

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

CONTINUOUS HYDROGEN GAS PRODUCTION
FROM WASTE MATERIALS IN AN UP-FLOW
PACKED BED REACTOR

by
Betül KIRLI

January, 2014
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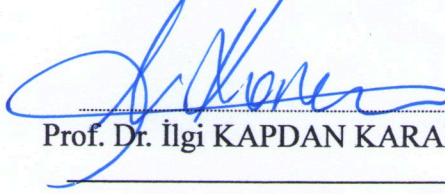
**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of
Dokuz Eylül University in Partial Fulfillment of the Requirements for the
Degree of Master of Science in
Biotechnology Department**

**by
Betül KIRLI**

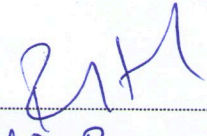
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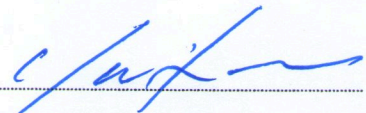
M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled “**CONTINUOUS HYDROGEN GAS PRODUCTION FROM WASTE MATERIALS IN AN UP-FLOW PACKED BED REACTOR**” completed by **BETÜL KIRLI** under supervision of **PROF. DR. İLĞİ KARAPINAR KAPDAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.


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CONTINUOUS HYDROGEN GAS PRODUCTION FROM WASTE MATERIALS IN AN UP-FLOW PACKED BED REACTOR

ABSTRACT

The objective of this thesis to investigate hydrogen production in immobilized systems by dark fermentation. The studies conducted for this purpose are support particle selection for immobilization, hydrogen production in repeated batch operation with immobilized bacterial and continuous operation of up-flow packed bed reactor containing selected support particle. The substrate was ground waste wheat which was hydrolyzed in dilute acid and at high temperature. Heat treated and hydrogen producing *Clostridium* sp containing mixed anaerobic waste sludge was used as microbial culture. Total gas production, percentage hydrogen in total gas, hydrogen gas volume, initial and final total sugar concentrations were monitored daily through out the experiments. The hydrogen production potentials of support particles, repeated batch and continuous operations were evaluated in terms of hydrogen production volume, yields and rates. Support particle selection studies were conducted by using plastic scouring sponge pad, plastic nylon sponge, black porous sponge, plastic scouring sponge pad with metal mesh, plastic nylon sponge with metal mesh. The operation strategy was repeated batch with different loading cycles varying between every 1 to 5 days. Hydrogen production yield was the main parameter in the selection of particle and the maximum yields were obtained with was obtained with metal mesh covered plastic scouring sponge pad for all repeated batch operation cycles times.

Continuous operation studies were carried out in an up flow packed bed reactor containing plastic scouring sponge pad with metal mesh. The effect of hydraulic retention time (HRT) on hydrogen production was investigated. Reactor was operated at HRTs between 2 and 13 h at constant initial total sugar concentration (5g/L). The highest production rate and yield were obtained as $V = 3500 \text{ L/d}$ and $Y = 1.6 \text{ mol/mol TS}$, respectively at $\text{HRT} = 2 \text{ h}$.

Keywords: Biohydrogen, waste wheat, repeated batch operation, packed bed, continuous operation, dark fermentation.

ATIK BUĞDAYDAN YUKARI AKIŞLI YATAK REAKTÖR İLE SÜREKLİ HİDROJEN GAZI ÜRETİMİ

ÖZ

Bu tez çalışmasında, bağlı büyüme sistemlerinde mezofilik şartlarda karanlık fermentasyon ile hidrojen gazı üretiminin incelenmesi amaçlanmıştır. Bu amaçla, destek taneciği seçimi, tekrarlı kesikli işletimde hidrojen gazı üretimi ve seçilen destek taneciği ile sürekli işletilen yukarı akışlı dolgulu kolon reaktörde hidrojen gazı üretimi çalışmaları yürütülmüştür. Asit ve yüksek sıcaklıkta hidrolize edilen öğütülmüş atık buğday substrat olarak kullanılmıştır. Karanlık fermentasyon mikroorganizma kültürü olarak ısıl işleme tabi tutulmuş ve hidrojen gazı üretimi yapan *Clostridium* karışık kültürünün bulunduğu anaerobik çamur kullanılmıştır. Deneysel çalışmalarda başlangıç ve son şeker derişimi, toplam gaz üretim hacmi, üretilen gazdaki hidrojen yüzdesi günlük olarak takip edilmiştir. Hidrojen üretim süreçlerinin performansları üretim hacmi, üretim hızı ve üretim verimi açısından değerlendirilmiştir. Destek taneciği seçimi çalışmaları plastik ovma sünger, metal örgü ile sarılı plastik ovma sünger, büyük boşluklu siyah sünger, naylon sünger, metal kafes ile sarılı naylon sünger kullanılarak tekrarlı kesikli işletimle gerçekleştirilmiştir. Başlangıç substrat derişimi sabit tutulmuş, her bir destek taneciği için 1-5 gün arasında değişen substrat yükleme döngüleri uygulanmıştır. Destek taneciği seçiminde hidrojen üretim verimi esas alınmış ve en yüksek hidrojen üretimi verimi metal kafes ile sarılı naylon sünger taneciği ile sağlanmıştır.

Sürekli işletilen dolgulu kolon reaktör çalışmalarında metal kafes ile sarılı naylon sünger destek taneciği kullanılarak hidrolik alıkonma süresinin hidrojen üretimine olan etkisi incelenmiştir. Sistem 13 saat ile 2 saat arasında değişen farklı alıkonma sürelerinde işletilmiştir. Başlangıç substrat derişimi 5g/L de sabit tutulmuştur. Sürekli sistemde en yüksek üretimin $HRT = 2$ saat ile alıkonma süresinde elde edilmiştir.

Anahtar Kelimeler: Biyohidrojen, atık buğday, tekrarlı kesikli işletim, dolgulu kolon, sürekli işletim, karanlık fermentasyon.

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CHAPTER ONE

INTRODUCTION

1.1 Biohydrogen Gas Production by Dark Fermentation

Hydrogen is considered as the ideal substitute for fossil fuels because of clean oxidation products generation after burning and its high energy contents (Pan et al., 2008). Hydrogen is one of the energy sources with the highest energy contents per unit mass of fuel among the other well known ones. Almost all countries are interested in finding a feasible hydrogen production methods (Xin Mei Guo et al., 2010). However, high costs of production and storage of hydrogen caused delaying in considering hydrogen as alternative fuel of future so far (Mei-Ling et al., 2009).

Among the various hydrogen-production methods, fermentation of organic wastes into hydrogen is a promising technology. Because, it does not only reduce pollution but also recovers renewable energy (Tao et al., 2008). One of the well known fermentation process for hydrogen production is dark fermentation (Davila-Vazquez et al., 2009). The microorganisms present in this fermentation approach are mainly *Clostridium*, *Bacillus* and *Enterobacter*, either in pure or mixed cultures (Davila-Vazquez et al., 2008).

Biological hydrogen production processes are listed as ; 1 - Biophotolysis of water with the help of algae and cyanobacteria (decomposition), 2-Photofermentation of organic acids and carbohydrates by photosynthetic bacteria (light fermentation) 3 – Dark fermentation of carbohydrates by anaerobic bacteria (Kapdan et al., 2006, Das & Veziroğlu 2001, Genç 2009).

Blue green algae break down the water molecules to hydrogen and oxygen by the direct and indirect biophotolysis. The use of renewable sources of water and CO₂, through algae for biohydrogen production can be considered as an economic and sustainable. However, the oxygen produced during algal biohydrogen production causes hydrogenase enzyme inhibition and hence cessation of gas generation. As a

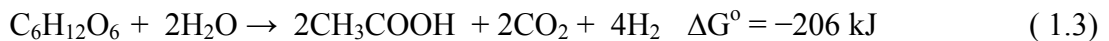
result, the process fails for the continuous production of hydrogen. Moreover, process is dependent on sunlight and also rate of production is rather slow compared to other biological production methods (Argun et al., 2008).

Another method used in the production of biological hydrogen is photofermentation. The most common organisms used in photofermentation are photoheterotrophic anaerobic bacteria such as *Rhodobacter*, *Rhodopseudomonas*. Biohydrogen production with this method is realized in the presence of light and organic acids as a carbon source. These organisms can also use simple sugars like glucose, fructose as well as sucrose as carbon source to produce hydrogen (Kapdan et al., 2009). The organic acid that will result in hydrogen generation by photofermentation are pure essential fatty acids such as lactic acid, acetic acid, butyric acid etc (Argun et al., 2008a, 2008b). The major problems in the photofermentation are the complex nutritional requirements (EDTA, Mo, Fe) of the *Rhodobacter* species, strict control requirements for environmental conditions ($T = 30\text{--}35\text{ }^{\circ}\text{C}$, $\text{pH} = 6.8\text{--}7.5$, light intensity = $4\text{--}6\text{ k lux}$) and inhibitions caused by high concentrations of VFAs ($>2500\text{ mg L}^{-1}$) and $\text{NH}_4\text{-N}$ ($>50\text{ mg L}^{-1}$). Most of the light fermentation studies were performed with pure VFAs such as acetic or butyric acids using different pure *Rhodobacter* species (Shi & Yu, 2004, Barbosa et al., 2001, Oh et al., 2004).

Hydrogen production by anaerobic bacteria occur in the absence of light and therefore process is called as dark fermentation. Bacteria grow on the organic substrate (heterotrophic growth), to provide the building blocks for growth and metabolic energy. Fermentation of glucose to hydrogen by dark fermentation follows the metabolic path as generation of pyruvate, oxidation of pyruvate to acetyl-CoA (1.1) and then conversion of acetyl-CoA into acetyl phosphate. Acetyl-CoA, finally is converted to metabolites such as ethanol, acetate, butyrate. During oxidation of pyruvate to acetyl-CoA, ferredoxin (Fd) is reduced. The reduced ferredoxin is oxidized via hydrogenase enzyme with the yield of molecular hydrogen (1. 2) (Nath et al., 2004).



Fermentation end products and fermentation conditions as pH and temperature are the major parameters to be considered in the yield of hydrogen formation. The main end products of the dark fermentative hydrogen generation are acetate and butyrate. Although acetate is the desired end product for high yield of formation, butyrate could be the dominant organic acid or more abundant in the organic acid mixture depending on fermentation conditions. Theoretically, when the end-product is acetic acid, 4 mol H₂ can be produced per 1 mol glucose consumed (reaction 3). If butyric acid is the end-product, 2 mol of H₂ per mole of glucose is obtained as shown in reaction 4 (Bartacek et al., 2007). Unlike acetic and butyric acid generations, propionate as an end product lowers the production of hydrogen. Propionic acid formation consumes 1 mol of H₂ per mole of propionic acid formed (Argun & Kargi, 2011). The other factors that cause decreasing in hydrogen generation is the presence of homoacetogens as *Clostridium aceticum*. These organisms consume hydrogen for acetic acid generation. Finally, some of the organic matter is used for growth and maintenance. Therefore, the theoretical yield of 4 mole H₂/mole glucose can not be realized in practice (Argun et al., 2009).



Temperature is another factor affecting the fermentative hydrogen production. The increase in the fermentation temperature in the certain ranges provides a parallel increase in the activity of hydrogen producing organisms and hence hydrogen production. However, temperatures out of this range cause a decrease in biological activity and so hydrogen production. Previous studies have shown that the optimum

temperature is not always the same for the different microbial cultures (Wang & Wan, 2009).

The effect of temperature on volumetric hydrogen production rate can be explained thermodynamically by considering the changes in Gibbs free energy and standart enthalpy of conversion of glucose to acetate and also theoretical yield of formation. Gibbs free energy and enthalpy change of acetate formation reaction from glucose are $\Delta G_o = -207 \text{ kJ / mol}$ and $\Delta H_o = +90.69 \text{ kJ / mol}$, respectively. These values indicate that the reaction is endothermic and the reaction may occur spontaneously. Van't Hoff equation explaining the effect of temperature on the equilibrium constant can be seen in equation 5. (Davila-Vazquez et al., 2008).

$$\ln \left(\frac{K_2}{K_1} \right) = -\frac{\Delta H^\circ}{R} \left[\frac{1}{T_2} - \frac{1}{T_1} \right] \quad (1.5)$$

The temperature increases, so does the kinetic constant equilibrium. Because, the reaction is endothermic. So, the incerase in glucose fermentation temperature will increase the volume of H₂. On the other hand, the thermal denaturation of proteins that affect the activity of micro-organisms can occure at high tempartures resulting in lowering in the hydrogen production. Another potential disadvantage of thermophilic processes is rise in energy costs.(Davila-Vazquez et al., 2008, Hallenbeck, 2009).

Microorganism type affects both end product and hydrogen generation rate. It is possible to obtain high yields of hydrogen by using pure cultures. Hovewer, it is not practical and applicable to use pure cultures in industrial scale. The pure culture can be easily contaminated by the other bacterial cultures from feed or other sources. Since, it is not economically feasible to do sterilization througout the operation, utilization of pure culture does not represent the real conditions (Hallenbeck et al., 2009b). The most common bacterial cultures for hydrogen production are *Clostridium sp.* and *Enterobacter sp.* The advantages of *Enterobacter sp.* over

Clostridium is its resistance to oxygen. However, *Clostridium* sp. extremely sensitivity to oxygen, this condition can lead to serious operation problems during hydrogen production. On the other hand, hydrogen production potential of *Clostridia* is substantially higher than *Enterobacter* sp. Moreover, selection and activation of *Clostridia* from the mixture of bacteria is simple and applicable. The spore forming *Clostridia* can be dominated in the bacterial mixture by keeping the culture in nutrient deficient media or exposing it to the extreme environmental conditions such as high temperature or acidic environment (Wang & Wan, 2009). Heat treatment of anaerobic sludge is the most common method to obtain hydrogen forming organisms.

Fermentation pH has got a substantial effect on hydrogen production. pH affects both metabolic pathway of organic acid production and hydrogenase enzyme activity. The most suitable pH range can change depending on bacterial culture. If the system is operated at the most suitable pH range regarding to the microbial culture, the hydrogen production rate and yield increases. High fermentation rate will increase the organic acid production during hydrogen generation. That will shift the pH of the media to the acidic conditions around pH=4.0-4.5. This acidic conditions could adversely affect the microbial culture in terms of shift in population from hydrogen producers to hydrogen consumer. Therefore pH of the fermentation media must be controlled throughout the operation for hydrogen generation.

1.2 Bioprocesses for Biohydrogen Production

Hydrogen production processes are under investigation. Batch hydrogen generation studies have been carried out long period of time and in detail. The recent studies concentrate on continuous processes. Suspended culture and immobilized bioprocess technologies can be used for hydrogen production. The studies indicated that immobilized systems are superior over suspended ones. The main questions are to determine microbial support particle types and operation conditions. Following sections briefly summarize immobilization techniques that can be used for hydrogen production.

1.2.1 Bio -Hydrogen Production by Immobilized Cell

Immobilization in biotechnology is “ the technique used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins (e.g. monoclonal antibodies) onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continues use. Therefore, it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free-cell counterparts.(Colin & Dervakos, 1996).

The other advantages of immobilized cell systems are; improved biological stability, biomass hold-up, product stability, product yields. Immobilization can be categorized according to the physical process involved. These are attachment, entrapment, containment and aggregation.

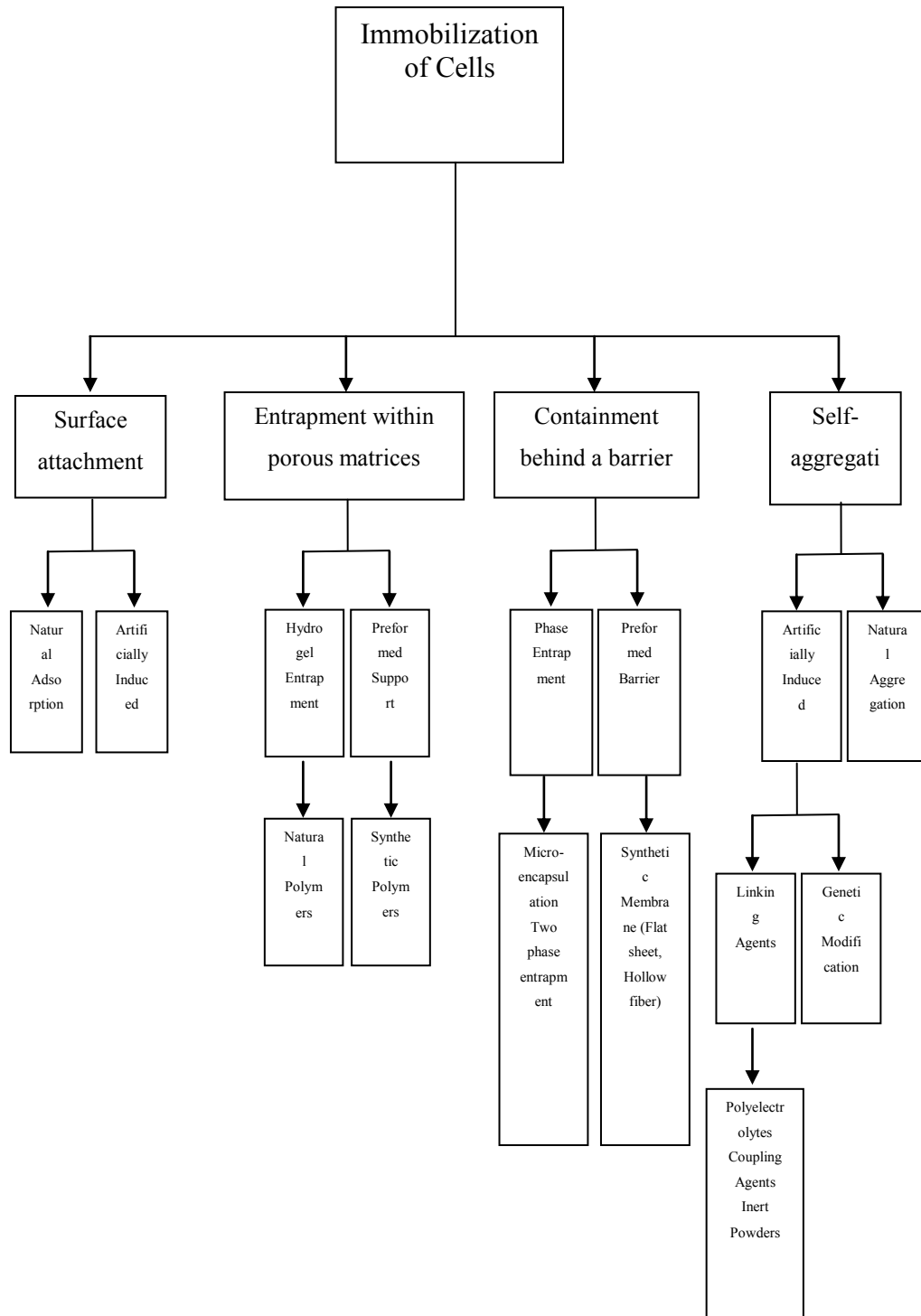


Figure1.1 Classification of immobilized cell systems according to the physical localization and the nature of micro environment.(Nedovic & Willaert, 1996)

1.2.1.1 Attachment

Attachment is the oldest and the simplest immobilization techniques. A carrier matrix on the surface of cells can be immobilized. Sand and coke, wood, etc. surfaces used in this technique as well as other active porous materials include (Vega et al., 1988). This immobilization can be by the aid of covalent bonding or adsorption. The surface attachment might be artificially induced using linking agents such as metal oxides. Cells begin to grow on the layer, after a period of time to form biofilm. Surface adsorption of hydrogen producing sludge has been examined by using different support materials such as activated carbon (Wu et al., 2005, Mohan et al., 2008), polyurethane-activated carbon (Mitchell et al., 2009), granular activated carbon (Zhang et al., 2007), glass beads (Zhang et al., 2006), a mesoporous pure silica SB15 (Vega et al., 1988), expanded clay (Shida et al., 2009), loofah sponge (Chang et al., 2002).

