have not observed any potential difference with non-oriented BR in PAG, but measured about 50 mV potential difference with oriented BR immobilized in PAG. This conclusion also explains why efficiency was so low in BR reconstituted synthetic membrane devices.

Another important point was to observe the effect of PAG surface on the variation of pH in

compartments. What is the isoelectric point of gel used? How the gel influences the net proton pumping behavior of BR? What is the contribution of PAG surface on protonation and deprotonation? Those are certain questions have to be answered. Unfortunately, in literature it was not possible to find any information to answer these questions. From Figure 4.3.1, it has been observed that if a disturbance of pH is given to the PAG surface, the re-establishment of steady-state pH takes about 40 minutes which can not be explained by the hydrodynamics of the cell. Protonation or deprotonation may occur on the PAG surface due to the existence of amide groups in PAG. Since it is a weak base, protonation is expected to be slow. Below isoelectric point of PAG, protonation is expected. Above this point, deprotonation is expected. It should be emphasized that protonation or deprotonation of PAG is light independent phenomena which has been justified by the experiments in this research.

Buffering capacity of BR is very high (Zabut, 1990). Therefore upon illumination, association is expected below pI values and dissociation is expected above pI value. Bacteriorhodopsin showed the same behavior in BR immobilized PAG membrane (see Figures 4.4.1 to 4.4.8). At pH 5, the dipole orientation of BR has been previously observed (Zabut, 1990), at which $[H^+]$ ion direction is altered. In the present study, experimental results indicate that pI value is around 7.

Upon illumination, dissociation was observed for initial pH values greater than 7 and association of [H^+] ion was observed for initial pH values less than 7.

Eroğlu *et al*(1990) have proposed a mechanistic model of [H^+] pumping rate for BR reconstituted liposomes under continuous illumination. The model was also applicable to the dark phase reactions. According to this model [H^+] is pumped from inner phase to the outer phase by active transport of BR during which BR exists in different intermediate forms. These intermediate components are produced due to the confirmational changes of the protein.These cyclic reactions are assumed to be reversible and have the following steps such as activation, proton dissociation, proton translocation, proton association and relaxation. During [H^+] ion pumping, all these steps are expected to occur.

However, referring to the results of experiments carried out with PM fragments, Zabut (1990) had postulated that the photocycle of BR does not complete when BR is in form of sheets. The cycle is divided into two; light phase reactions and dark phase reactions. At pH around 7, dissociation was observed in light phase and association was observed in dark phase.

The results of the present work were in accordance

with the results obtained with PM fragments except that the association and dissociation of protons were not reversible in BR immobilized in PAG system. That might have resulted from the interference of protonation and deprotonation of PAG to the light induced protonation of BR.

Referring to Figure 4.4.1 and 4.4.2, one may state that upon 150 W illumination proton dissociated from excited BR increases up to a certain level, then proton association starts which might be resulted from PAG protonation or proton association to BR. It is very difficult to separate two effects from each other. It is much likely that BR dissociates protons upon illumination and reaches to an end value in accordance with the results obtained with fragments. and reaches to a maximum value. However, this pH variation causes a disturbance on PAG surface. Then, the light independent protonation of PAG takes place which has been verified by Figure 4.3.1.

By increasing the light intensity, the number of excited BR molecules might be increased. As it is seen in Figures 4.4.3 and 4.4.4, upon illumination with 1000 W proton association rate of BR is much faster than the rate of protonation of PAG. Therefore at first pH decreased and then a steady state was attained, which might indicate same association and dissociation rates.Consequently, protons dissociated from BR during

photoreaction might have protonated to the gel. When the steady state was attained BR, relaxation cycle might have been disturbed because BR remained as unprotonated. That might be the reason why further illumination did not give any response.

CHAPTER 6

CONCLUSION

In this research, the bacteria *Halobacterium halobium* has been grown and its purple plasma membrane which contains light sensitive retinal protein BR has been isolated. Growth curve of *Halobacterium halobium* was plotted and as well BR has been characterized. By means of activity tests, it was found that BR isolated was photoactive. When BR was immobilized in polyacrylamide gel, it did not lose its photoactivity.

During the experiments, it was observed that there existed no significant simple diffusion. However rupture of PAG has been observed when pH was below 3.0. The light had no significant effect on PAG. However protonation and deprotonation of PAG has been observed as a result of pH disturbance in the medium.

It was interesting to deduce that, although pH varied with illumination of BR, same behavior was observed in two chambers both with oriented and non-oriented BR immobilized in PAG. Therefore it has been concluded that $[H^+]$ ion did not transfer from one compartment to the other. The pH change with illumination of BR is attributed to the dissociation of $[H^+]$ from BR if initial pH greater than 7 and association of $[H^+]$ from BR if the initial pH is less than 7.

CHAPTER 7

RECOMMENDATIONS

While carrying out the experiments with biological systems, the measurable quantities lie in the microscopic scale. The measuring system has to be developed to have higher sensitivity and it has to be modified to measure photoelectric signals. For this reason a computer analog sensor for the system parameters will result a more sensitive and continuous data.

By the data taken from the experiments carried in this research a further study on the kinetics and modeling of the system should be performed.

REFERENCES

Abdulaev,N.G. et al,1978. "Product of Limited Proteolysis of BR generate a Membrane Potential", FEBS Letters,90,190-194.

Blaurock,A.E., 1975 "Bacteriorhodopsin, at trans-Membrane Pump Containing α-Helix", Journal of Molecular Biology, 93,139-158.