1.2.1.2 Entrapment

Materials which have pores of different sizes such as, ceramics, sintered glass, activated carbon, reticulated foam, stainless steel wire , can be used for entrapment of cells (Zhen-Peng Zhang et al., 2007). Immobilization efficiency varies depending on cell types and support material type. Different from attachment type biofilm formation, cells which have been entrapped in a preformed porous supports are preserved from the shear field outside the particles but, like attached cells, they are not confined to the particle by any carrier. The major advantage of this technique is that the immobilized biomass hold-up can be controlled such that cells growing beyond the boundaries of the particle are removed by abrasion, either by the flow area surrounding the particle or by particle-particle contact (Wang & Wan 2009).

1.2.1.3 Containment

These systems are highly useful when cell separation from the effluent is required or when some high-molecular-weight or specific product needs to be separated from

the effluent. Cells might be immobilized by interception behind a barrier, either preformed or formed in situ. The barrier might be as simple as interface between two immiscible liquids in which case the cell suspension is emulsified with an organic solvent and re-suspended as droplets in an aqueous phase or may include semi permeable membranes used for microfiltration and ultrafiltration (Rupp, 1985, Chang, 1987).

1.2.1.4 Self-Aggregation

Self-Aggregation named as " Cell to cell attachment". In other words, increasing the density of the cells by aggregation. If the flocs can be separated from the bulk liquid by coarse screen or rapid sedimentation, thus flocculated cells in the form of large aggregates can be considered to be immobilized. Self flocculation of biomass procure certain advantageous over entrapment and surface adsorption procedure. It is support particle free, cost effective and more viable to the grand scale hydrogen production systems. The granules formed by self flocculation are constant during the long term operation (Azbar& Levin 2012).

1.3 Literature Review

Biohydrogen production studies have been accelerated since about year 2000. Physical and chemical hydrogen production methods have been already in use for generation of hydrogen for industrial purpose. However, limitations in energy sources, strict international regulations about CO₂ emissions and air pollution control made the hydrogen as alternative and a promising energy source. Therefore, studies devoted to improvement of existing hydrogen generation methods to increase production capacity at lower cost in order to satisfy energy need. These existing technologies require high temperature and that's why it is not much possible to reduce cost of production to make the process economical. Alternatively, biological hydrogen production methods are realized at mild environmental conditions with the use of waste materials as feed. Therefore, biological methods are cost effective and economical. The limitation with the biological hydrogen production is rate and yield

of production. Due to these fact, biological hydrogen generation can hardly compete with other methdos. The process needs improvement.

Considerable amount of effort have been given to selection of bacterial culture and determination of environmental or media composition by batch experiments in the literature. The recent studies have been devoted to investigation of different immobilized or suspended growth bioprocess technologies with continuoues operation. Type of bioprocess used for continuous fermentative biyohidrogen production are continuous stirred tank reactor (CSTR), the membrane bioreactor (MBR) (S.E. Oh et al., 2004), packed bed bioreactor (fixed / packed-bed) (Peixoto et al., 2011), fluidized bed bioreactor (fluidized-bed) (Barros et al., 2010), up-flow anaerobic sludge bed bioreactor (UASB) (Carrillo-Reyes et al., 2012) and trickling filter bioreactor (trickling BIOFILTER) (Arriaga et al., 2011). The main parameters that have been studied so far are comparison of immobilized and suspended culture systems, selection of particle type for immobilized systems and also determination of best operation conditions such as hydraulic retention time (HRT) and organic loading rate (OLR). Some of these studies have been summorized as follows.

One of the main parameter that affect the hydrogen production yield an rate is hydrauolic retention time (HRT). immobilization matrix of Polyoxyethylene (POE) was used into CSTR system They worked hydrogen producer when operating at HRT= 8h -2h. Maximum yield and rate were obtained as 1.18 mol H₂/ mol hexose and 37 mmol/h/L when was operated HRT =4 h and 20 g COD/L. (Azbar& Levin 2012)

Kumar et. al, (2001) studied on hydrogen production in an immobilized sytem by using lignocellulosic materials like rice straw, bagasse and coir as support material. The effect of HRT on hydrogen production was investigated on production rate. The results inidcated that that hydrogen production rate increased with increasing dilution rate to $D = 0.9 \text{ h}^{-1}$ (HRT = 1 h) for all matrixes and the maximum rate was obtained as 62 mmol/ h L with coir. Recirculation of effluent escalated the production rate to 75. 6 mmol/ h L with 9.4 h^{-1} dilution rate.

Lee et. al, (2007) used a membrane bioreactor (MBR) with a continuous flow stirred tank reactor (CSTR) to produce hydrogen. Three different carbon sources (glucose, sucrose, and fructose) were used. Hydraulic retention time (HRT) was varied between 2-4 h. Fermentation was carried out at a temperature of 35°C and at agitation rate of 150 rpm. pH was conserved within the range of 6.2–6.8. MBR system was operated at an extremely low HRT of 1 h with an optimal steady-state H₂ production rate HPR of 1.48, 2.07, and 2.75 l/h/l, for glucose, sucrose, and fructose as the sole carbon source, respectively. The maximum yield was calculated as 1.36 mol H₂/mol hexose for fructose.

Lima et. al (2013) , investigated the effect of substrate type like glucose, sucrose and feeding rate on hydrogen production in an anaerobic fixed bed reactor. The temperature of the system was conserved at 25 °C. Hydraulic retention time (HRT) was 2 h. Reactors were fed with a chemical oxygen demand (COD) of 2000 mg/L, sucrose (1781,24 mg/L) or glucose (1875,00 mg/L) as the main carbon source. Low-density polyethylene used as support particle. Maximum hydrogen yields were obtained as 3.22 mol H₂/mol sucrose and 1.51 mol H₂/mol glucose.

Azbar et. al, (2011) used ceramic ferrule and pumice stone as immobilization material in a packed bed system. Process was operated at different HRT (1.5, 3, 6, 12, 24 hours). Hydrogen production yields were 2.98 L H₂/L/day at HRT = 1.5 h with ceramic ring, 2.28 L H₂/L/day for pumice stone at HRT = 3 hr. A CSTR was operated in paralel to packed bed reactor, the maximum rate of production was 0,5 L H₂/L/day at HRT = 24 hours. This study indicated that immobilized systems are superior over CSTR in terms of hydrogen generation.

Chojnacka et al. (2011) operated immobilized reactor for hydrogen production. The study was designed to compare the performances of two porceses. One with granites stone as support particle and immobilization technique. The other one contained granite stone and granular sludge together to investigate the performance of both adhesion and self granulation together for hydrogen production. Sugar beet molasses was used as the substrate in packed bed reactors. HRT was varied between

10-18 h. Reactor with only granite stone generated 35.7 % hydrogen while the other reactor produced about 48.6 % with the production yield of 5.48 mol H₂/mol sucrose. It was concluded that granite with granular sludge is a better reactor media for hydrogen generation.

Chang et. al, (2002) investigated the effect of hydrogen production by using three different packed bed materials as gourd fiber, expanded clay, activated carbon in a continuously operated packed bed reactor. HRT was varied between 0.5h and 5 h. The reactor was operated at mesophilic conditions (35 °C) with carbon source of sucrose (20 g COD /L). Biomass holding capacities of the immobilization materials were compared. It was observed that pumpkin fiber (0.94 g VSS / L) and expanded clay (VSS = 1.85 g / L) were not as good as activated carbon (5.53 g VSS / L) in terms of biomass holding capacity. The maximum hydrogen production rates with expanded clay and activated carbon were 0.415 L / hr / L at HRT = 2 and 1.32 L/h/L HRT=1 h, respectively.

Jo et. al, (2008) worked on utilization of glucose for hydrogen production with *Clostridium tyrobutyricum* JM1 strain isolated from waste food treatment process. Polyurethane foam is used as the support material in packed bed bioreactor. Glucose was the main carbon source at 5 g/L concentration. Reactor fed with growth medium at HRT of 24, 18, 12, 9, 5, 4, 3, 2, 1 hr. The effect of HRT on H₂ content of bio-gas and H₂ production rate was investigated. Hydrogen production rate increased when HRT was decreased from 24 hours to 2 hours. Maximum H₂ production rate was obtained as 7.2 L H₂/L/day at HRT = 2 hr.

Cristiane Marques dos Reis et al,(2011) operated two anaerobic fluidized-bed reactor. Influence of upflow velocity (V_{up}) applied to an anaerobic fluidized-bed reactor on hydrogen production was studied. Reactors were operated at 1.24cm/s (R124) and 1.88 cm/s (R188) up flow velocities. The highest hydrogen production was obtained at the minimum fluidization velocity (1.24 cm/s). The best hydrogen yield was 2.55 mol H₂ /mol glucose for an HRT of 2 h. The maximum hydrogen production rate was 2.21 L/L/h, when HRT was 1h .

Chi-Neng Lin et. al, (2006) studied on hydrogen production in a draft tube fluidized bed reactor (DTFBR) containing SC-immobilized cells as the biocatalyst. The DTFBR system was operated at HRT= 2.2–8.9 h and an influent sucrose concentration of 5–40 g COD/L. Culture was subjected to thermal treatment at a temperature of 70–80 °C. The maximum volumetric and specific H₂ production rate of 2.27 L/h/L (2.23mol H₂/l/d) and 0.777 L/h/g-VSS, respectively, were obtained at HRT > 3 h and optimal yield was 4.98mol H₂/mol sucrose.

Gavala et.al,(2005), compare CSTR and UASB reactors for hydrogen production. Hydraulic retention time was varied between 2 and 12 h. Biohydrogen production from glucose in theUASB at mesophilic temperature range was studied. CSTR was operated at both mesophilic (35°C) and thermophilic (55°C) conditions. Thermophilic operation resulted in high hydrogen production rate (104 mmole H₂/h/L/g VSS), but in the mesophilic environment hydrogen yield was 12 mmole H₂/h/L/g VSS at 6h HRT. Besides, the hydrogen production rate in the UASB reactor was remarkably higher compared to that of the CSTR at low retention times (19.05 and 8.42 mmole H₂/h/L respectively at 2h HRT).

Another study about utilization of UASB for hydrogen production was conducted by Chang et al (2004). The system was operated at HRTs between 24–4 h. The maximum rate of production was 47. 2 L/d with 42 % hydrogen gas content at 20 g COD/L of sucrose and HRT=8 hours. Sucrose depletion was enhanced when HRT was increased. However, it was concluded that consumed carbon was diverted to biomass synthesis rather than hydrogen production.

1.4 Scope and Objective

The major objective of this work, to investigate hydrogen production in immobilized bioprocess systems from hydrolyzed wheat starch by dark fermentation. The specific objectives were as follows;

- To select the microbial support material to be used in immobilized system,

- To investigate the hydrogen gas production potential of repeated batch operation of immobilized system,
- To determine cycle time in repeated batch operation for the highest hydrogen production yield,
- To investigate the effects of HRT on hydrogen production in continuously operated up-flow packed bed reactor.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Dark Fermentation

2.1.1 Organisms

The mixed microbial culture was obtained from Pak-Maya Baker's Yeast Industry anaerobic wastewater treatment plant. In order to obtain spore forming- hydrogen producing bacterial culture, heat treatment was applied to anaerobic treatment sludge. Sludge was boiled for an hour at 100°C at pH= 5.9. Argon gas was passed through the bottles before incubation. The bottles were plugged with gas-tight silicone stoppers and screw cap.

2.1.2 Preparation of the Substrate

Waste wheat was used as a source of carbon. Wheat was first ground and then particle size was adjusted to 70 μm (less than 200 mesh). The ground wheat was hydrolyzed by dilute acid and heat treatment method. Ground wheat was first acidified to pH=3 by H_2SO_4 and then hydrolyzed at 90°C for 15 min in an autoclave. Solid phase of the hydrolyzate was separated by centrifugation at 8000 g for 20 min and the supernatant sugar solution was neutralized to pH = 6.5 by addition of 10 M NaOH.

Sugar solution from hydrolyzed wheat starch was nitrogen (N) and phosphorous (P) and Fe(II) deficient. Nitrogen, phosphorus and Fe(II) were supplemented to yield N/P/Fe/C ratio of 2/0.8/1.5/100 by using KH_2PO_4 and FeSO_4 as nitrogen, phosphorus and iron source, respectively. Maximum hydrolysis yield was found to be 0.94 for 35g wheat powder.

2.1.3 Analytical Methods

2.1.3.1 Sample Preparation

Samples removed from the liquid phase everyday were centrifuged at 8000 rpm and the clear supernatants were used for analysis of residual sugar (TS)

2.1.3.2 Total Sugar Determination

Initial and residual sugar concentrations were tested by the acid-phenol spectrophotometric method (Dubois et al., 1956).

2.1.3.3 Total Gas Volume Measurement

The total gas volume was determined by water displacement method everyday by using sulfuric acid (2%) and NaCl (10%) containing solution.

2.1.3.4 H₂ Gas Measurements

Hydrogen gas was sampled from the head space of the bottles by using gas-tight glass syringes. Hydrogen gas concentration in the gas phase was measured by using a gas chromatograph (HP Agilent 6890). The column was Alltech, Hayesep D 80/100 6" × 1/8" × 085". Nitrogen gas was used as carrier with a flow rate of 30 ml min⁻¹ and the head pressure was 22 psi. Temperatures of the oven, injection, detector, and filament were 35 °C, 120 °C, 120 °C, 140 °C, respectively.

2.2 Experimental Set Up and Procedure

2.2.1 Repeated Batch Dark Fermentation

Repeated batch experiments were carried out in 250 ml serum bottles. Plastic scouring sponge pad, plastic nylon sponge, black porous sponge ,plastic scouring

sponge pad with metal mesh, plastic nylon sponge with metal mesh were used as immobilization material. The support particle size was adjusted to $L=1$ cm, $D=1$ cm and $W=0.5$ cm. Total particle number was as follows; plastic scouring sponge pad, $N_1=60$, plastic nylon sponge $N_2=8$, black prous sponge $N_3=10$ in the reaction volume of 200 ml. Bottles containing support particles were inoculated with 90 ml of heat treated sludge culture. The initial total sugar concentration was 10 g/L. Sludge, carbon source and immobilization material containing bottles were incubated for at least 7 days to provide immobilization of microorganisms on the support materials. Repeated batch operation was started when biofilm formation was observed on the support particles. Total sugar concentration was 10 g/L in repeated batch operation studies. In order to investigate the hydrogen production with repeated batch operation, the liquid phase of bottles were poured out after biofilm formation was achieved. Biomass was separated from the liquid phase by centrifugation. Then the bottles were reloaded with fresh media and the biomass obtained after centrifugation at each repeated cycle. The operation was terminated when cumulative hydrogen production volume remained almost constant. A control experiment was conducted in parallel to immobilized repeated batch operation. Control bottles contained only sludge and fermentation medium without support particle. The serum bottles were sparged with argon gas to provide anaerobic conditions. The experiments were conducted at mesophilic conditions (37 °C). pH of the fermentation media was adjusted to pH=6 when required.

2.2.2 Continous Operetion of Up-flow Packed Bed Reactor

The highest hydrogen yield was obtained with plastic nylon sponge covered with metal mesh support material in repeated batch experiments. Therefore, this particle was used as support material in continuously operation packed bed reactor. Particle number in the reactor was 85 with dimentions of $L=1$, $W=1$, $D=0,5$ cm. Packed bed reactor was made up of stainless steel. The reactor was 57 cm in length, 8 cm in diameter with liquid volume of 2100 ml. There was 4 sampling port along the reactor. The gas was collected from the top and given to the gas collection unit trough gas tight tubing. The system was fed from the botton during continuous

CHAPTER THREE

RESULTS AND DISCUSSIONS

Hydrogen production studies in immobilized systems were carried out in two parts. The first part of the study contains repeated batch experiments with different support particles in order to select the most suitable particle for hydrogen production by dark fermentation. The second part includes continuous operation of up-flow reactor packed with selected particle in repeated batch experiments.

3.1 Selection of Support Material for Hydrogen Production by Repeated Batch Operation

Repeated batch experiments were conducted at different cycle times from 1 day to 5 days. Hydrogen production potentials of different particles were investigated at these repeated batch intervals. The particle types were plastic scouring sponge pad (PSP), plastic nylon sponge (PNS), black porous sponge (BPS), plastic scouring sponge pad with metal mesh (PSPM) and plastic nylon sponge with metal mesh (PNSM). The pore sizes and also porosities of the particles were different from each other. Surface area of a support particle is one of the main parameters to be able to compare the effectiveness of the particle in immobilization of microorganisms and hence hydrogen production. However, the method used to measure the surface area of the particles is not appropriate to apply the particles used in this study. Since, the particles were not resistant to the heat used in the measurement method. Therefore, particle size ($L=1$ cm, $D=1$ cm and $W=0.5$ cm) and particle number ($N_1=60$, $N_2=8$, $N_3=10$), in the reaction volume (200 ml) were kept constant instead of keeping surface area constant. Initial total sugar concentration was 10 ± 1 g/L. Hydrogen production potentials of different particles were evaluated through cumulative hydrogen production, hydrogen production yield (mol H_2 /mol TS and g H_2 /g TS) and also total sugar consumption at each cycle of the repeated batch operations. Control experiments without particles were carried out in parallel to the experiments with particle to evaluate the effect of immobilization on hydrogen production.

3.1.1 Repeated Batch Operation with 5 Days Cycle Time

3.1.1.1 Plastic Scouring Sponge Pad Particle

Figure 3.1 indicates variation of cumulative hydrogen volume with 5 days repeated batch cycles for plastic scouring sponge pad particle. Cumulative hydrogen volume (CHV) for the first cycle was around CHV=73, 6 ml. The volume increased to CHV= 131 ml at the end of the second cycle. However, the total production volume for the second cycle was only V_{H_2} =54 ml. Hydrogen production slowed down for the next two cycles and provided V_{H_2} =31 ml and 17 ml, respectively. The total volume produced at the end of the 4 cycles was CHV=185 ml. Production yield is directly related with conversion of organic substance to organic acids as acetic and butyric acid. Variation of hydrogen production yields as ml H_2 /g TS and mol/mol glucose were given in Figure 3.2 and Figure 3.3, respectively. The yields were almost the same for the first three cycles as around 30 ml H_2 /g TS (0, 25 mol H_2 / mol glucose). However, it drastically decreased to 12, 4 ml H_2 /g TS (0, 09 mol H_2 / mol glucose). Initial total sugar concentration for cycles varied between TS= 11 g/L-8, 6 g/L. The amount of sugar consumed for the first two cycles was around 97%. Then, it decreased to 84% for the rest of cycles.

These results indicate that plastic scouring sponge pad is a quite efficient microorganism support material to be used in hydrogen production. Although, hydrogen volume decreased slightly with the cycle, the yield of production did not vary substantially until the last cycle. High sugar removal efficiencies indicate that microorganisms immobilized on or in the particles were active even at the last cycle. The reasons for the decrease in the yield at the last cycle could be diversion of microbial population from hydrogen producers to non-hydrogen producers or hydrogen consumers. The microbial culture could devote their metabolism to the growth due to highly available substrate rather than organic acid production which generates hydrogen. In addition, organic acid produced at the final cycle could be propionic acid rather than hydrogen generating organic acids like acetic or butyric

acid. Finally, it can be concluded that the system reaches to the steady state in terms of hydrogen production after five times loading.