Block,M.C. and Van Dan K.,1978. "Association of BR Containing Phospholipid Vesicles with Phospholipid Impregnated Millipore Filters", Biochim. Biophys. Acta, 507,48-61.

Block,M.C. et al,1977. "Reconstitution of BR in a Millipore System" FEBS Letters, 76,45-50.

Drachev,L.A. et al, 1974. "Direct Measurement of Electric Current Generation by Cytochrome Oxidase, H^+-ATPase and Bacteriorhodopsin", Nature, 249,321-324.

Eisenbach,M. et al,1977. "Bacteriorhodopsin Loaded Charged Synthetic Membranes", FEBS Letters, 81(1),

77-80.

Eroğlu,İ. et al,1990 Unpublished Paper.

Gerber,G.E. et al,1977. "Orientation of Bacteriorhodopsin in *Halobacterium halobium* as Studied by Selected Proteolysis", Proc. Natl. Acad. Sci., 74(12), 5530-42.

Govindjee,R. et al,1980. "The Quantum Efficiency of Proton Pumping by the Purple Membrane of *Halobacterium halobium*" BioPhys J. 30,231-242.

Hong,F.T. and Montal,M.,1979 "Bacteriorhodopsin in Model Membranes", Biophys. J. 25,465-472.

Hristova,S.G. et al,1986. "Effect of pH on the Photocycle and Electric Signal Kinetics in Purple Membrane Subjected to Digestion with Proteolytic Enzymes", Photobiochem. and Photophys. 12,231-234.

Huang,S.B. et al,1978. "Transient Photovoltages in Purple Membrane Multilayers", Biochim. Biophys. Acta, 509,300-317.

Kalisky,O. et al, 1981. "Environmental Effects on the Formation and Photocycle of M_{412} Photoproduct of

BR. Implication for the Mechanism of Proton Pumping", 20,649-655.

Karoly,N.,1978. "Photoelectric Activity of Dried Oriented Layers of PM from *Halobacterium halobium*", Biochem. Biophys. Res. Comm. 85,383-390

Kayushin,L.P., Skulachev,V.P. 1974, "Bacteriorhodopsin as an Electrogenic Proton Pupp; Reconstitution of BR Protesliposomos Generating / and /pH", FEBS Letters, 30 (1) 39-42.

Khorana, H.G., Gerber, G.E., Herlihy, W.C., Gray, C.P., Andereg, R.J., Nihei, K., Biemman, K. K., 1979, Proc. Matl. Acad. Sci., 76, 5046-5050.

Korenstein, R., Hess, B.,1978. "Analysis of Photocycle and Orientation in Thin Layers", Methods in Enzymology", 180-193.

Korenstein, R., Hess, B.,1978. "Immobilization of Bacteriorhodopsin and Orientation of Its Transition Moment in Purple Membrane", FEBS Letters, 89,15-20.

Kunugi,S.,et al,1987. "Oriented Immobilization of Bacteriorhodopsin in Synthetic Polymer Membranes by Use of Electrostatic Field", Polymer Bull. 18,87-90.

Liu,S.Y. and Ebrey,T.G.,1988. "Photocurrent Measurements of the Purple Membrane Oriented in a Polyacrylamide Gel", Biophys. J. 54,321-329.

Liu,S.Y et al,1990. "Light Induced Current from Oriented Purple Membrane. II. Proton and Cation Contributions to the Photocurrent", Biophys. J. 57,951-963.

Marinetti,T., Mauzerall,D. 1983. "Absolute Quantum Yields and Proof of Proton and non-Proton Transient Release and Uptake in Photoexcited BR". Proc.Natl.Acad. Sci. 80,178-180.

Mukohata,Y., Sugiyama,Y. "Isolation of White Membrane of Crystalline Bacterio-opsin from Halobacterium halobium R_1mV Lacking Carotenoid.", Methods in Enzymology, 88,407-411

Oesterhelt, D., Stoeckenius, W., 1971, "Rhodopsin-Like Protein From the Purple Membrane of Halobacterium halobium", Nature, New. Biol., 233.

Ormos,P., et al,1978 "Mechanism of Genetraion and Regulation of Photopotential by Bacteriorhodopsin in Bimolecular Lipid Membrane.", Biochim. Biophys.

Acta, 503,304-315.

Ort,D.R., Parson,W.W.,1979. "The Quantum Yield of Flash Induced Proton Released by BR Containing Membrane Fragments", BioPhys J., 25,341-354.

Ovchinnicov, Y.A.,1979. "The Structural Basis of the Function of Bacteriorhodopsin" FEBS LETT. 100(2),219-224.

Packer,L. et al,1977. "Conformational Changes in Bacteriorhodopsin Accompanying Ionophore Activity", Fed.Proc. 36,1819-1823).

Packer,L. et al, 1987. "Proton Translocation by Chemically Modified Bacteriorhodopsin", Biochem.Int. 14,977-985.

Singh, K., Caplan, R.S., 1980, The Purple Membrane and Solar Energy Conversion TIBS, 63.

Slifkin,M.A., et al,1986. "Chemical Relaxation in Dark and Light Adapted BR Subjected to Irradiation without Modulated Heat", Biochim. Biophys. Acta. 883,169-171

Spudich,F.L.,1983. "Spectroscopic Discrimination of the three Rhodopsin-Like Pigments in *Halobacterium*

halobium Membrane", Biophysical Society, 43,243-246.

Stoeckenius, W., 1980. "Purple Membrane of Halobacteria: A New Light-Energy Converter", Accounts of Chemical Research 13(10),337-344.