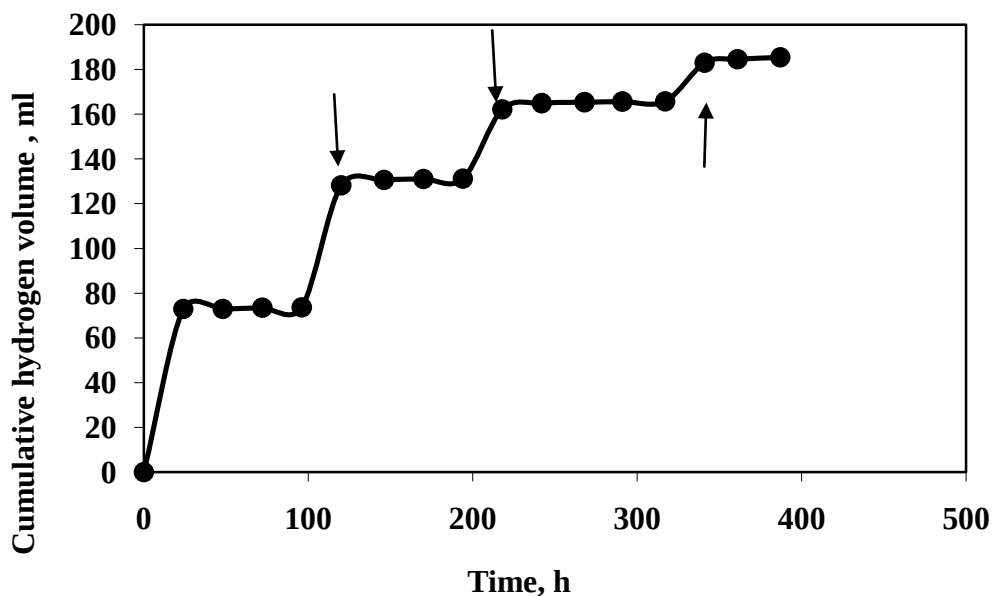


Figure 3.1 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad at 5 days cycle time repeated batch operation.

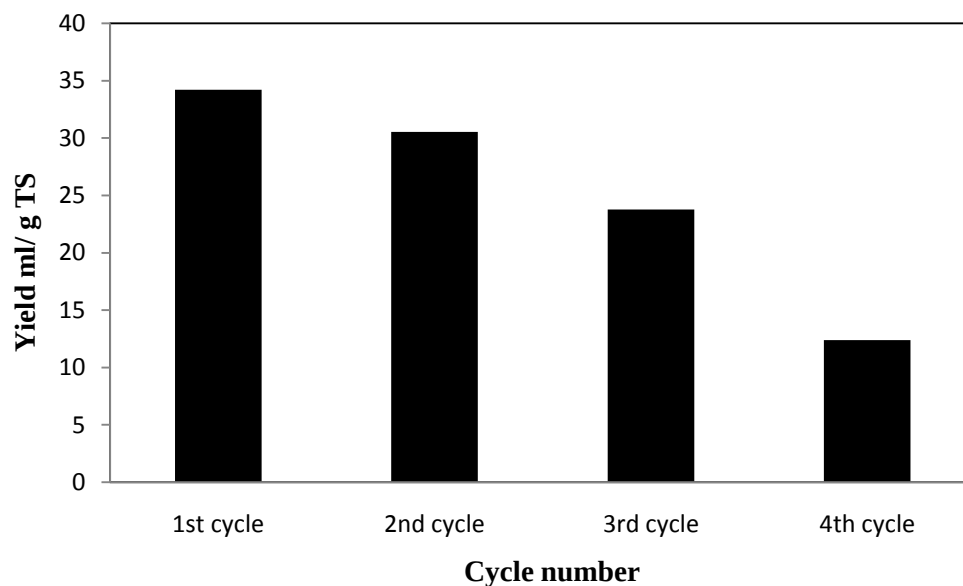


Figure 3.2 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic scouring sponge pad at 5 days cycle time repeated batch operation.

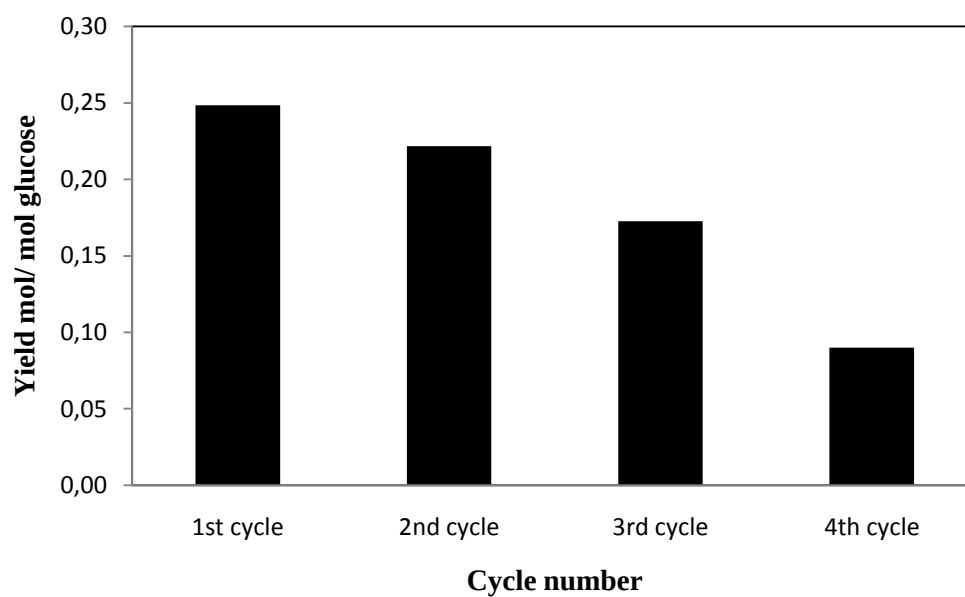


Figure 3.3 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic scouring sponge pad at 5 days cycle time repeated batch operation.

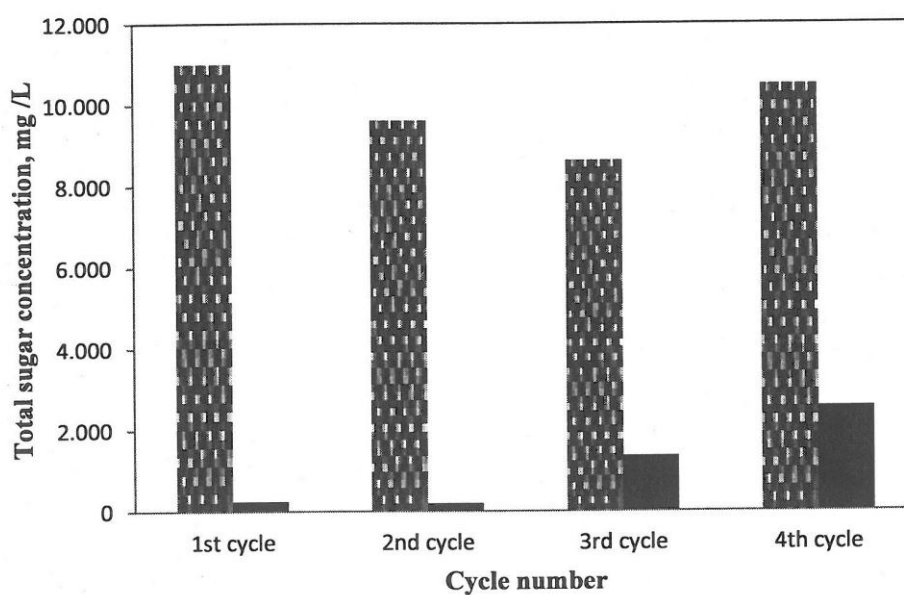


Figure 3.4 Initial and final TS concentrations for different cycles of plastic scouring sponge pad support particle at 5 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.1.2 Plastic Scouring Sponge Pad with Metal Mesh Particle

Plastic scouring sponge pads were covered with metal mesh to increase the density of the particle. The first cycle resulted in CHV = 74 ml cumulative hydrogen volume (Figure 3.5). A slight increase in the second cycle was observed with the final volume of around 126.5 ml. The third cycle was not very efficient and resulted in only 87 ml hydrogen gas production. Finally 4th cycle provided 348.5ml in cumulative hydrogen volume. Variation of hydrogen production yields as ml H₂/g TS and mol H₂/ mol TS were given in Figure 3.6 and Figure 3.7, respectively. The main increase in the yield of hydrogen formation was observed at 3rd cycle and it remained almost constant for the 4th cycle. The resulting yield were 66.76 ml H₂ / g TS (0. 48 mol H₂/mol glucose) and 51 ml H₂/g TS (0. 37 mol H₂/mol glucose) for the 3rd and 4th cycles, respectively. This result indicates that system reached to almost steady state condition after 3rd cycle and no need to repeat the operation further. Sugar consumption was investigated at each cycle. The maximum sugar consumption was obtained at second cycle with 97.5 % removal efficiency (Figure 3.8).

According to these results, support material with the wire mesh is a more stable support material compared to the sponge particle without wire mesh. In addition, wire mesh might have provided an extra surface area for immobilization of microorganisms. The increases in the yield of hydrogen formation and cumulative hydrogen volume as the fresh substrate is loaded indicate increase in the biofilm thickness on the support particle.

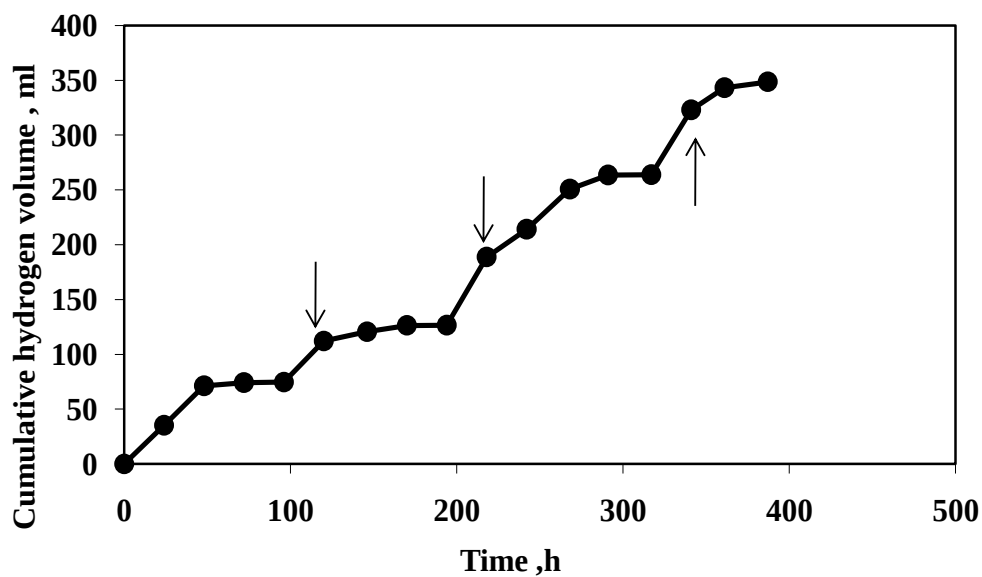


Figure 3.5 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad with metal mesh at 5 days cycle time repeated batch operation.

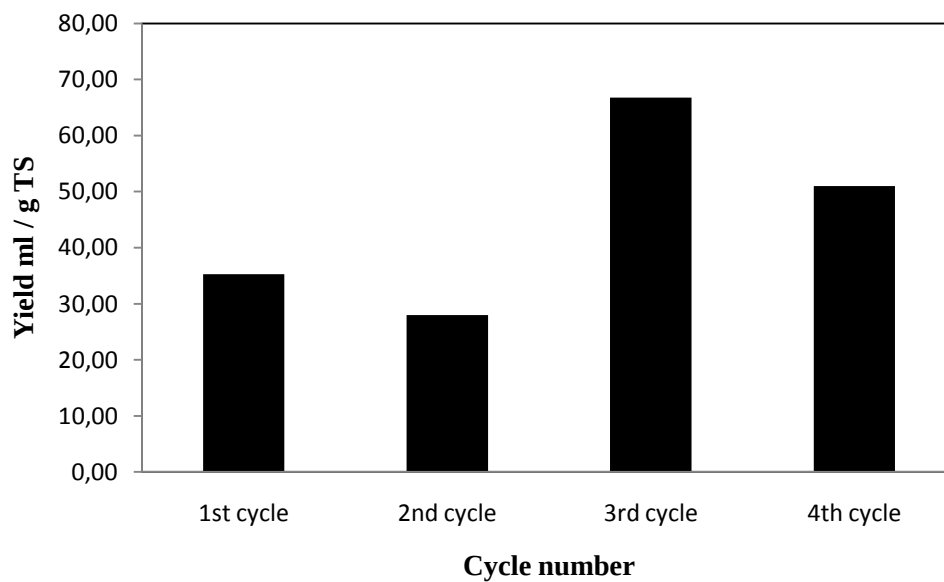


Figure 3.6 Hydrogen production yields, ml H_2 / g TS, at different cycles of 5 days cycle repeated batch operation with plastic scouring sponge pad with metal mesh.

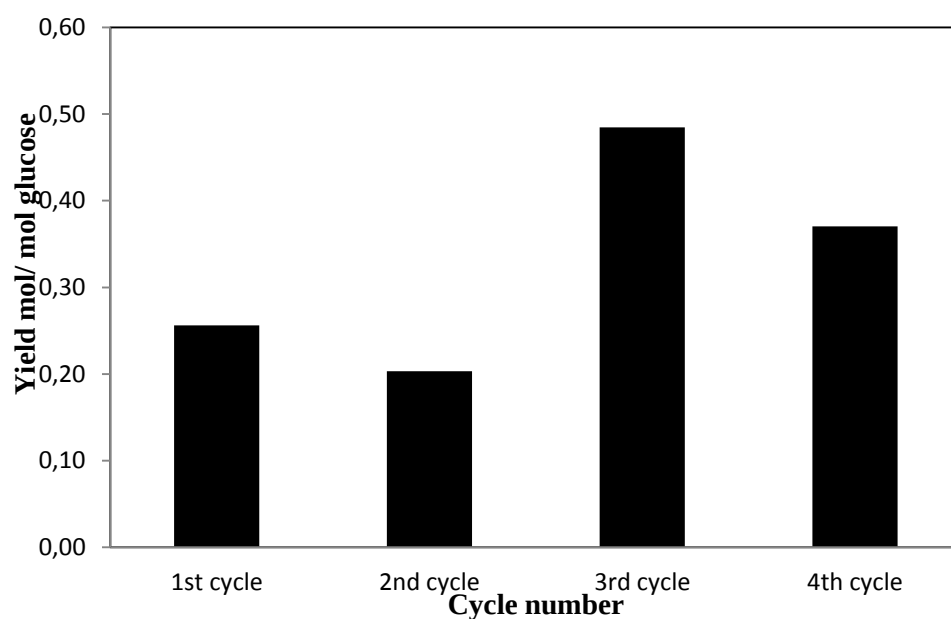


Figure 3.7 Hydrogen production yields, mol H₂/ mol glucose, at different cycles of 5 days cycle repeated batch operation with plastic scouring sponge pad with metal mesh.

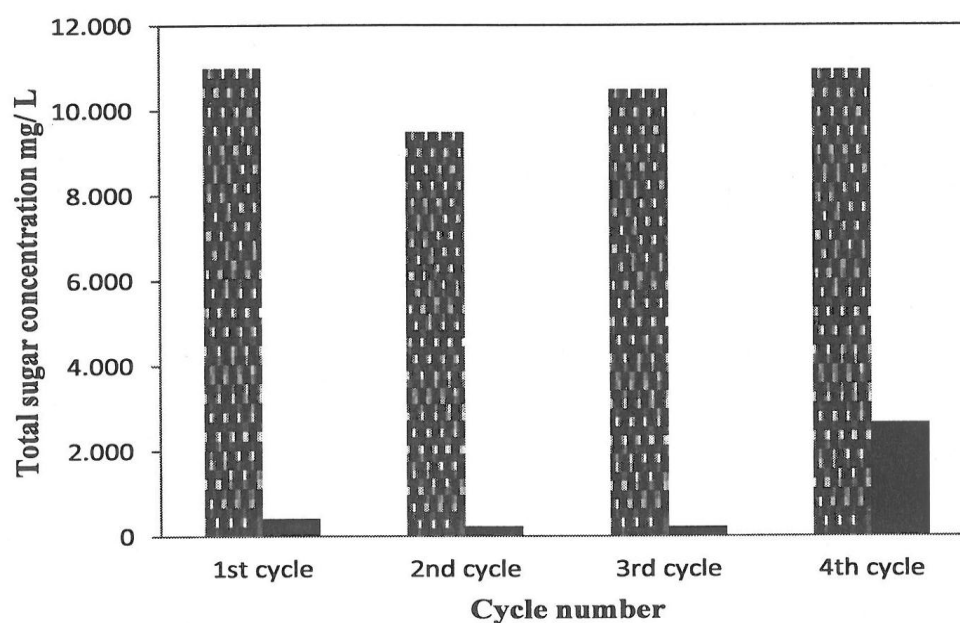


Figure 3.8 Initial and final TS concentrations for different cycles of plastic scouring sponge pad with metal mesh at 5 days cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.1.3 Plastic Nylon Sponge

Figure 3.9 depicts the variation of cumulative hydrogen gas volume with time for plastic nylon sponge at 5 days cycle repeated batch operation. The highest hydrogen production was achieved in the first cycle with CHV= 82 ml. However, no efficient hydrogen production was observed for the next cycles. In average 33 ml of hydrogen was produced for the cycles between 3rd and 4th. The total hydrogen volume produced at the end of the 4th cycle was CHV=157 ml. Figure 3.10 shows variation of hydrogen production yield (ml H₂/ g TS) with cycles for plastic nylon sponge. At the end of the first cycle, hydrogen yield was 38.15 ml H₂/g TS (0,28 mol H₂/ mol glucose). Total sugar consumption was around 97% (Figure 3.12)

Hydrogen volume and production yield showed a decreasing trend as system was loaded with fresh media. The main reason for the decrease in the production could be the low biomass or biofilm formation on the plastic nylon particles due to large pore size or porosity.

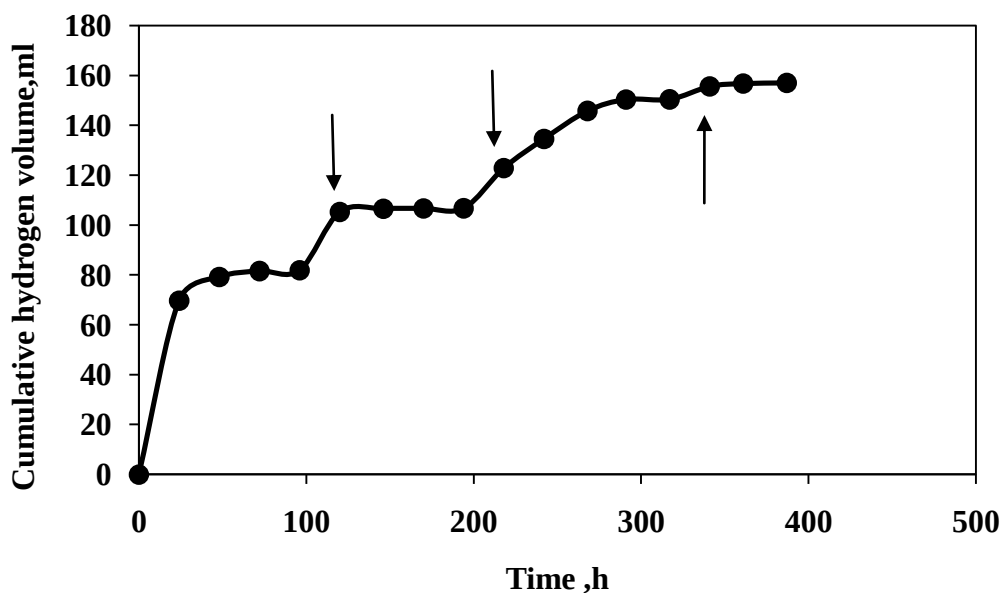


Figure 3.9 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge at 5 days cycle time repeated batch operation.