Stoeckenius, W., Bogomolni, A.R.,1982 "BR Oriented Pigments of Halobacteria", Annual Review of Biochemistry, Vol. 52, 587-616.

Sumper,M., "The Brown Membrane of *Halobacterium halobium*, the Biosynthetic Precursor of the Purple Membrane", Methods in Enzymology, 88, 391-395.

Van Dijek, P.W., Van Dan, K., 1982, "Bacteriorhodopsin in Phospholipid Vesicles", Methods in Enzymology, 88, 447-463.

Wallace, B.A., 1982 "Comparison of BR and Rhodopsin Molecular Structure", Methods in Enzymology, 88, 447-463.

Yücel, M., Eroğlu, I., 1987, Güneş Enerjisi Dönüsümünde Biyomimetrik Sistemler", Isı Bilimi ve Tekniği Dergisi, 12, 17-24.

Yücel, M., Zabut, B.M., Eroğlu, I., 1989, "Biolojik Model

Sistemlerde Işık Enerjisinin Elektrik Enerjisine Dönüşümü, Doğa TU Biol., p. 113

Zabut,B., 1987, MSc. Thesis, METU, Ankara.

Zabut,B., 1990. Ph.D. Thesis, METU, Ankara

APPENDICES

APPENDIX A

PREPARATION OF SOLUTIONS

A.1. COMPOSITION OF GROWTH MEDIUM

250.0 g; NaCl

20.0 g; $MgSO_4.7H_2O$

3,64 g; Trisodium Citrate 5.5 H_2O

2.0 g; KCL

10.0 g; Oxoid bacteriological peptone L-37

A.2. PREPARATION OF PAG

0.5 ml; 7.5% PAG solution

0.725 ml; 1% w/v Ammonium persulphate

0.775 ml; Distilled water (or BR)

3 µl; TEMED

APPENDIX B

EXPERIMENTAL DATA AND GRAPHICS

Table B-1. Growth of Halobacterium Halobium S-9 Strain. Absorbance Value is given for samples from 125 ml culture medium.

Time	Abs.	Abs
(Min)	560	660
0.00	0.12	0.11
2.38	0.24	0.22
9.20	0.23	0.19
20.58	0.46	0.36
25.77	0.64	0.50
33.48	0.92	0.70
44.87	1.20	0.90
53.90	1.14	0.85
75.77	1.09	0.81
84.75	1.06	0.79
93.17	1.04	0.79
104.30	1.02	0.77
116.30	1.01	0.76
127.69	0.98	0.74

Table B-2. pH versus time data for PM fragments upon 1000 W of illumination in activity test.

Time	pH	[H+]
(Min)		x E-7
0.00	6.65	2.24
0.33	6.64	2.29
1.08	6.63	2.34
1.75	6.62	2.40
2.42	6.61	2.46
2.92	6.60	2.51
3.42	6.59	2.57
4.25	6.58	2.63
6.33	6.57	2.69
7.42	6.56	2.75
8.42	6.55	2.82
9.50	6.54	2.88
10.75	6.53	2.95
11.00	6.53	2.95
12.00	6.54	2.88
12.67	6.55	2.82
13.42	6.56	2.75
14.08	6.57	2.69

Table B-2 (Cont'd)

Time	PH	[H+]
(Min)		x E-7
15.50	6.59	2.57
16.17	6.60	2.51
17.00	6.61	2.46
17.92	6.62	2.40
18.75	6.63	2.34
20.17	6.64	2.29
21.50	6.65	2.24

Table B-3. Diffusion test data through PAG with a pulse of diluted 5 µl HCl in AGTCC.

Time	pHA	pHB	[H+] A	[H+] B
(Min)			x E-7	x E-7
0.00	6.94	6.22	0.72	1.15
5.00	6.94	6.25	0.69	1.15
10.00	6.94	6.31	0.63	1.15
15.00	6.94	6.38	0.56	1.15
20.00	6.94	6.41	0.53	1.15
25.00	6.94	6.48	0.46	1.15
30.00	6.94	6.50	0.44	1.15
35.00	6.94	6.49	0.45	1.15
40.00	6.94	6.51	0.43	1.15
45.00	6.94	6.51	0.43	1.15
50.00	6.94	6.58	0.36	1.15
55.00	6.94	6.59	0.35	1.15
60.00	6.94	6.59	0.34	1.15
65.00	6.93	6.59	0.35	1.15
70.00	6.93	6.60	0.33	1.18
75.00	6.94	6.60	0.34	1.15
80.00	6.94	6.60	0.34	1.15
85.00	6.95	6.61	0.34	1.12
90.00	6.94	6.61	0.33	1.15

Table B-4. Diffusion test data through PAG pulse of 5 µl HCl in JTCC.

Time	pHA	pHB
(Min)		
0.00	6.94	6.94
1.00	6.94	3.51
11.00	6.94	3.42
24.00	6.94	3.40
31.00	6.94	3.40
32.55	6.93	3.40
35.62	6.93	3.40
44.15	6.92	3.39
50.58	6.91	3.39
60.00	6.91	3.39
60.68	6.90	3.39
67.87	6.89	3.39
73.63	6.88	3.39
82.40	6.87	3.39

Table B-4. (Cont'd)

Time	pHA	pHB
(Min)		
90.78	6.86	3.39
97.68	6.85	3.39
107.25	6.84	3.39
116.25	6.83	3.39
126.45	6.82	3.39
134.22	6.81	3.39
147.90	6.80	3.39
153.08	6.80	3.40
154.18	6.80	3.40
164.05	6.79	3.40
172.28	6.78	3.40
176.22	6.77	3.40
193.13	6.76	3.40
195.43	6.75	3.40

Table B-5. pH versus time data for non-oriented BR immobilized in PAG upon illumination of 150 W in AGTCC.