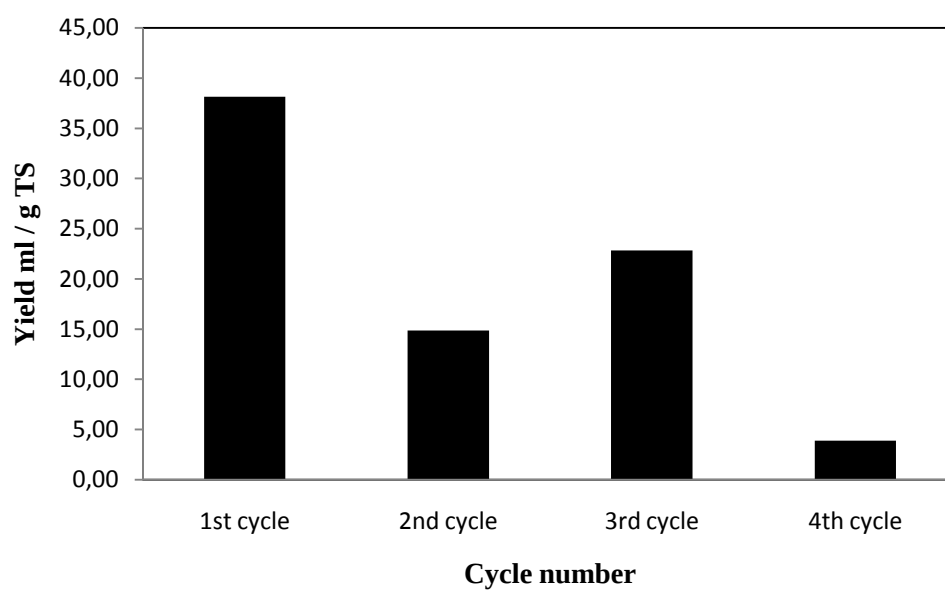


Figure 3.10 Hydrogen production yields, ml H₂/ g TS, at different cycles of 5 days cycle repeated batch operation with plastic nylon sponge.

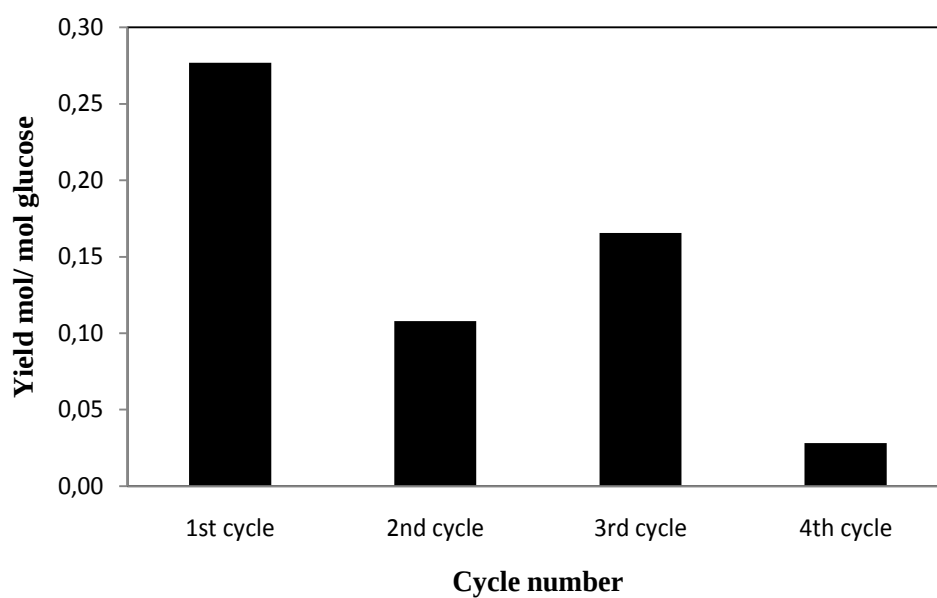


Figure 3.11 Hydrogen production yields, mol H₂/ mol, at different cycles of 5 days cycle repeated batch operation with plastic nylon sponge.

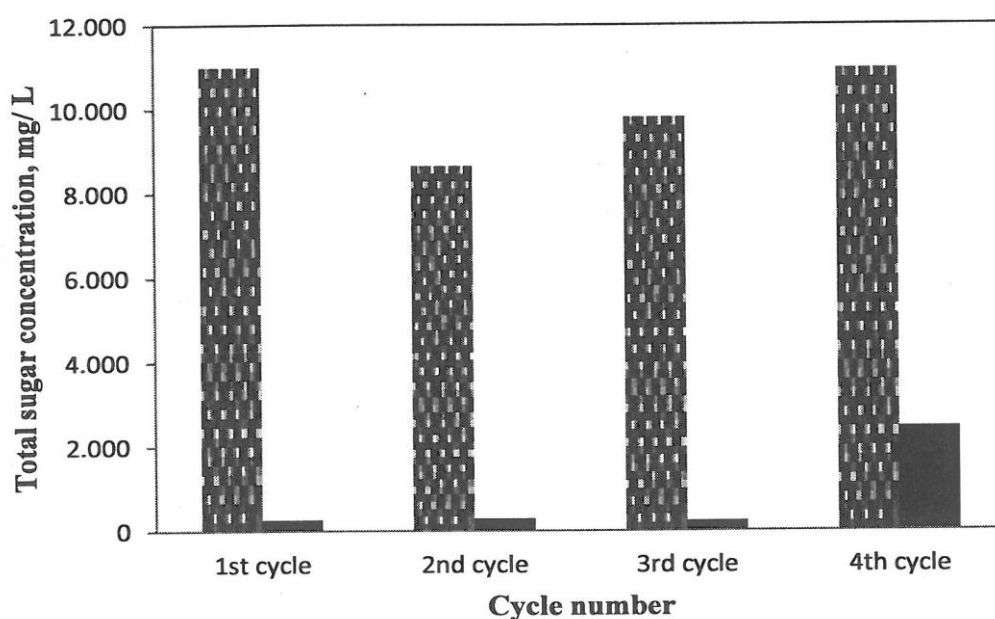


Figure 3.12 Initial and final TS concentrations for different cycles of plastic nylon sponge at 5 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.1.4 Plastic Nylon Sponge with Metal Mesh Particle

The pore size of plastic nylon was reduced by covering the particles with metal mesh. Figure 3.13 shows time variation of cumulative hydrogen volumes obtained with this particle. A steady increase in volume for the first three cycles was observed. About 100 ml hydrogen was produced at each cycle up to 4th one. The last cycle provided only $V_{H_2}=37$ ml hydrogen production. The total volume produced was $CHV=391$ ml at the end of process. Figure 3.14 and Figure 3.15 show yields of the hydrogen production for different cycles. The highest yield was around 76 ml H_2 /g TS (0.55 mol H_2 / mol glucose) obtained at the second cycle. The reason for the decrease in the yield at the last cycles could be diversion of microbial population from hydrogen producers to non-hydrogen producers or hydrogen consumers. Initial total sugar concentration of cycles varied between $TS=11$ g/L- 9.5 g/L. The rate of consumption of sugar declined with fresh media loading. Maximum amount of sugar consumed at the first cycle was nearly 97% and decreased to 76 % at the end of operation.

Covering plastic nylon sponge with metal mesh improved the hydrogen production due to reduction in pore size and hence providing better environment for immobilization. Hydrogen production this particle was higher than that of obtained with the others.

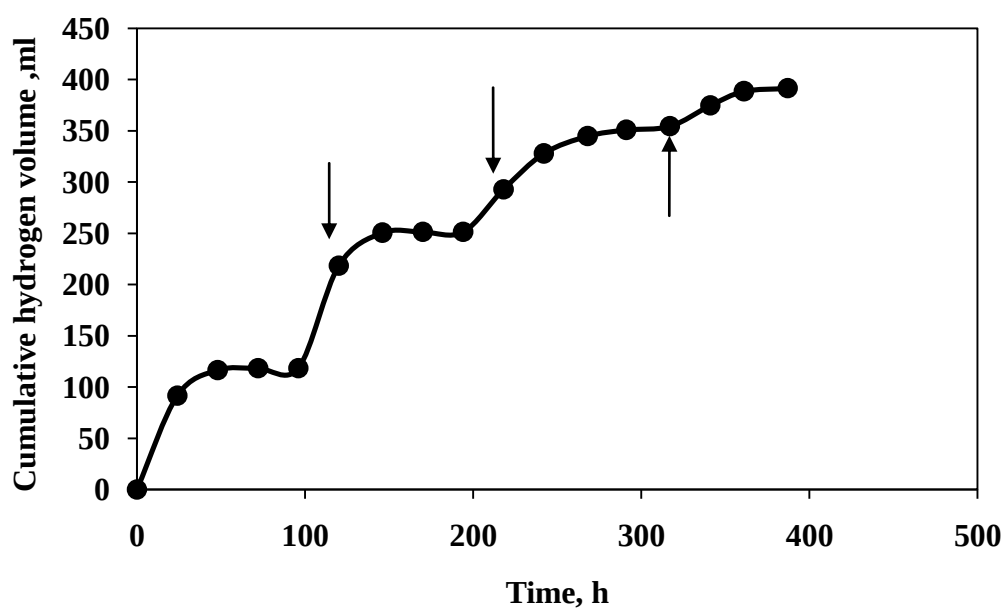


Figure 3.13 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge with metal mesh at 5 days cycle repeated batch operation.

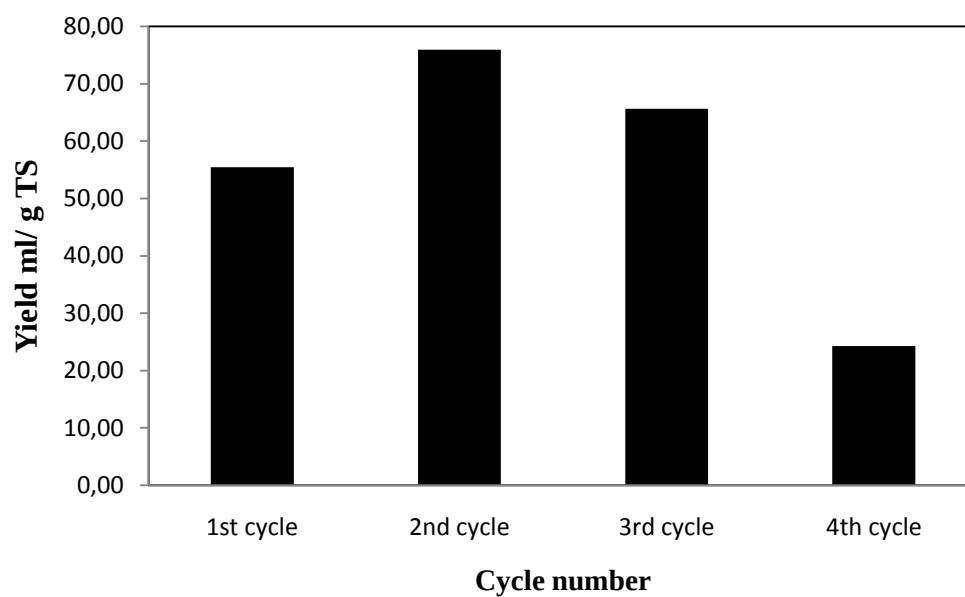


Figure 3.14 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic nylon sponge with metal mesh at 5 days cycle repeated batch operation.

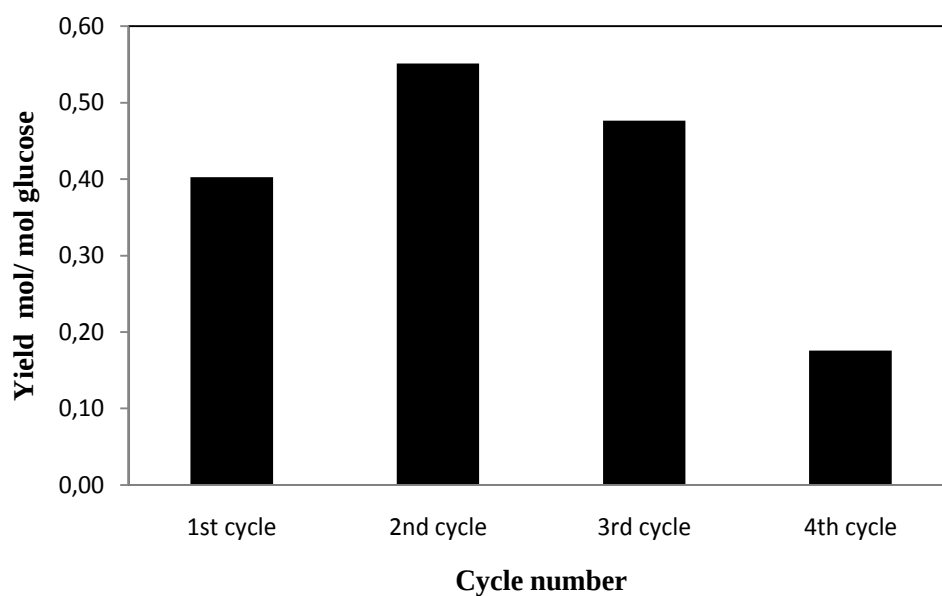


Figure 3.15 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic nylon sponge with metal mesh at 5 days cycle time repeated batch operation.

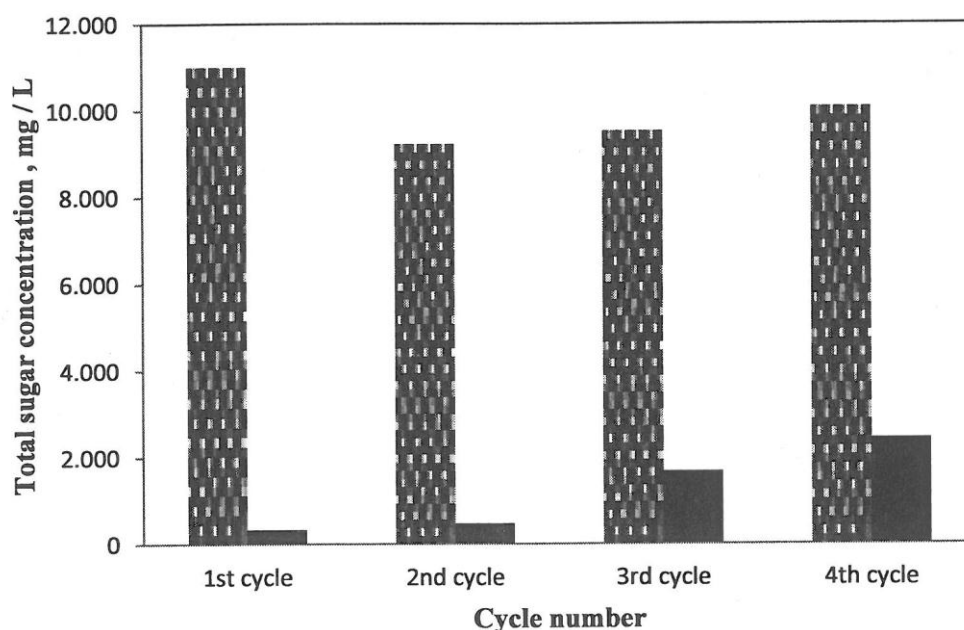


Figure 3.16 Initial and final TS concentrations for different cycles of plastic nylon sponge with metal mesh at 5 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.1.5 Black Porous Sponge

The last particle used in this study was called as black porous sponge used in Material Engineering. Repeated batch operation with this particle resulted in CHV= 221 ml (Figure 3.17) The highest production was achieved at the first two cycles with volume of around 100 ml at each. The last cycle produced only 2 ml rise in cumulative hydrogen volume. Hydrogen production yields as ml H₂/g TS and mol/mol glucose were given in Figure 3.18 and Figure 3.19, respectively. Hydrogen yields decreased gradually. The highest yield was obtained at the end of the first cycle (47.18 ml H₂/ g TS). It was 31.1 ml H₂/ g TS (0.23 mol H₂/ mol glucose) at the end of second cycle. Since there was no significant gas production for the last ones, the yields were negligible. The amount of sugar consumed for the first three cycles was around 96% and decreased to 70% for the last ones. .

This support material degraded due to low pH value of fermentation media. Although the volume of production was high in the first cycle, particles were

destroyed as exposed to new media and to low pH values during the repeated batch operation. Particle degradation resulted in destruction in immobilization of microorganisms and hence decrease in the total biomass concentration in the process. Therefore, the decrease in the yield of hydrogen was observed. Low sugar utilization at the last cycle supports this conclusion.

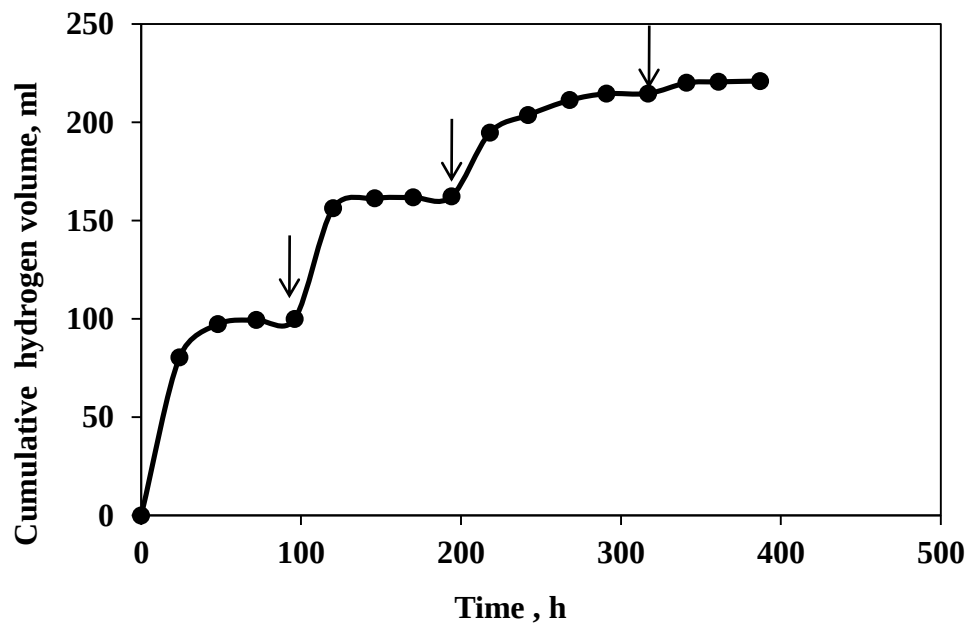


Figure 3.17 Variation of cumulative hydrogen gas volume with time for black porous sponge at 5 days cycle time repeated batch operation

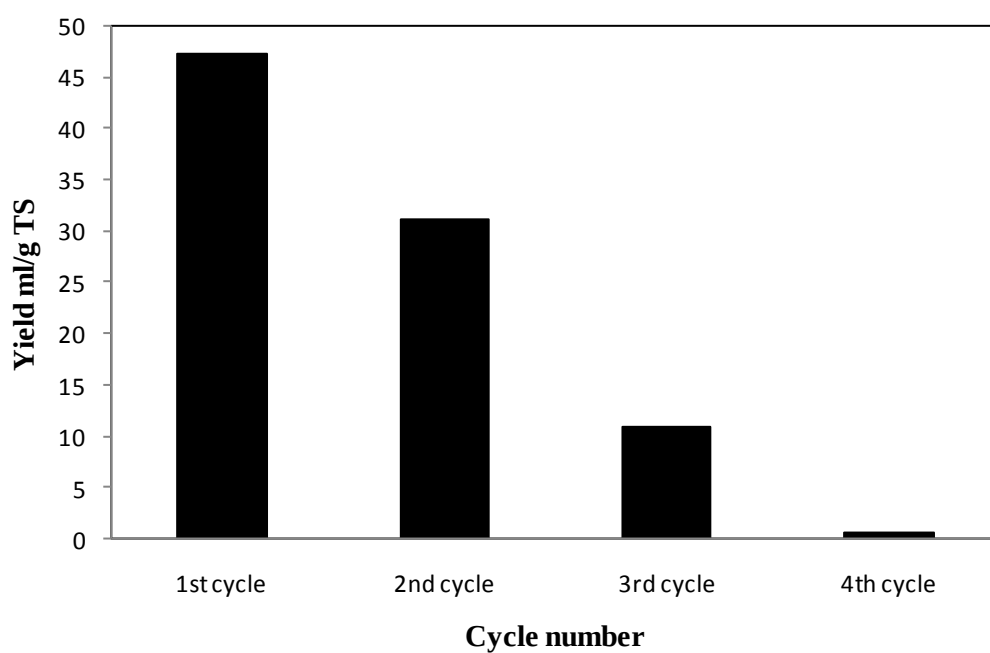


Figure 3.18 Hydrogen production yields, ml H₂/ g TS, at different cycles of 5 days cycle repeated batch operation with black porous sponge.

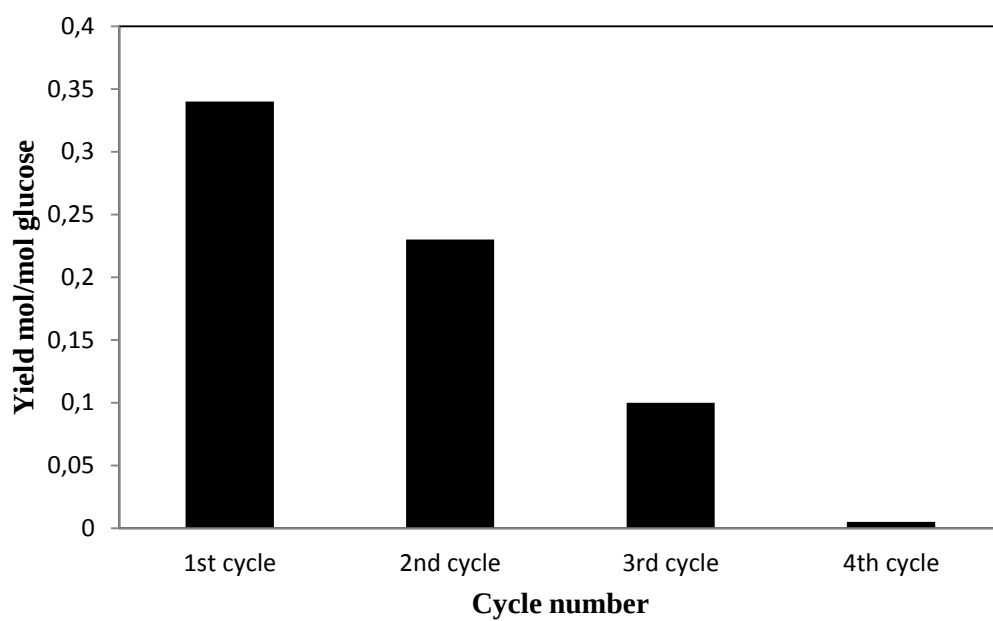


Figure 3.19 Hydrogen production yields, mol H₂/ mol, at different cycles of 5 days cycle repeated batch operation with black porous sponge.

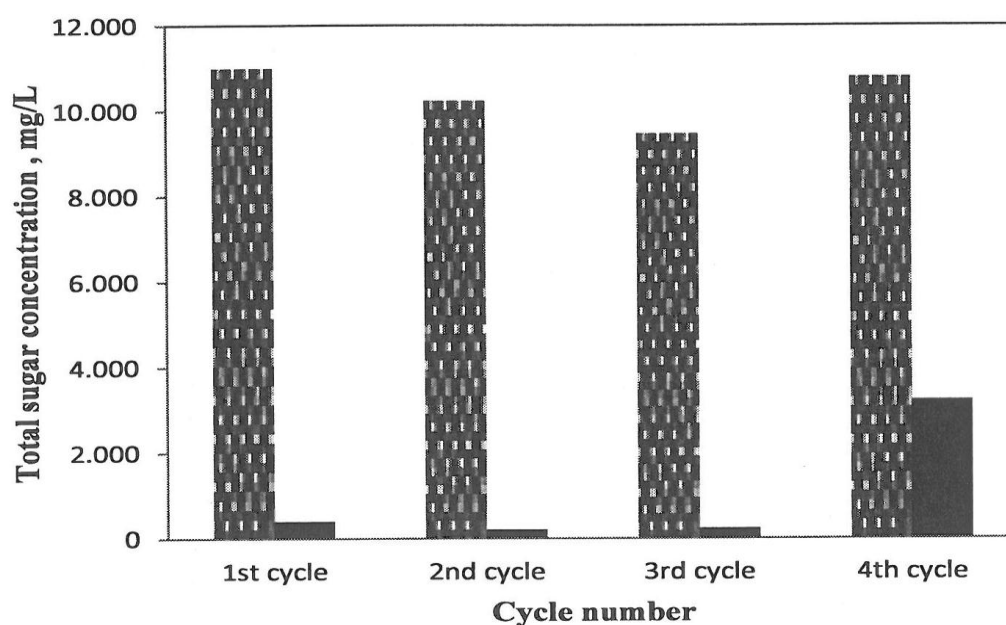


Figure 3.20 Initial and final TS concentrations for different cycles of black porous sponge support particle at 5 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.1.6 Control Experiments without Support Material

Figure 3.21 shows time variation of cumulative hydrogen volume for control experiments without support material. The first cycle resulted in CHV = 74.45 ml. Production gradually decreased for second (CHV=30 ml) and third cycles (CHV = 20 ml). However, final one provided only 2 ml rise in cumulative hydrogen volume. In total hydrogen volume was CHV=134 ml at the end of repeated batch operation. Figure 3.22 and Figure 3.23 show yield of the hydrogen production in suspended culture (control experiments). The maximum yield calculated to first cycle 35.39ml/ g TS (0.26 mol/mol glucose) The lowest hydrogen yield was 0.01 mol H₂/mol glucose at the end of 4th cycle. The amount of sugar consumed for all cycles was around 98%.

The yield of hydrogen formation in control experiments which represent suspended growth systems was lower compared to the immobilized systems. This result indicated that immobilization of microorganisms enhances hydrogen production.

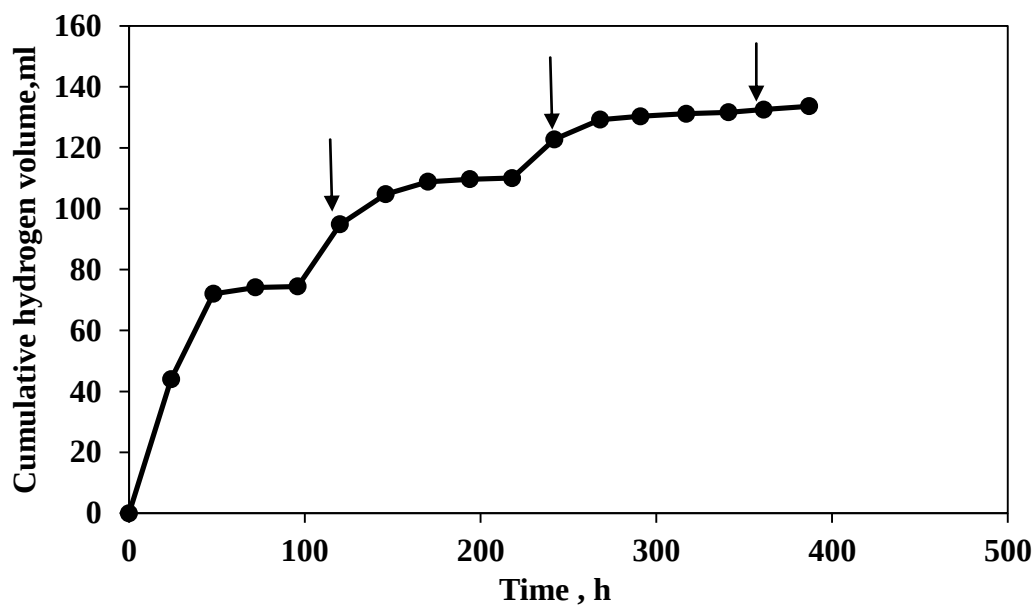


Figure 3.21 Hydrogen production yields, ml H₂/ g TS, at different cycles of 5 days cycle repeated batch operation with control experiment.

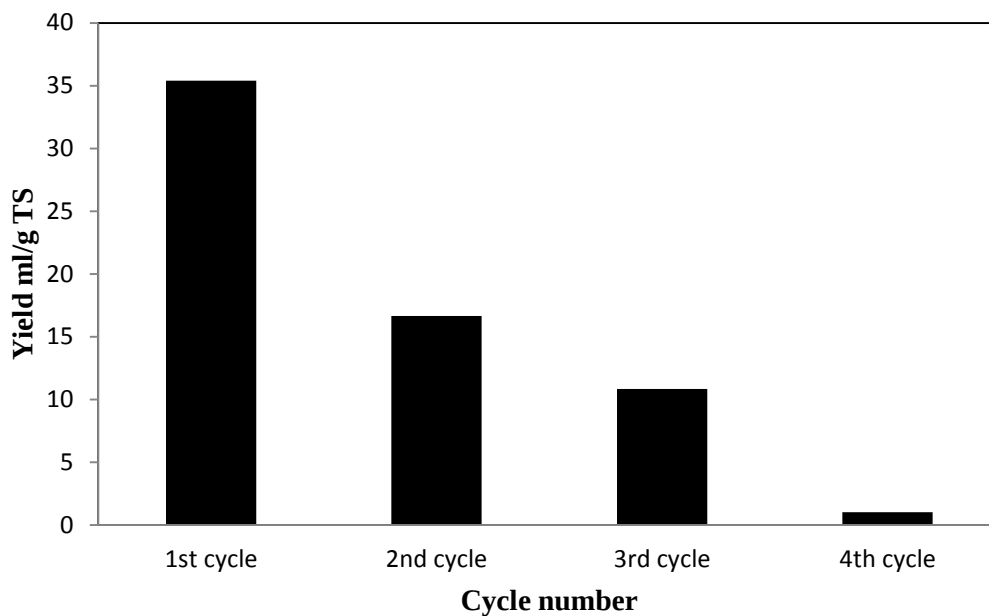


Figure 3.22 Hydrogen production yields, ml H₂/ g TS, at different cycles of 5 days cycle repeated batch operation with control experiment.

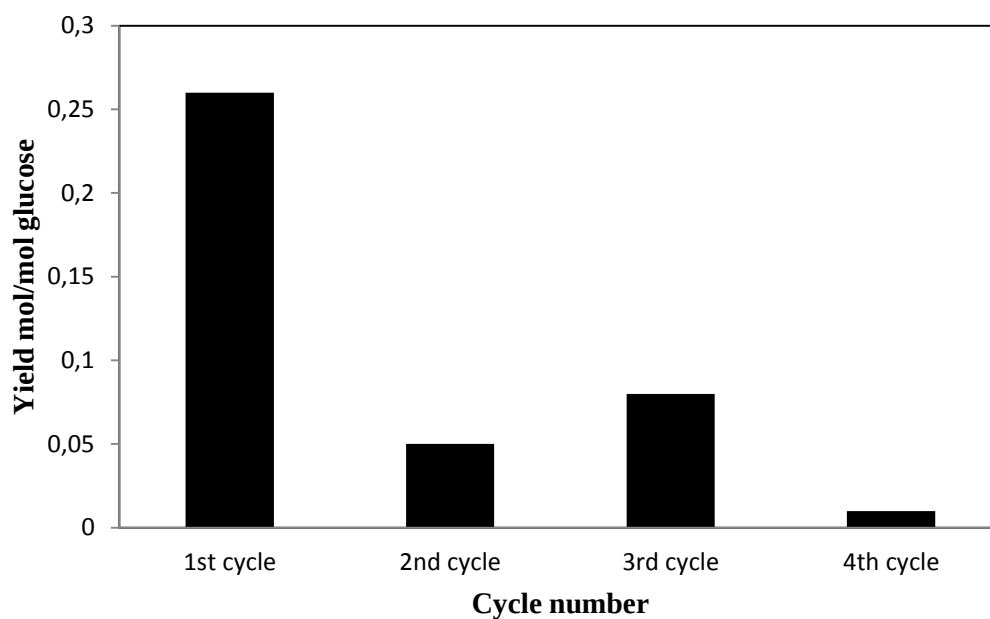


Figure 3.23 Hydrogen production yields, mol H₂/ mol, at different cycles of 5 days cycle repeated batch operation with control experiment.

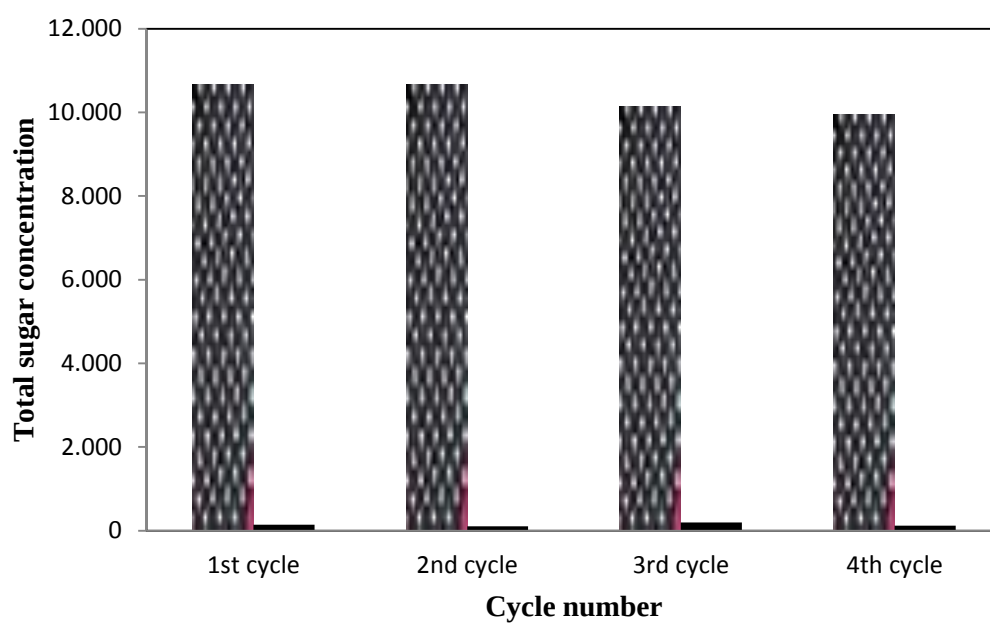


Figure 3.24 Initial and final TS concentrations for different cycles of control experiments without support material at 5 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.2 Repeated Batch Operation with 3 Days Cycle Time

Repeated batch cycle was reduced to 3 days to investigate the effect of frequent substrate loading on hydrogen production performance of organisms. Decreasing cycle time could divert the metabolism of organisms to hydrogen production rather than biomass synthesis. In addition, the growth phase could be kept at exponential phase at which effective hydrogen production can be achieved. Moreover, decreasing cycle time closes the system to continuous operation. The batch reactors were loaded with fresh media 5 times. In other words, the batch process was repeated five times to reach the steady state condition in terms of hydrogen production.

3.1.2.1 Plastic Scouring Sponge Pad Particle

Figure 3.25 shows that variation of cumulative hydrogen volume with 3 days repeated batch cycles for plastic scouring sponge pad particle. Cumulative hydrogen volume for the first cycle was around CHV=110 ml. A slight increase in the second cycle was observed with the final volume of around 176 ml. However, there was no substantial hydrogen production for the following cycles. Final volume was CHV=286 ml. Variation of hydrogen production yields as ml H₂/g TS and mol/mol glucose was given in Figure 3.26 and Figure 3.27 the maximum hydrogen yield was observed at the first cycle as 43.73 ml H₂/ g TS (0.35 mol H₂/ mol glucose). Hydrogen yield was calculated as 35 ml H₂/ g TS (0.25 mol H₂/ mol glucose) at the end of second cycle. The lowest hydrogen yield was calculated at the end of the third cycle as 19.77 ml H₂/g TS (0.14 mol H₂/ mol glucose). Total sugar consumption was around over 90% for the first 3 repeated batch operation and decreases to around 75% for the following loadings.

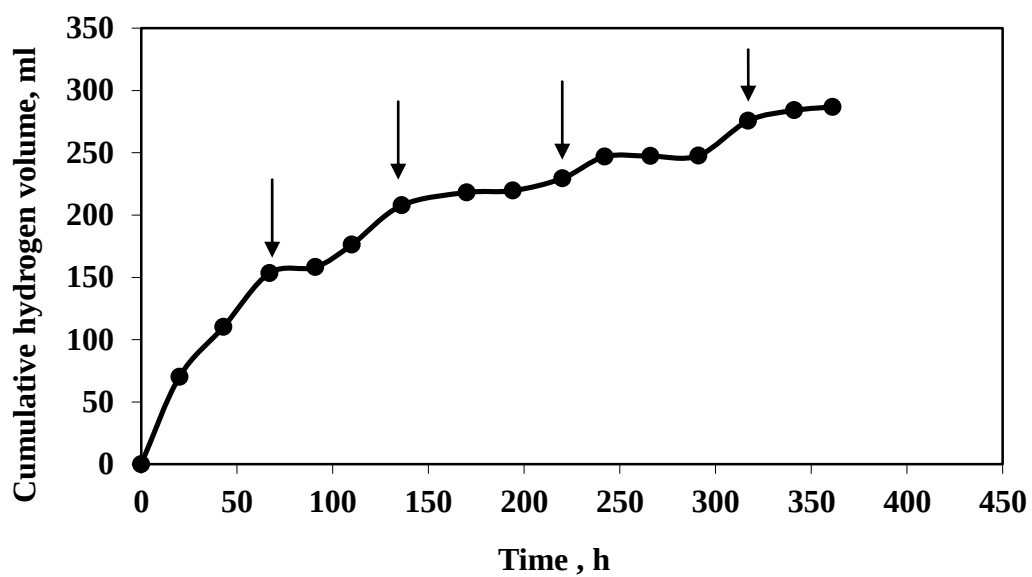


Figure 3.25 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad at 3 days cycle time repeated batch operation

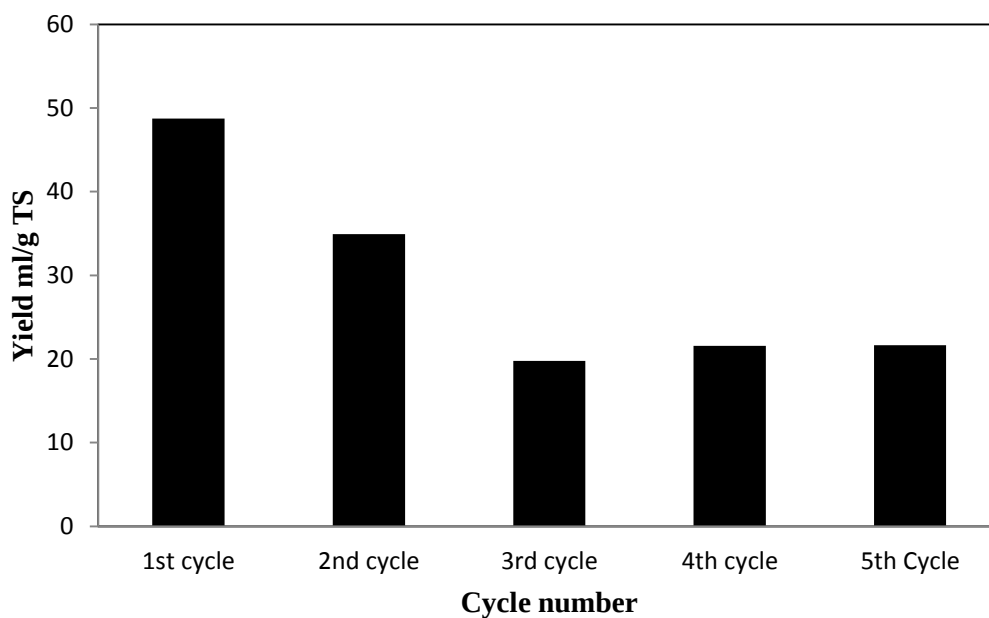


Figure 3.26 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic scouring sponge pad at 3 days cycle time repeated batch operation.