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB
(Min)			x E-7	x E-7	
0.00	6.97	6.97	1.07	1.07	0.00
0.77	6.96	6.97	1.10	1.07	-0.01
2.22	6.95	6.97	1.12	1.07	-0.02
2.72	6.95	6.96	1.12	1.10	-0.01
3.33	6.94	6.96	1.15	1.10	-0.02
3.67	6.94	6.95	1.15	1.12	-0.01
4.25	6.93	6.95	1.18	1.12	-0.02
4.78	6.93	6.94	1.18	1.15	-0.01
5.13	6.92	6.94	1.20	1.15	-0.02
6.13	6.92	6.93	1.20	1.18	-0.01
6.27	6.91	6.93	1.23	1.18	-0.02
7.50	6.90	6.93	1.26	1.18	-0.03
7.70	6.90	6.92	1.26	1.20	-0.02
9.70	6.90	6.91	1.26	1.23	-0.01
9.90	6.89	6.91	1.29	1.23	-0.03
10.37	6.88	6.90	1.32	1.26	-0.02
11.95	6.88	6.89	1.32	1.29	-0.01
12.07	6.87	0.00	1.35	0.00	-0.02
13.57	6.87	6.88	1.35	1.32	-0.01

Table B-5. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB
(Min)			x E-7	x E-7	
13.83	6.86	6.88	1.38	1.32	-0.02
15.00	6.86	6.87	1.38	1.35	-0.01
15.62	6.85	6.87	1.41	1.35	-0.02
16.80	6.85	6.86	1.41	1.38	-0.01
17.50	6.84	0.00	1.45	0.00	-0.02
18.45	6.84	6.85	1.45	1.41	-0.01
19.28	6.83	6.85	1.48	1.41	-0.02
20.20	6.83	6.84	1.48	1.45	-0.01
21.30	6.82	6.84	1.51	1.45	-0.02
22.15	6.82	6.83	1.51	1.48	-0.01
23.53	6.81	6.83	1.55	1.48	-0.02
24.28	6.81	6.82	1.55	1.51	-0.01
25.88	6.81	6.82	1.55	1.51	-0.01
26.70	6.81	6.81	1.55	1.55	0.00
27.00	6.80	6.81	1.59	1.55	-0.01
28.85	6.79	6.81	1.62	1.55	-0.03
30.10	6.79	6.80	1.62	1.59	-0.01
32.30	6.78	6.80	1.66	1.59	-0.02
33.63	6.78	6.79	1.66	1.62	-0.01
34.85	6.77	6.79	1.70	1.62	-0.02
40.00	6.77	6.79	1.70	1.62	-0.02
41.42	6.77	6.80	1.70	1.59	-0.03
43.00	6.78	6.80	1.66	1.59	-0.02

Table B-5. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB
(Min)			x E-7	x E-7	
45.28	6.78	6.81	1.66	1.55	-0.03
47.12	6.79	6.81	1.62	1.55	-0.03
48.40	6.79	6.82	1.62	1.51	-0.02
50.62	6.80	6.82	1.59	1.51	-0.02
52.33	6.80	6.83	1.59	1.48	-0.03
55.30	6.81	6.83	1.55	1.48	-0.02
58.25	6.81	6.84	1.55	1.45	-0.30
60.65	6.82	6.84	1.51	1.45	-0.02
64.63	6.82	6.85	1.51	1.41	-0.03
65.72	6.83	6.85	1.48	1.41	-0.02
71.33	6.84	6.85	1.45	1.41	-0.01
74.53	6.84	6.86	1.45	1.38	-0.02
78.05	6.85	6.86	1.41	1.38	-0.01
84.40	6.85	6.87	1.41	1.35	-0.02
84.78	6.86	6.87	1.38	1.35	-0.01
89.37	6.87	6.87	1.35	1.35	0.00
89.77	6.87	6.88	1.35	1.32	-0.01
94.43	6.88	6.88	1.32	1.32	0.00
95.40	6.88	6.89	1.32	1.29	-0.01
105.00	6.88	6.89	1.32	1.29	-0.01
105.00	6.88	6.89	1.32	1.29	-0.01
115.00	6.88	6.89	1.32	1.29	-0.01
116.62	6.89	6.89	1.29	1.29	0.00
122.00	6.89	6.89	1.29	1.29	0.00

Table B-6. pH versus time data for non-oriented BR immobilized in PAG upon illumination of 150 W in AGTCC (pH_o=6.99).

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
0.00	6.99	6.99	1.02	1.02	0.00	20.50
0.77	6.98	6.98	1.05	1.05	0.00	20.70
1.87	6.97	6.98	1.07	1.05	-0.01	20.70
3.50	6.97	6.97	1.07	1.07	0.00	20.80
4.30	6.97	6.96	1.07	1.10	0.01	20.80
5.33	6.96	6.96	1.10	1.10	0.00	20.80
7.00	6.95	6.96	1.12	1.10	-0.01	20.80
8.08	6.95	6.96	1.12	1.10	-0.01	20.90
8.53	6.95	6.95	1.12	1.12	0.00	20.90
9.95	6.94	6.95	1.15	1.12	-0.01	21.00
11.17	6.94	6.94	1.15	1.15	0.00	21.00
13.07	6.93	6.94	1.18	1.15	-0.01	21.00
13.60	6.93	6.93	1.18	1.18	0.00	21.10
15.70	6.92	6.93	1.20	1.18	-0.01	21.10
16.07	6.92	6.92	1.20	1.20	0.00	21.10
18.70	6.91	6.92	1.23	1.20	-0.01	21.10
19.12	6.91	6.91	1.23	1.23	0.00	21.10
24.45	6.90	6.91	1.23	1.23	-0.01	21.20
27.07	6.90	6.90	1.26	1.26	0.00	21.30

Table B-6. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
40.50	6.89	6.89	1.29	1.29	0.00	21.50
45.50	6.89	6.89	1.29	1.29	0.00	21.50
50.50	6.88	6.89	1.32	1.29	-0.01	21.30
60.50	6.88	6.89	1.32	1.29	-0.01	21.45
67.50	6.88	6.89	1.32	1.29	-0.01	21.80
72.28	6.87	6.89	1.35	1.29	-0.02	21.80
79.62	6.88	6.89	1.32	1.29	-0.01	22.00
83.33	6.88	6.90	1.32	1.26	-0.02	22.20
91.87	6.89	6.91	1.29	1.23	-0.02	22.10
95.05	6.89	6.92	1.29	1.20	-0.03	22.20
96.23	6.90	6.92	1.26	1.20	-0.02	22.20
97.98	6.90	6.93	1.26	1.18	-0.03	22.20
99.88	6.91	6.93	1.23	1.18	-0.02	22.20
100.70	6.91	6.94	1.23	1.15	-0.03	22.20
103.70	6.92	6.94	1.20	1.15	-0.02	22.20
105.28	6.92	6.95	1.20	1.12	-0.03	22.20
107.33	6.93	6.95	1.18	1.12	-0.02	22.20
108.12	6.93	6.96	1.18	1.10	-0.03	22.30
111.08	6.94	6.96	1.15	1.10	-0.02	22.30
111.87	6.94	6.97	1.15	1.07	-0.03	22.20
114.55	6.95	6.97	1.12	1.07	-0.02	22.00
115.85	6.95	6.98	1.12	1.05	-0.03	21.90
118.43	6.96	6.98	1.10	1.05	-0.02	21.60

Table B-6. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
121.78	6.96	6.99	1.10	1.02	-0.03	21.50
121.88	6.97	6.99	1.07	1.02	-0.02	21.50
122.67	6.97	7.00	1.07	1.00	-0.03	21.50
128.50	6.97	7.00	1.07	1.00	-0.03	21.50

Table B-7. pH versus time data for non-oriented BR immobilized in PAG upon illumination of 1000 W in JTCC (pH_o=6.53).

Time	pHA	pHB	[H+]A	[H+]
(Min)			x E-7	x E-7
0.00	6.53	6.63	2.95	2.34
4.00	6.53	6.64	2.95	2.29
7.83	6.54	6.64	2.88	2.29
8.17	6.54	6.65	2.88	2.24
15.72	6.54	6.66	2.88	2.19
18.58	6.55	6.66	2.82	2.19
20.17	6.55	6.67	2.82	2.14
24.25	6.55	6.68	2.82	2.09
28.67	6.56	6.68	2.75	2.09
29.17	6.56	6.69	2.75	2.04
33.63	6.56	6.70	2.75	2.00
39.72	6.56	6.71	2.75	1.95
43.83	6.57	6.71	2.69	1.95
46.58	6.57	6.72	2.69	1.91
53.08	6.57	6.73	2.69	1.86
61.17	6.58	6.73	2.63	1.86
64.97	6.58	6.72	2.63	1.91
69.25	6.58	6.73	2.63	1.86
80.83	6.58	6.74	2.63	1.82

Table B-7. (Cont'd)

Time	pHA	pHB	[H+]A	[H+]B
(Min)			x E-7	x E-7
81.58	6.59	6.74	2.57	1.82
98.33	6.60	6.73	2.51	1.86
98.33	6.60	6.73	2.51	1.86
104.17	6.60	6.78	2.51	1.66
112.00	6.60	6.79	2.51	1.62
118.83	6.60	6.80	2.51	1.59
130.25	6.61	6.81	2.46	1.55
140.17	6.61	6.82	2.46	1.51
140.17	6.61	6.82	2.46	1.51
141.83	6.61	6.82	2.46	1.51
147.22	6.62	6.82	2.40	1.51
158.22	6.62	6.83	2.40	1.48
189.22	6.62	6.84	2.40	1.45
224.22	6.63	6.84	2.34	1.45
224.22	6.63	6.84	2.34	1.45
238.97	6.63	6.85	2.34	1.41
263.97	6.63	6.85	2.34	1.41

Table B-8. pH versus time data for non-oriented BR immobilized in PAG upon illumination of 1000 W in AGTCC (pH_o=7.19).