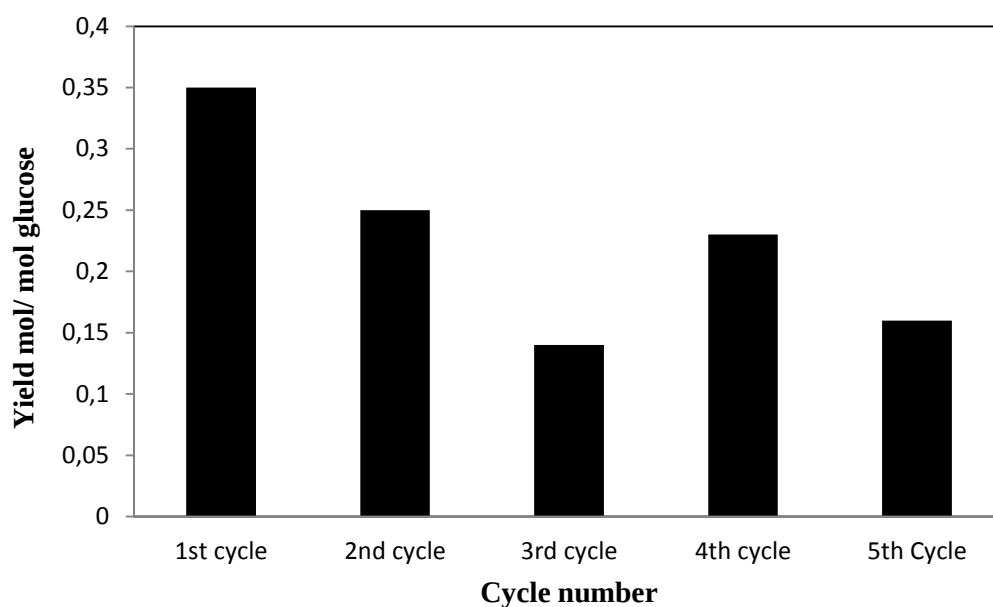


Figure 3.27 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic scouring sponge pad at 3 days cycle time repeated batch operation.

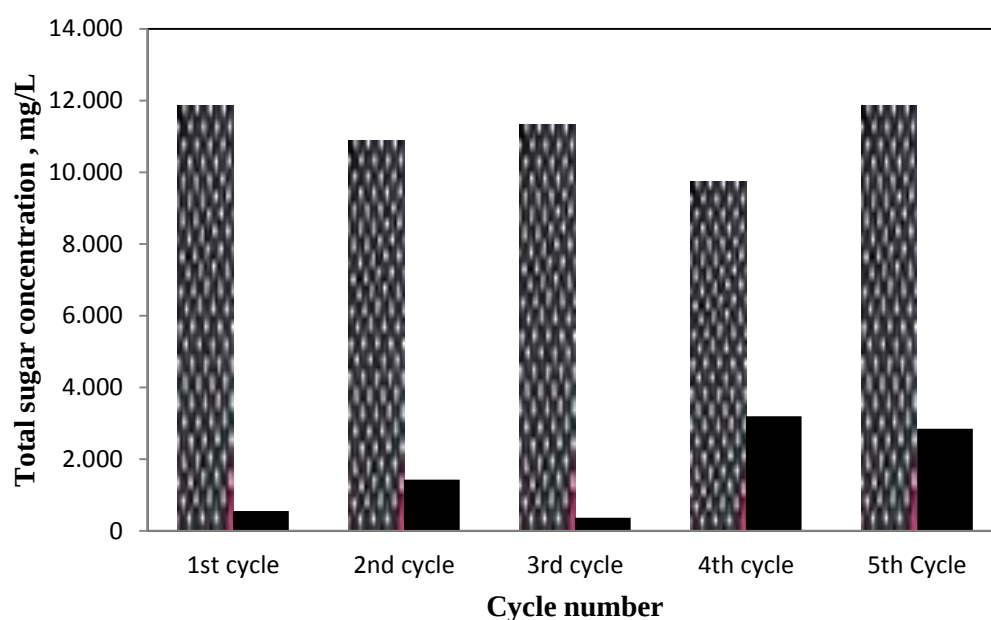


Figure 3.28 Initial and final TS concentrations for different cycles of plastic scouring sponge pad at 3 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ — , Final TS concentration).

3.1.2.2 Plastic Scouring Sponge Pad with Metal Mesh Particle

Cumulative hydrogen gas volume drastically increased to around CHV=300 ml at the end of 3rd cycle with this particle. After the third cycle, no significant hydrogen production was observed. Total volume obtained after 5th cycle was CHV=314 ml. The system reached to almost steady state after the 3rd cycle. Depending on the decrease in the volume, the yield of production also decreased (Figure 3.30 and Figure 3.31). The maximum yield calculated at first cycle was 80.91 ml/g TS (0.59 mol/mol glucose). The lowest hydrogen yield was 2.31 ml/g TS at the end of the fourth cycle. Maximum amount of sugar consumed for the first cycle was nearly 94% but decreased to 62% at third cycle. Low substrate utilization may be concluded as low biomass activity, or wash out of organisms as system was loaded. Low biomass concentration could be the reason for low hydrogen production at last cycles.

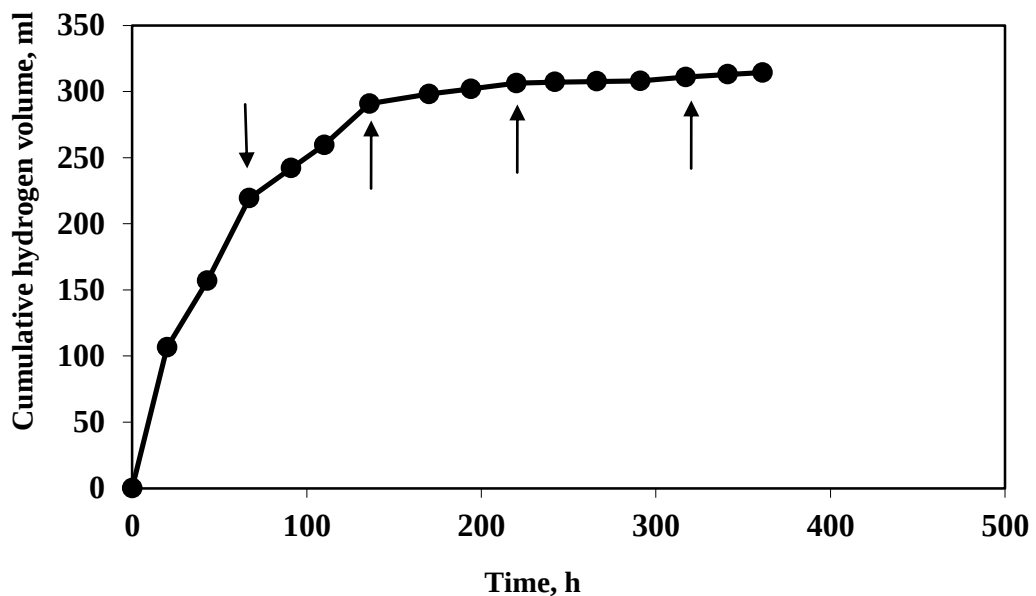


Figure 3.29 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad with metal mesh particle at 3 days cycle time repeated batch operation

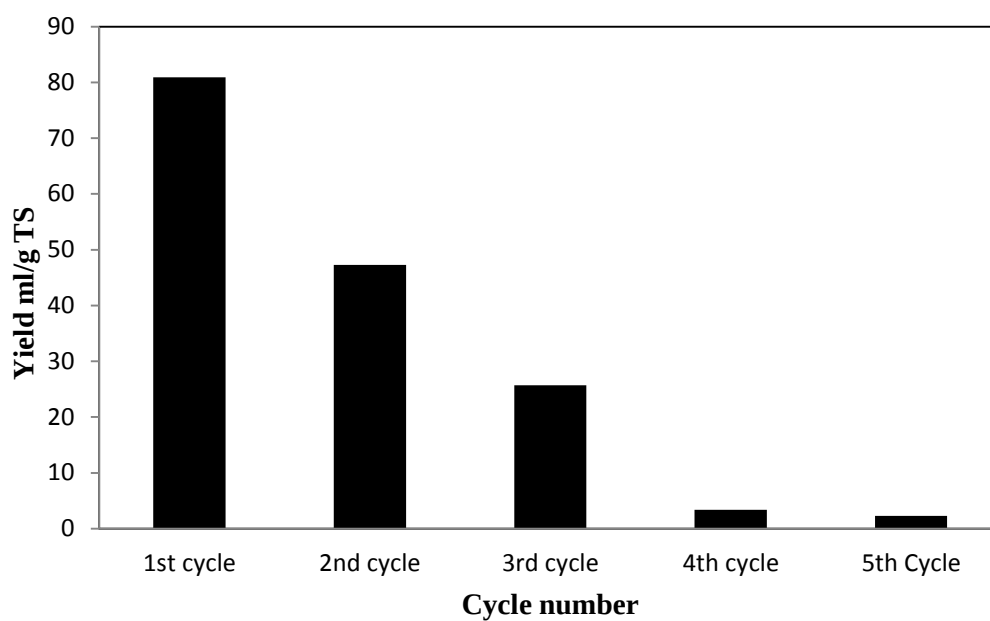


Figure 3.30 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic scouring sponge pad metal mesh particle at 3 days cycle time repeated batch operation.

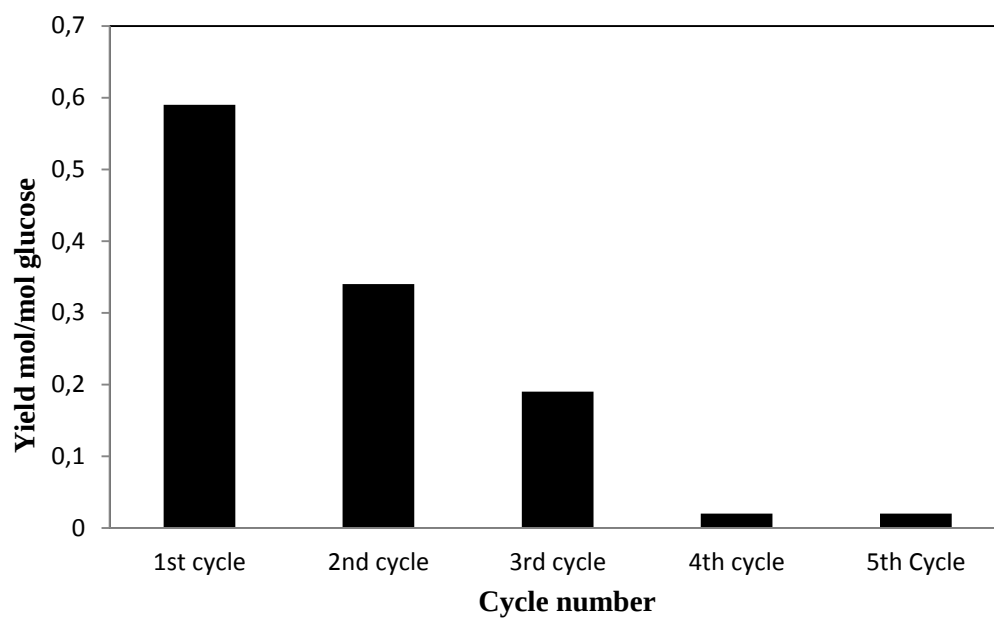


Figure 3.31 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic scouring sponge pad with metal mesh particle at 3 days cycle time repeated batch operation.

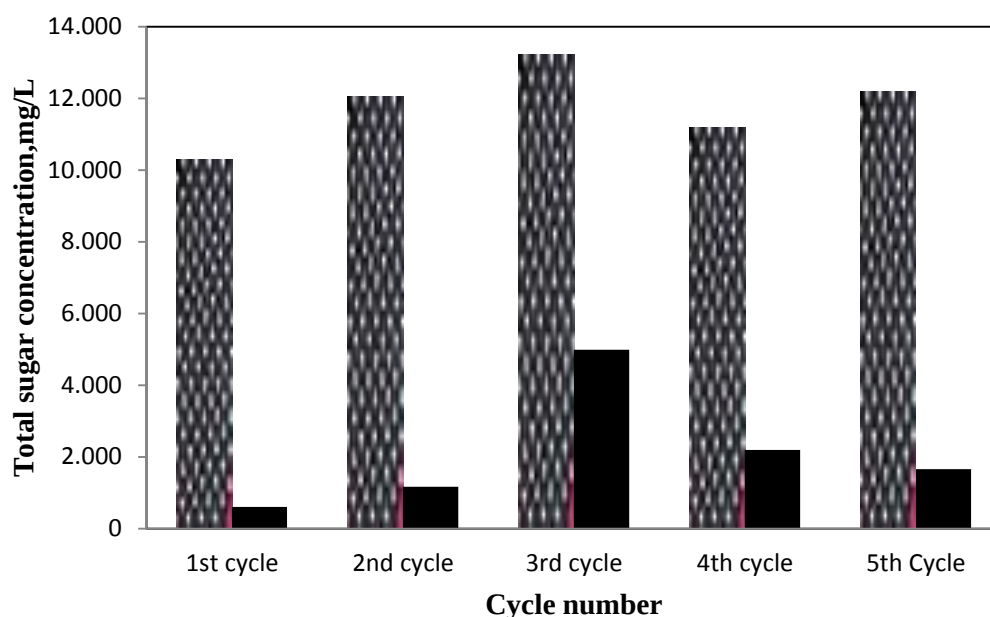


Figure 3.32 Initial and final TS concentrations for different cycles of plastic scouring sponge pad with metal mesh particle at 3 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.2.3 Plastic Nylon Sponge

Cumulative hydrogen production with plastic nylon sponge at 3 days repeated batch operation is given in Figure 3.33. The first cycle resulted in CHV = 192 ml cumulative hydrogen volume. The second and third cycle cumulative hydrogen volume was 275 ml and 324 ml, respectively. No significant increase in the hydrogen volume was observed after the 3rd cycle. The total volume of production at the end of repeated batch operation was 358 ml. Similar to the previous particle types, system reaches to steady state after the 3rd cycle. Hydrogen production yield, ml H₂/ g TS, of different cycles is given in Figure 3.34. Maximum hydrogen yield was calculated to 69.25 ml H₂/g TS. The yields were almost the same for the second and third cycles as around 35 ml H₂/g TS (0.25 mol H₂/ mol glucose). Yield at the fifth cycle was considerably low. The amount of sugar consumed for the first three cycles was around 96 %. But decreased to 60 % at the last cycle. Low sugar consumption indicates that biomass was not active enough to convert organic substance to hydrogen.

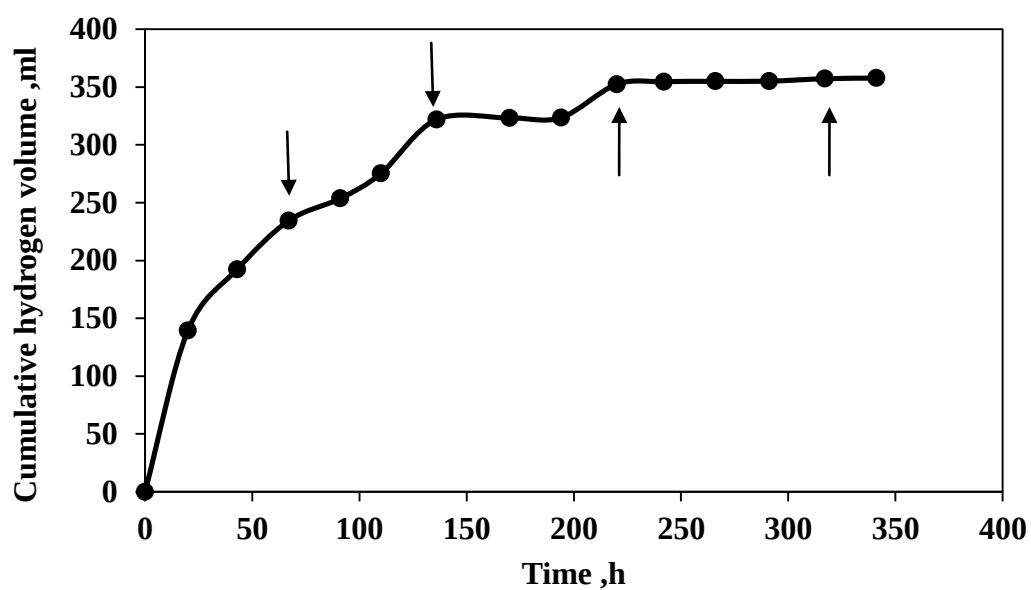


Figure 3.33 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge particle at 3 days cycle time repeated batch operation.

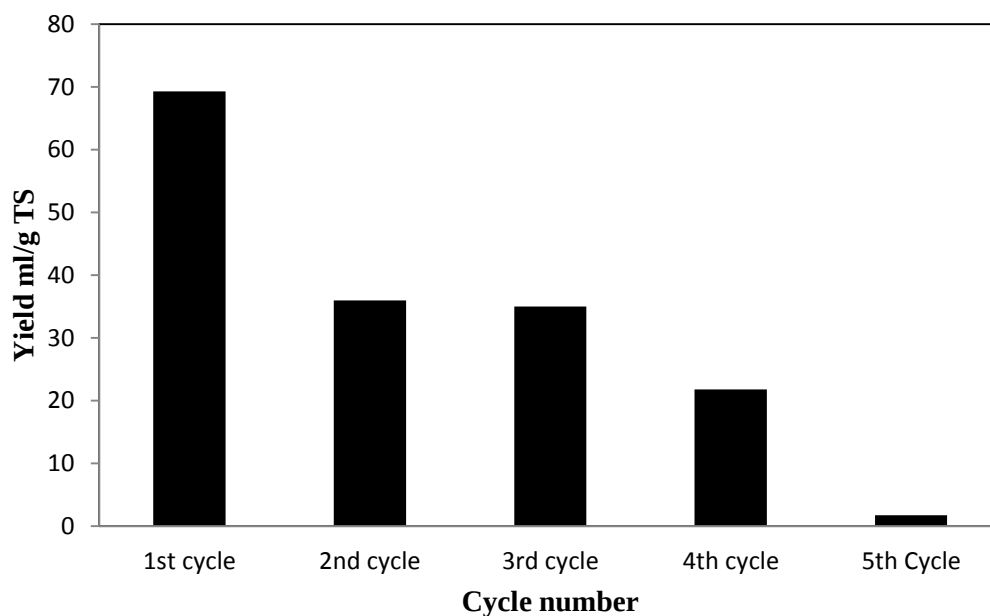


Figure 3.34 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic nylon sponge particle at 3 days cycle time repeated batch operation.

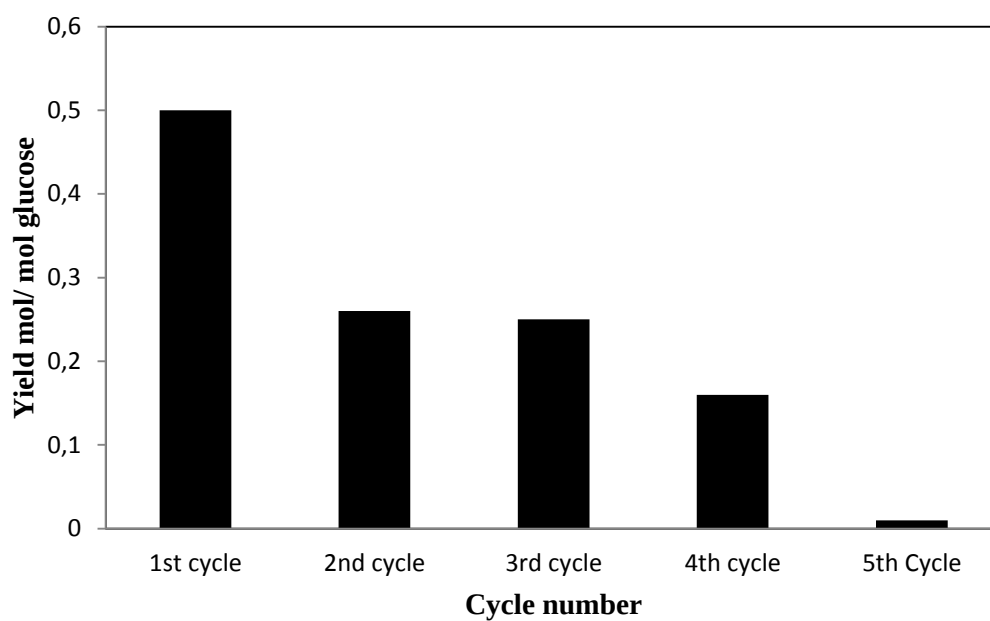


Figure 3.35 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic nylon sponge particle at 3 days cycle time repeated batch operation.

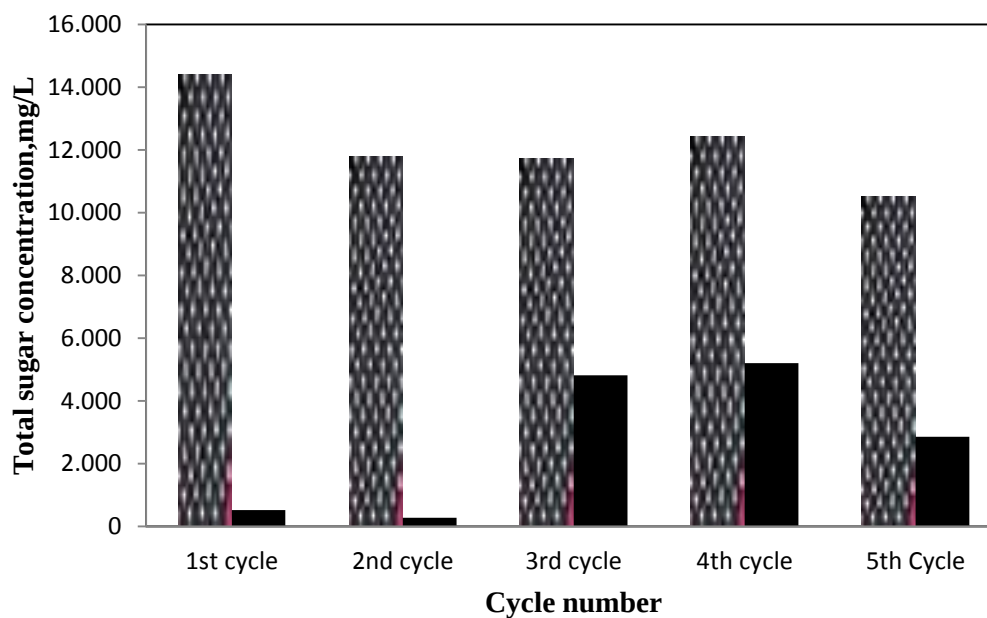


Figure 3.36 Initial and final TS concentrations for different cycles of plastic nylon sponge particle at 3 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.2.4 Plastic Nylon Sponge with Metal Mesh

The cumulative hydrogen gas volume obtained by plastic nylon sponge is shown in Figure 3.37. The volume (CHV) for the first and second cycle was CHV=214 ml, CHV =367 ml, respectively. Only 100 ml hydrogen was produced at the third cycle. The total hydrogen volume produced at the end of the fifth cycle was CHV=613 ml which is the highest hydrogen volume achieved in 3 days cycle repeated batch operation. The first cycle hydrogen yield was calculated as 75.04 ml H₂/ g TS (0.54 mol H₂/ mol glucose) (Figure 3.38 and Figure 3.39, respectively). In latter cycles, yield did not vary substantially and remained almost constant. The lowest hydrogen yield was obtained as 0.15 mol H₂/mol glucose at the end of the fourth cycle. Sugar consumption was 96% of the first cycle and decreased to 60% for the last cycle. These results indicated that plastic nylon sponge with metal mesh is a quite efficient microorganism support material to be used in hydrogen production. That could be due to good immobilization of microorganisms, better contact with biomass and substrate or metabolic pathway devoted to acetic acid production rather than butyric and propionic acid.

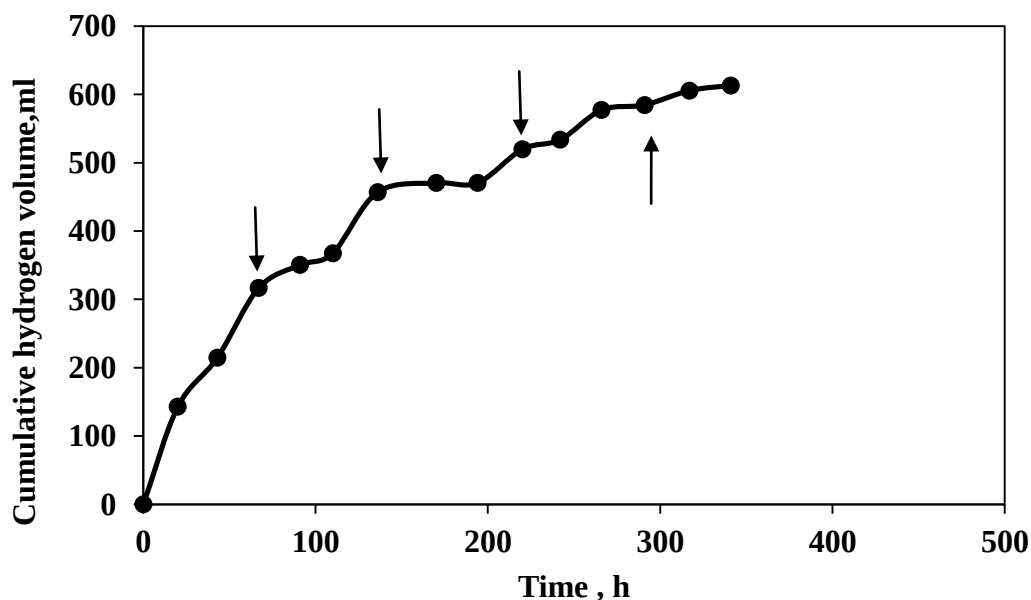


Figure 3.37 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge with metal mesh particle at 3 days cycle time repeated batch operation.

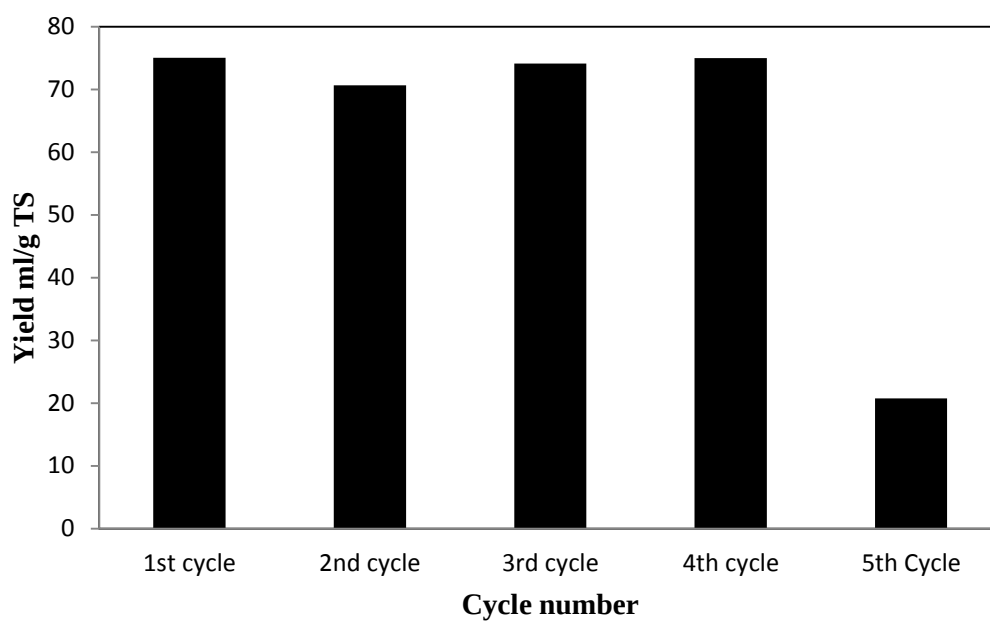


Figure 3.38 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic nylon sponge with metal mesh particle at 3 days cycle time repeated batch operation.

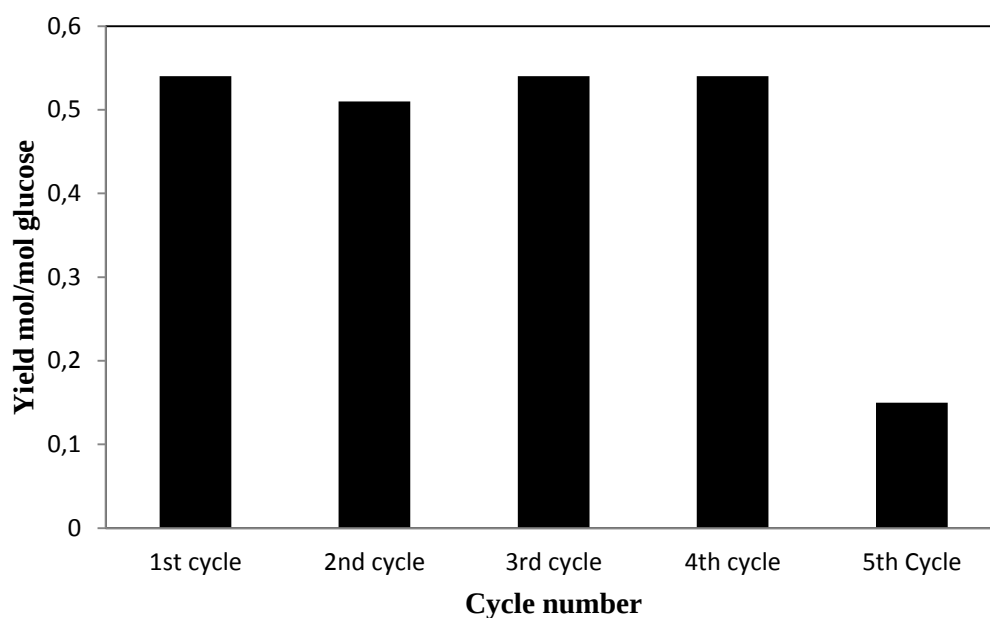


Figure 3.39 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic nylon sponge with metal mesh particle at 3 days cycle time repeated batch operation.

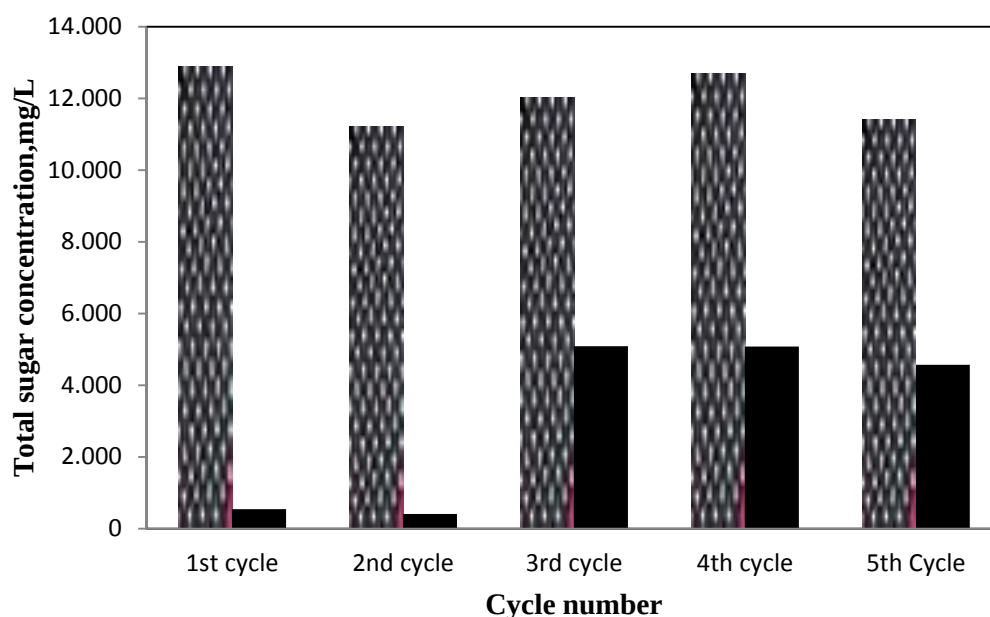


Figure 3.40 Initial and final TS concentrations for different cycles of plastic nylon sponge with metal mesh particle at 3 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.2.5 Black Prous Sponge

No efficient hydrogen production was observed with black porous sponge particle at 5 days cycle repeated batch operation due to deterioration of particles or immobilization surface. Similarly, cumulative hydrogen production with 3 days cycle was not very high. (Figure 3.41). The first cycle resulted in CHV = 111 ml cumulative hydrogen volume. The volume increased to CHV= 273 ml at the end of the second cycle. The amount of hydrogen produced at the end of the third cycle was only 20 ml. The total hydrogen volume produced at the end of the operation was CHV=346 ml. The yield of production was 46.31 ml H₂/ g TS first cycle. Then it reached to its maximum value of 59 ml H₂/ g TS at the second cycle. A substantial decrease in the yield was observed at 3rd cycle probably due to uncontrolled environmental conditions like temperature or pH. However, 4th cycle resulted in increasing in the yield to 30 ml H₂/ g TS with the strict control of environmental conditions. Total sugar consumption was around 90% for the first cycles but decreased to 40% for the last one. These results indicate that microorganisms were not

active enough to consume substrate and to produce hydrogen or washed out due to support material deterioration.

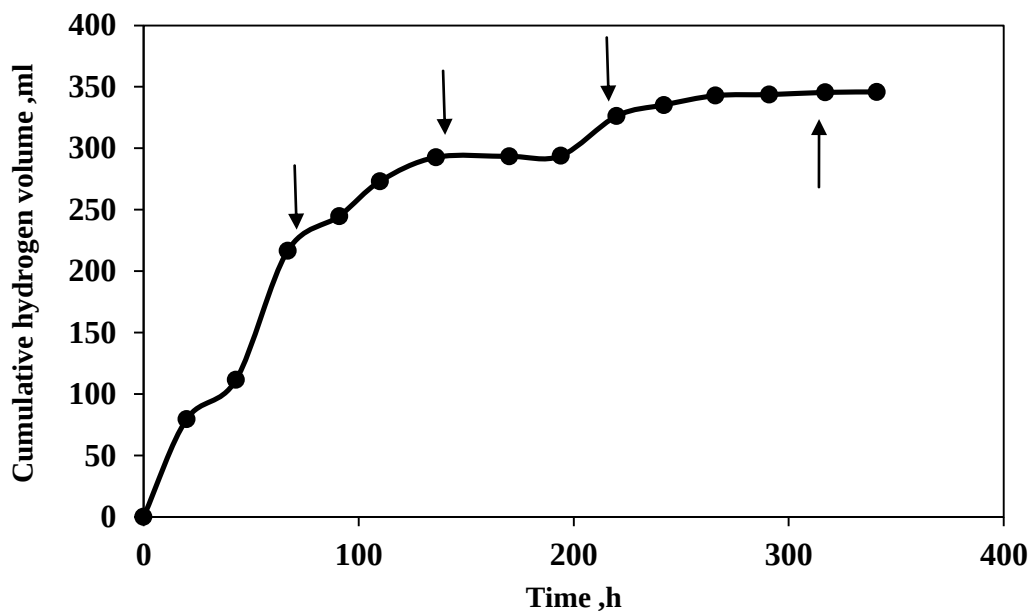


Figure 3.41 Variation of cumulative hydrogen gas volume with time for black prous sponge particle at 3 days cycle time repeated batch operation.

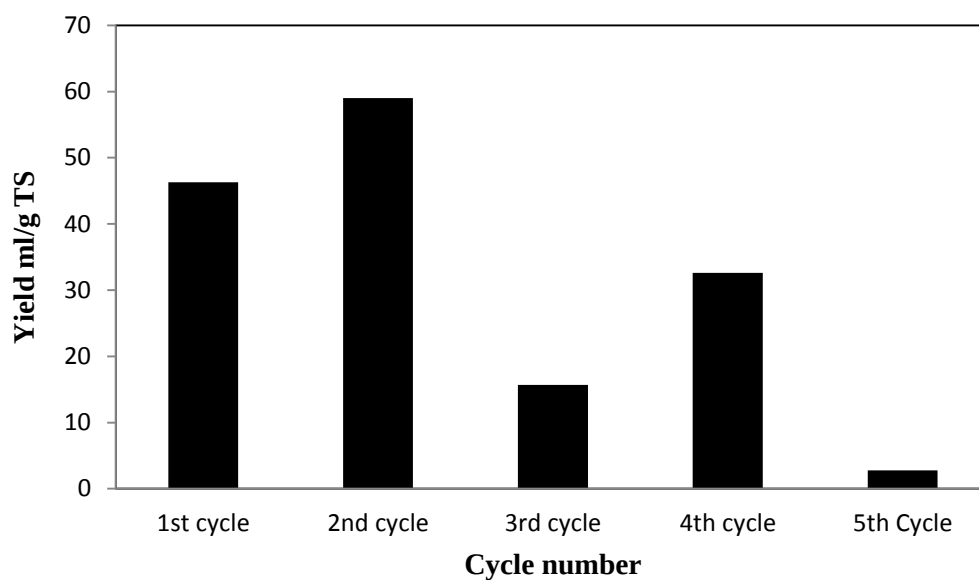


Figure 3.42 Variation of hydrogen production yield ml H₂/ g TS with cycles for black prous sponge particle at 3 days cycle time repeated batch operation.

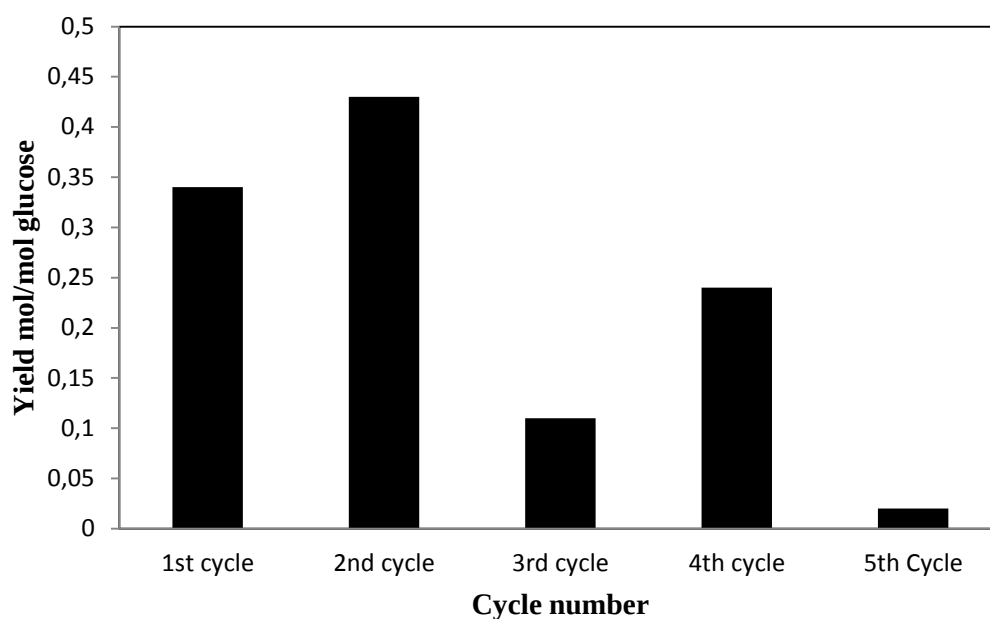


Figure 3.43 Variation of hydrogen production yield mol H_2 / mol glucose with cycles for black prous sponge particle at 3 days cycle time repeated batch operation.