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|
(Min)			x E-7	x E-7	
0.00	7.05	7.16	0.89	0.69	0.11
2.25	7.05	7.15	0.89	0.71	0.10
4.17	7.05	7.14	0.89	0.72	0.09
4.58	7.04	7.14	0.91	0.72	0.10
6.10	7.04	7.13	0.91	0.74	0.09
7.70	7.03	7.13	0.93	0.74	1.00
7.83	7.03	7.12	0.93	0.76	0.09
9.30	7.03	7.11	0.93	0.78	0.08
10.00	7.02	7.11	0.96	0.78	0.09
11.22	7.02	7.10	0.96	0.79	0.08
11.92	7.01	7.10	0.98	0.79	0.09
13.50	7.01	7.09	0.98	0.81	0.08
13.97	7.00	7.09	1.00	0.81	0.09
15.78	7.00	7.09	1.00	0.81	0.09
16.17	6.99	7.09	1.02	0.81	0.10
17.38	6.99	7.08	1.02	0.83	0.09
18.45	6.98	7.08	1.05	0.83	0.10
20.85	6.97	7.08	1.07	0.83	0.11
22.40	6.97	7.07	1.07	0.85	0.10

Table B-8. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|
(Min)			x E-7	x E-7	
23.30	6.96	7.07	1.10	0.85	0.11
26.03	6.95	7.07	1.12	0.85	0.12
26.25	6.95	7.06	1.12	0.87	0.11
29.07	6.94	7.06	1.15	0.87	0.12
31.55	6.94	7.05	1.15	0.89	0.11
33.50	6.93	7.05	1.18	0.89	0.12
39.08	6.92	7.05	1.20	0.89	0.13
40.07	6.92	7.04	1.20	0.91	0.12
44.50	6.92	7.03	1.20	0.93	0.11
44.70	6.91	7.03	1.23	0.93	0.12
48.33	6.91	7.02	1.23	0.96	0.11
49.17	6.90	7.02	1.26	0.96	0.12
49.35	6.89	7.01	1.29	0.98	0.12
49.97	6.89	7.00	1.29	1.00	0.11
57.28	6.88	7.00	1.32	1.00	0.12
58.67	6.88	6.99	1.32	1.02	0.11
60.97	6.87	6.99	1.35	1.02	0.12
62.77	6.87	6.98	1.35	1.05	0.11
64.57	6.86	6.98	1.38	1.05	0.12
67.23	6.86	6.97	1.38	1.07	0.11
68.37	6.85	6.97	1.41	1.07	0.12
73.35	6.84	6.97	1.45	1.07	0.13
75.00	6.84	6.97	1.45	1.07	0.13

Table B-8. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|
(Min)			x E-7	x E-7	
79.85	6.84	6.98	1.45	1.05	0.14
94.75	6.84	6.97	1.45	1.07	0.13
100.00	6.84	6.97	1.45	1.07	0.13
100.43	6.84	6.98	1.45	1.05	0.14
100.78	6.83	6.98	1.48	1.05	0.15
120.00	6.83	6.98	1.48	1.05	0.15

Table B-9. pH versus time data for oriented BR immobilized in PAG upon illumination of 1000 W in AGTCC.

Time	pHA	pHB	T
(Min)			(°C)
0.00	6.49	3.24	21.90
0.67	6.50	3.16	21.90
1.33	6.51	3.09	21.90
2.17	6.52	3.02	21.90
2.58	6.53	2.95	22.30
3.83	6.54	2.88	22.30
4.92	6.55	2.82	22.40
6.25	6.56	2.75	22.50
7.92	6.57	2.69	22.50
9.75	6.58	2.63	22.60
11.58	6.59	2.57	22.50
13.50	6.60	2.51	22.40
23.25	6.61	2.46	22.40
24.50	6.62	2.40	22.40
26.67	6.63	2.34	22.20
29.83	6.64	2.29	22.40
32.08	6.65	2.24	22.60
33.83	6.66	2.19	22.70
36.00	6.67	2.14	22.90

Table B- (Cont'd)

Time	pHA	pHB	T
(Min)			(°C)
42.00	6.70	2.00	23.40
42.17	6.71	1.95	23.40
44.00	6.72	1.91	23.60
46.08	6.73	1.86	23.70
47.92	6.74	1.82	23.90
50.00	6.75	1.82	24.10
51.83	6.75	1.78	24.20
52.75	6.76	1.74	24.70
63.17	6.77	1.70	24.50
64.25	6.77	1.70	24.30
64.25	6.77	1.70	24.00
79.75	6.78	1.66	23.20
87.95	6.79	1.62	22.20
105.22	6.82	1.51	23.00
120.58	6.81	1.55	23.10
124.65	6.80	1.59	23.10
127.47	6.79	1.62	23.10
129.50	6.78	1.66	23.10
131.97	6.77	1.70	23.10
135.05	6.76	1.74	23.10
147.25	6.76	1.74	23.05
147.67	6.77	1.70	23.05

Table B-10. pH versus time data for oriented BR immobilized in PAG upon illumination of 1000 W in AGTCC.

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
0.00	6.96	6.96	1.10	1.10	0.00	25.10
2.00	6.95	6.96	1.12	1.10	-0.01	25.20
3.17	6.95	6.95	1.12	1.12	0.00	25.20
4.17	6.94	6.95	1.15	1.12	-0.01	25.20
5.83	6.94	6.94	1.15	1.15	0.00	25.20
6.58	6.93	6.94	1.17	1.15	-0.01	25.20
8.25	6.93	6.93	1.17	1.17	0.00	25.30
9.12	6.92	6.93	1.20	1.17	-0.01	25.30
11.25	6.92	6.92	1.20	1.20	0.00	25.40
12.00	6.91	6.92	1.23	1.20	-0.01	25.30
15.00	6.91	6.91	1.23	1.23	0.00	25.40
16.67	6.90	6.91	1.26	1.23	-0.01	25.50
20.00	6.90	6.90	1.26	1.26	0.00	25.60
22.25	6.89	6.90	1.29	1.26	-0.01	25.60
25.17	6.89	6.89	1.29	1.29	0.00	25.60
28.00	6.89	6.89	1.29	1.29	0.00	25.80
30.50	6.88	6.89	1.32	1.29	-0.01	25.90
33.33	6.88	6.88	1.32	1.32	0.00	25.90

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Table B-10. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
35.00	6.88	6.88	1.32	1.32	0.00	25.90
37.17	6.87	6.88	1.35	1.32	-0.01	25.90
38.83	6.87	6.87	1.35	1.35	0.00	25.80
40.33	6.86	6.87	1.38	1.35	-0.01	25.90
42.50	6.86	6.86	1.38	1.38	0.00	25.90
50.00	6.86	6.86	1.38	1.38	0.00	26.00
60.33	6.87	6.86	1.35	1.38	0.01	25.60
60.83	6.86	6.87	1.38	1.35	0.00	25.40
66.33	6.85	6.86	1.41	1.38	0.00	25.40
67.33	6.85	6.86	1.41	1.38	-0.01	25.40
72.00	6.85	6.86	1.41	1.38	0.00	25.40
74.33	6.86	6.87	1.38	1.35	-0.02	25.40
76.00	6.86	6.87	1.38	1.35	-0.01	25.60
79.17	6.87	6.88	1.35	1.32	-0.02	25.70
79.83	6.88	6.88	1.32	1.32	-0.01	25.70
82.08	6.89	6.89	1.29	1.29	-0.01	25.80
84.50	6.89	6.89	1.29	1.29	0.00	25.90
88.50	6.90	6.90	1.26	1.26	-0.01	25.90
89.00	6.91	6.90	1.23	1.26	0.00	25.90
92.17	6.92	6.90	1.20	1.26	0.01	26.10
97.00	6.92	6.90	1.20	1.26	0.02	25.90
100.00	6.91	6.89	1.23	1.29	0.03	26.10

Table B-10 (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
100.25	6.92	6.89	1.20	1.29	0.02	26.20
101.83	6.92	6.90	1.20	1.26	0.02	26.10
104.17	6.92	6.91	1.20	1.23	0.01	26.10
106.00	6.92	6.91	1.20	1.23	0.01	26.10
109.00	6.92	6.92	1.20	1.20	0.01	26.10
112.00	6.92	6.92	1.20	1.20	0.00	26.10
112.08	6.93	6.92	1.17	1.20	0.01	26.10
112.17	6.94	6.92	1.15	1.20	0.02	26.10
114.00	6.94	6.92	1.15	1.20	0.02	26.30
114.50	6.93	6.92	1.17	1.20	0.01	26.30
116.42	6.93	6.93	1.17	1.17	0.00	26.30
118.00	6.93	6.93	1.17	1.17	0.00	26.30
119.50	6.93	6.92	1.17	1.20	0.01	26.40
120.50	6.92	6.92	1.20	1.20	0.00	26.60
122.00	6.92	6.92	1.20	1.20	0.00	26.60
122.67	6.91	6.92	1.23	1.20	-0.01	26.60
123.17	6.91	6.91	1.23	1.23	0.00	26.50
124.75	6.90	6.91	1.26	1.23	-0.01	26.60
126.33	6.90	6.90	1.26	1.26	0.00	26.60
127.00	6.90	6.90	1.26	1.26	0.00	26.60
129.67	6.90	6.90	1.26	1.26	0.00	27.00
132.25	6.90	6.91	1.26	1.23	-0.01	27.30

Table B-10. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	pHA-pHB	T
(Min)			x E-7	x E-7		(°C)
132.83	6.91	6.91	1.23	1.23	0.00	27.30
135.00	6.91	6.91	1.23	1.23	0.00	27.30
135.92	6.91	6.92	1.23	1.20	-0.01	27.30
136.83	6.92	6.92	1.20	1.20	0.00	27.30

Table B-11. pH versus time data for oriented BR immobilized in PAG upon illumination of 1000 W in JTCC.

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|	T
(Min)			x E-7	x E-7		(°C)
0.00	7.94	8.07	0.11	0.09	0.13	26.00
4.17	7.94	8.06	0.11	0.09	0.12	26.00
6.67	7.93	8.05	0.12	0.09	0.12	26.00
9.83	7.94	8.04	0.11	0.09	0.10	26.00
13.33	7.94	8.03	0.11	0.09	0.09	26.00
15.83	7.93	8.03	0.12	0.09	0.10	26.00
16.08	7.91	8.03	0.12	0.09	0.12	26.00
16.33	7.91	8.02	0.12	0.10	0.11	26.00
16.83	7.90	8.02	0.13	0.10	0.12	26.00
17.33	7.89	8.02	0.13	0.10	0.13	26.00
18.67	7.88	8.02	0.13	0.10	0.14	26.00
19.42	7.88	8.01	0.13	0.10	0.13	26.00
20.17	7.87	8.01	0.13	0.10	0.14	26.00
22.67	7.86	8.01	0.14	0.10	0.15	26.00
23.58	7.86	8.00	0.14	0.10	0.14	26.00
23.83	7.85	8.00	0.14	0.10	0.15	26.00
25.67	7.84	8.00	0.14	0.10	0.16	26.00
26.08	7.84	7.99	0.14	0.10	0.15	26.00
27.25	7.83	7.98	0.15	0.10	0.16	26.00

Table B-11. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|	T
(Min)			x E-7	x E-7		(°C)
32.00	7.83	7.98	0.15	0.10	0.15	26.00
33.97	7.83	7.97	0.15	0.11	0.14	26.00
36.00	7.83	7.96	0.15	0.11	0.13	26.00
38.75	7.82	7.96	0.15	0.11	0.14	26.00
39.33	7.82	7.95	0.15	0.11	0.13	26.00
44.42	7.82	7.94	0.15	0.11	0.12	26.00
44.75	7.81	7.94	0.16	0.11	0.13	26.00
47.75	7.80	7.93	0.16	0.12	0.13	26.00
50.58	7.80	7.92	0.16	0.12	0.12	26.00
53.25	7.80	7.91	0.16	0.12	0.11	26.00
53.42	7.79	7.91	0.16	0.12	0.12	26.00
56.25	7.79	7.90	0.16	0.13	0.11	26.00
60.08	7.78	7.89	0.17	0.13	0.11	26.00
62.50	7.78	7.88	0.17	0.13	0.10	26.00
64.50	7.77	7.88	0.17	0.13	0.11	26.00
66.08	7.77	7.87	0.17	0.14	0.10	26.00
68.00	7.76	7.87	0.17	0.14	0.11	26.00
69.17	7.76	7.86	0.17	0.14	0.10	26.00
70.67	7.75	7.86	0.18	0.14	0.11	26.00
72.50	7.74	7.85	0.18	0.14	0.11	26.00
74.00	7.73	7.85	0.19	0.14	0.12	26.00
76.58	7.73	7.84	0.19	0.14	0.11	26.00
81.25	7.73	7.83	0.19	0.15	0.10	26.00

Table B-11. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|	T
(Min)			x E-7	x E-7		(°C)
86.42	7.73	7.82	0.19	0.15	0.09	26.00
93.00	7.72	7.81	0.19	0.16	0.09	26.00
97.17	7.71	7.80	0.19	0.16	0.09	26.00
99.08	7.70	7.80	0.20	0.16	0.10	26.00
101.83	7.69	7.79	0.20	0.16	0.10	26.00
103.42	7.67	7.79	0.21	0.16	0.12	26.00
104.75	7.67	7.78	0.21	0.17	0.11	26.00
105.75	7.66	7.78	0.22	0.17	0.12	26.00
107.00	7.65	7.78	0.22	0.17	0.13	26.00
108.17	7.65	7.77	0.22	0.17	0.12	26.00
112.83	7.66	7.77	0.22	0.17	0.11	26.00
118.00	7.65	7.76	0.22	0.17	0.11	26.00
121.05	7.65	7.75	0.22	0.18	0.10	26.00
122.63	7.64	7.75	0.23	0.18	0.11	26.00
125.38	7.64	7.74	0.23	0.18	0.10	26.00
128.30	7.63	7.74	0.23	0.18	0.11	26.00
131.70	7.63	7.73	0.23	0.19	0.10	26.00
133.33	7.62	7.73	0.24	0.19	0.11	26.00
140.87	7.60	7.73	0.25	0.19	0.12	26.00
141.83	7.60	7.71	0.25	0.19	0.13	26.00
146.43	7.60	7.70	0.25	0.20	0.11	25.90
151.22	7.60	7.69	0.25	0.20	0.10	25.90
155.13	7.59	7.69	0.26	0.20	0.09	25.90

Table B-11. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|	T
(Min)			x E-7	x E-7		(°C)
157.87	7.59	7.68	0.26	0.21	0.10	26.00
161.57	7.59	7.67	0.26	0.21	0.09	26.00
163.30	7.58	7.67	0.26	0.21	0.08	26.00
165.00	7.58	7.67	0.26	0.21	0.09	26.00
166.08	7.58	7.66	0.26	0.22	0.09	26.00
168.45	7.57	7.66	0.27	0.22	0.12	26.00
172.38	7.57	7.65	0.27	0.22	0.09	25.90
178.30	7.56	7.65	0.28	0.22	0.08	25.90
179.17	7.56	7.64	0.28	0.23	0.09	25.80
184.33	7.56	7.63	0.28	0.23	0.08	25.70
186.28	7.55	7.63	0.28	0.23	0.07	25.70
187.62	7.54	7.63	0.29	0.23	0.08	25.70
189.12	7.53	7.63	0.29	0.23	0.09	25.70
189.38	7.53	7.62	0.29	0.24	0.10	25.70
191.55	7.52	7.62	0.30	0.24	0.09	25.70
196.42	7.52	7.61	0.30	0.25	0.10	25.70
199.27	7.51	7.61	0.31	0.25	0.09	25.60
203.68	7.51	7.60	0.31	0.25	0.09	25.60
208.12	7.50	7.60	0.32	0.25	0.10	25.60
212.82	7.50	7.59	0.32	0.26	0.09	25.60
216.50	7.49	7.59	0.32	0.26	0.10	25.60
219.72	7.49	7.58	0.32	0.26	0.09	25.60
219.28	7.48	7.58	0.33	0.26	0.10	25.50

Table B-11. (Cont'd)

Time	pHA	pHB	[H+] A	[H+] B	|pHA-pHB|	T
(Min)			x E-7	x E-7		(°C)
225.00	7.48	7.58	0.33	0.26	0.10	25.60
228.08	7.47	7.57	0.34	0.27	0.10	25.60
239.80	7.47	7.56	0.34	0.28	0.09	25.60
246.22	7.47	7.55	0.34	0.28	0.08	25.60
319.00	7.41	7.49	0.39	0.32	0.08	25.60
379.00	7.40	7.44	0.40	0.36	0.04	25.60

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