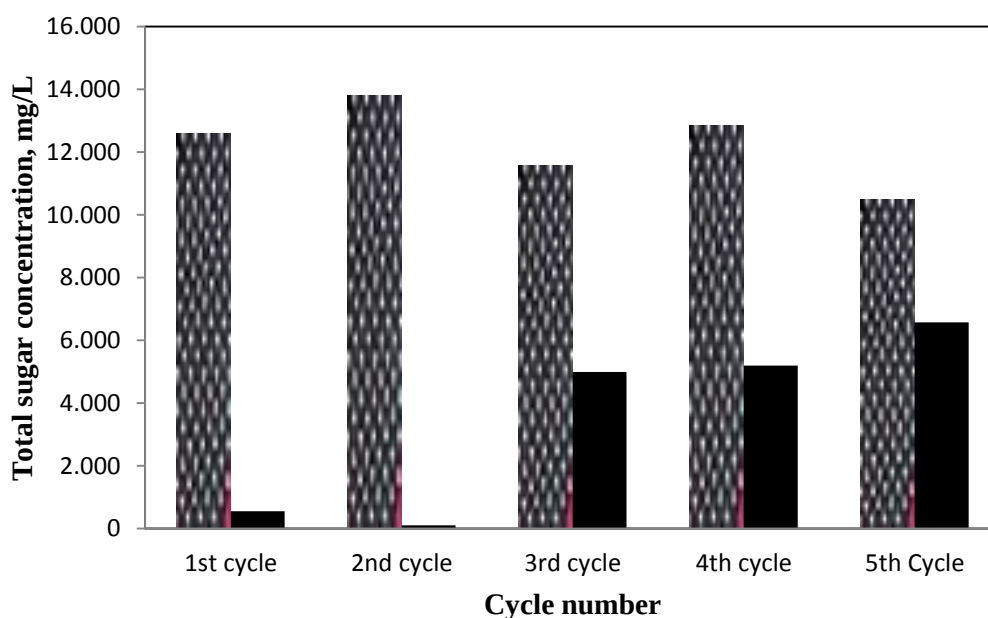


Figure 3.44 Initial and final TS concentrations for different cycles of black prous sponge particle at 3 days cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.2.6 Control Experiments without Support Material

Figure 3.45 depicts cumulative hydrogen gas volume from control experiments. The first cycle cumulative hydrogen production was 50 ml and at the end of the second cycle CHV was calculated 67 ml. Until the last cycle the amount of hydrogen produced was not significantly changed. The total hydrogen volume produced at the end of the fifth cycle was CHV=75.128 ml. The highest hydrogen yield was 22.41 ml H₂/ g TS at the first cycle (Figure 3.46) The total hydrogen yield at the end of the fifth cycle was only 0.2 ml H₂/ g TS. These results state that utilization of support material substantially increases hydrogen production volume and yield.

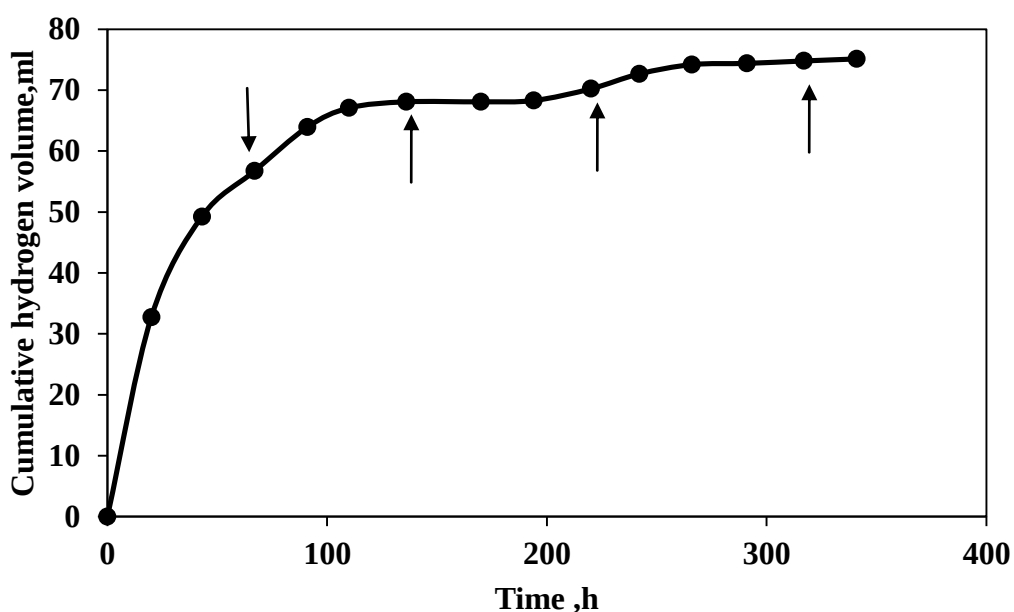


Figure 3.45 Variation of cumulative hydrogen gas volume with time for control experiments without support material at 3 days cycle time repeated batch operation.

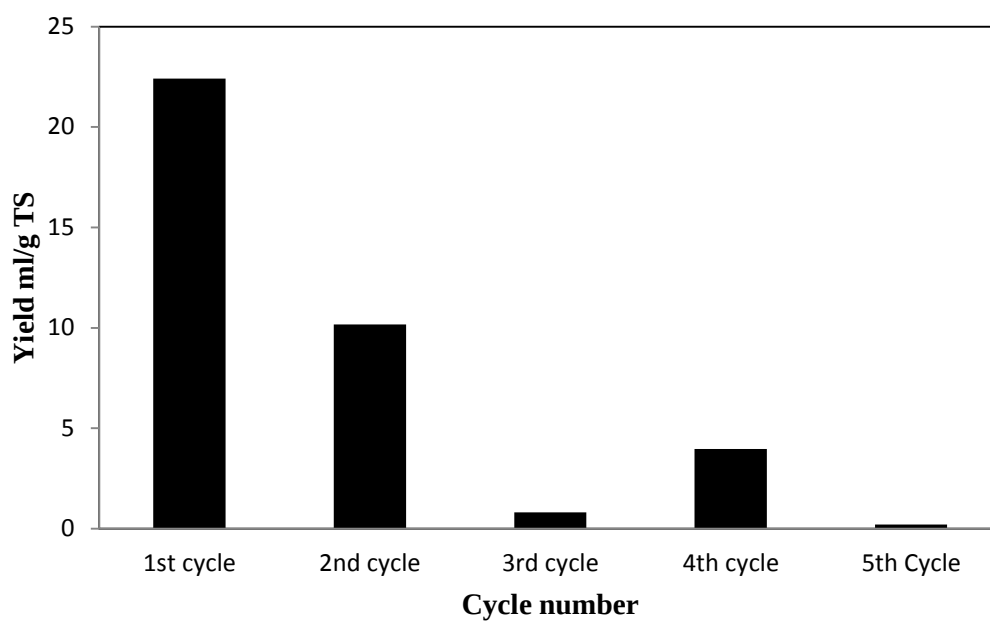


Figure 3.46 Variation of hydrogen production yield ml H₂/ g TS with cycles for control experiments without support material at 3 days cycle time repeated batch operation.

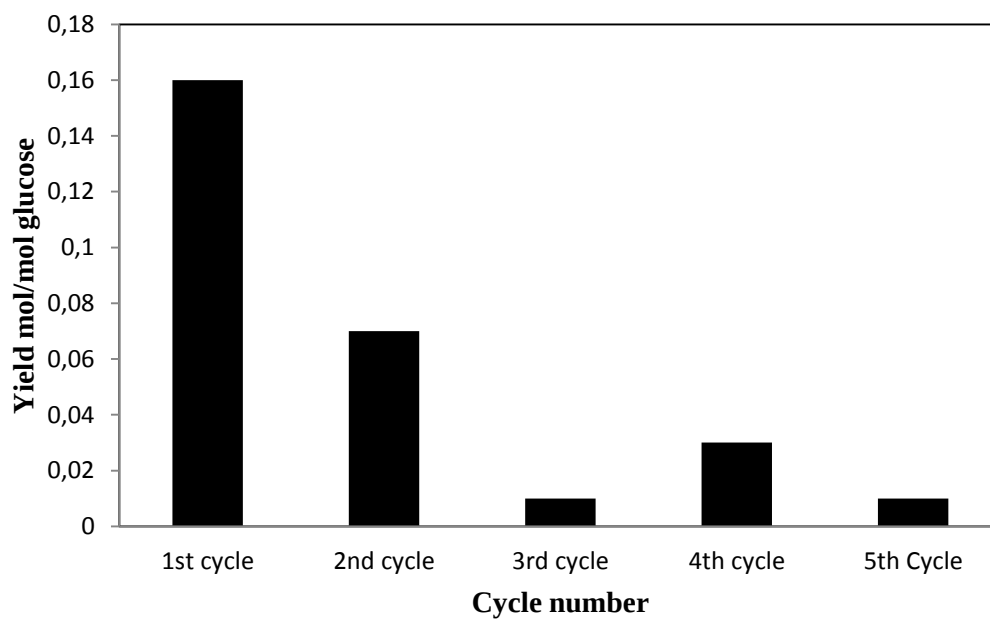


Figure 3.47 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for control experiments without support material at 3 days cycle time repeated batch operation.

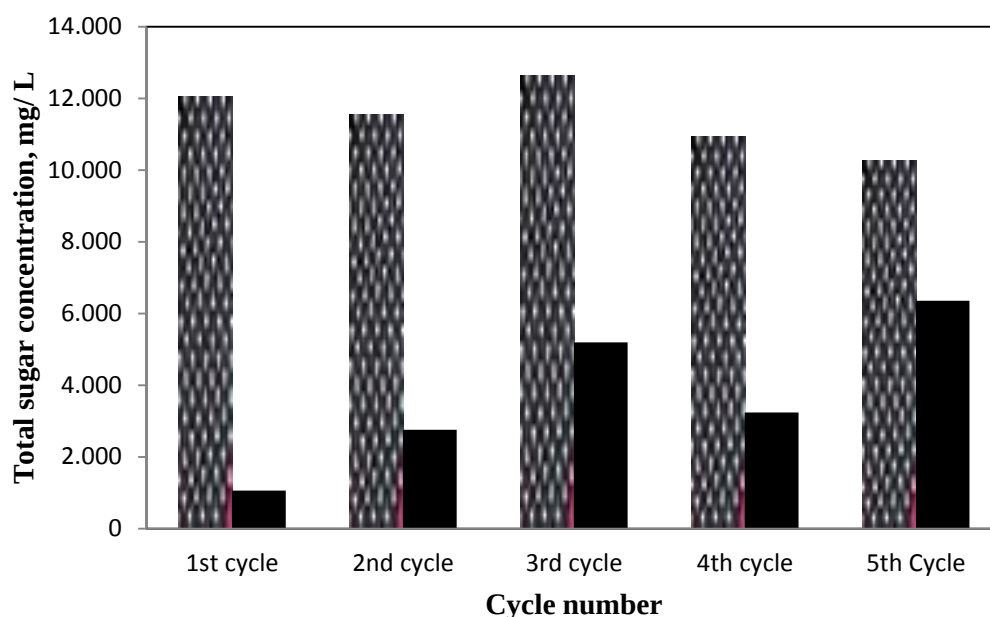


Figure 3.48 Initial and final TS concentrations for different cycles of control experiments without support material at 3 days cycle time repeated batch operation (— ■ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.3 Repeated Batch Operation with 1 Day Cycle Time

The cycle time represent the contact time of the reaction volume with the microorganisms. In this case, the contact time was 24 hours. The organisms were pushed to faster metabolic activity. Batch experiments were repeated at least 5 times or system was loaded with fresh media 5 times.

3.1.3.1 Plastic Scouring Sponge Pad Particle

Figure 3.49 shows cumulative hydrogen volume with 1 day repeated batch cycles for plastic scouring sponge pad particle. Cumulative hydrogen production was 129 ml for the first twenty-four hours. The volume increased to CHV= 238 ml at the end of the second cycle. The total hydrogen volume produced at the end of the fifth cycle was CHV=386 ml. Figure 3.49. The first cycle hydrogen production yield was 69. 28 ml H₂/ g TS (0.5 mol H₂/ mol glucose) .Afterwards, yield for second and third cycle reached to calculated 105. 61 ml H₂/ g TS (0.8 mol H₂/ mol glucose) and 136 ml H₂/ g TS (1 mol H₂/mol glucose), respectively. The highest hydrogen yield was 181,86

ml H₂/ g TS (1.32 mol H₂/ mol glucose) at the fifth cycle. The amount of sugar consumed for all cycles was around 85 % (Figure 3.50)

These results stated that decreasing cycle time provided a substantial increase in the yield and production volume. The main reasons for this improved production are well controlled pH by loading fresh media with pH=7, no accumulation of organic acids which inhibit hydrogen production at high concentrations. High substrate consumption indicates that microorganisms were active as well.

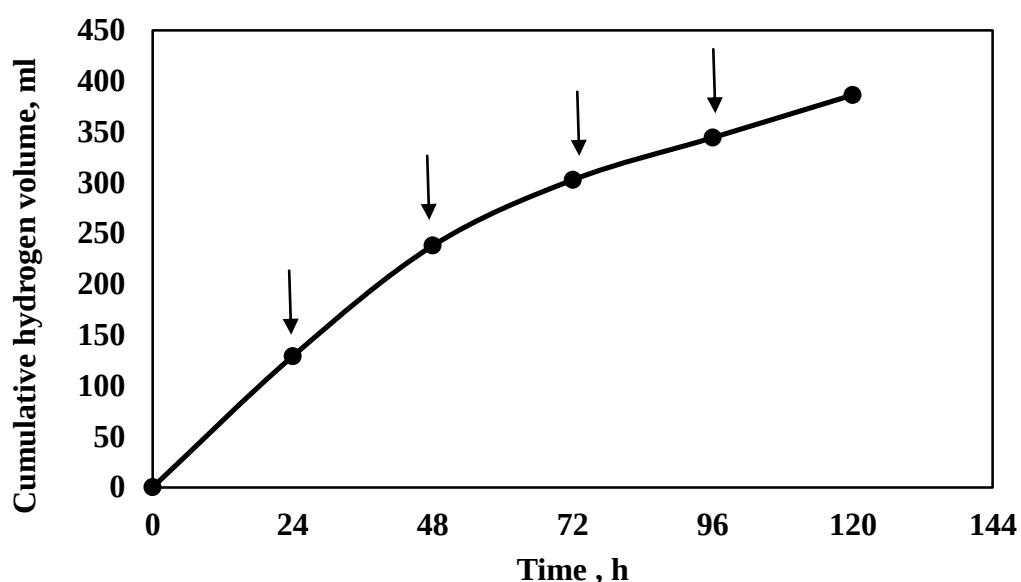


Figure 3.49 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad particle at 1 day cycle time repeated batch operation.

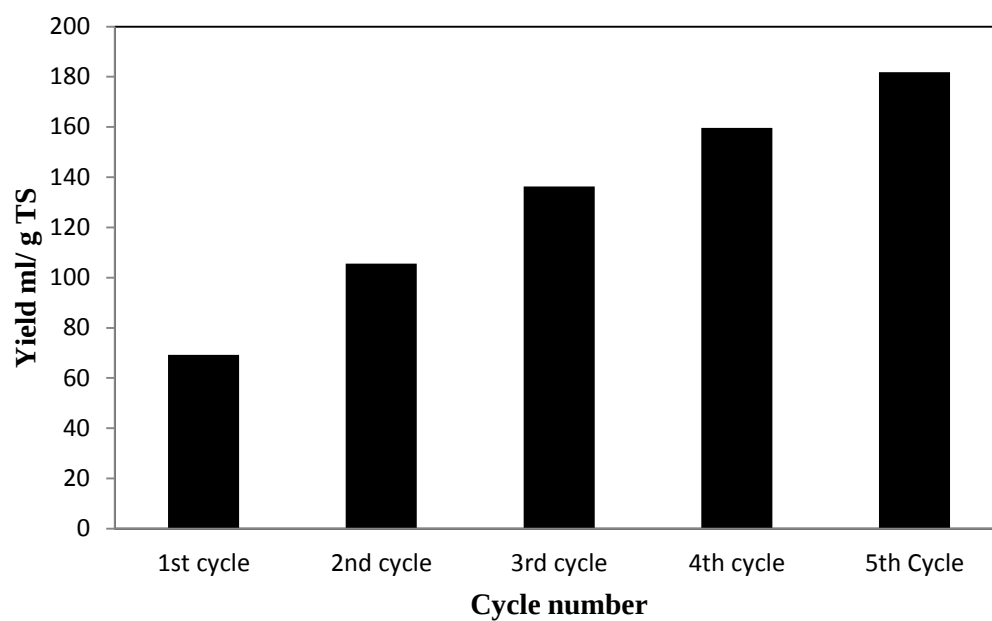


Figure 3.50 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic scouring sponge pad particle at 1 day cycle time repeated batch operation.

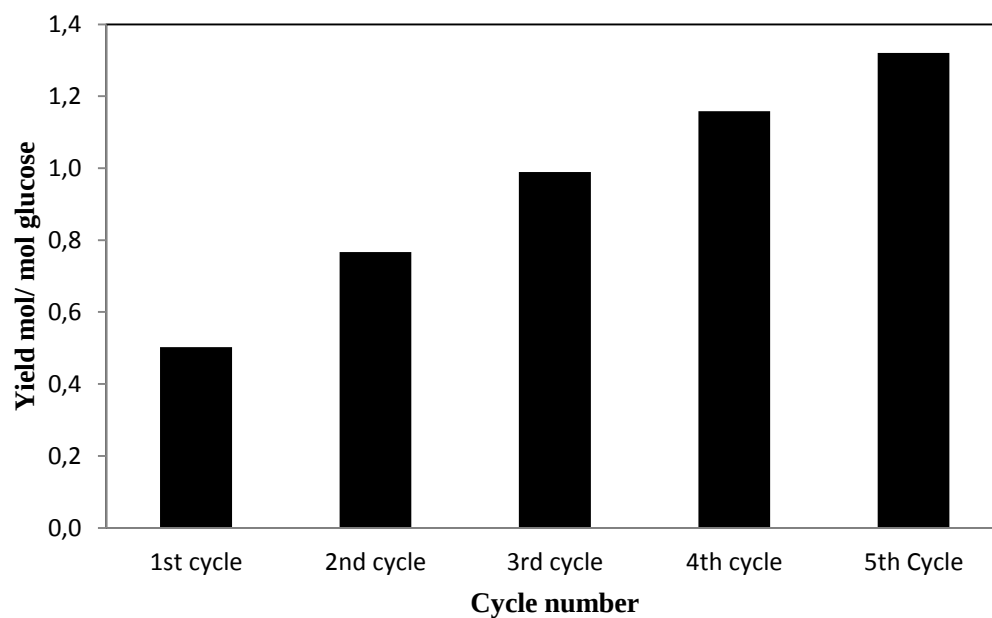


Figure 3.51 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic scouring sponge pad particle at 1 day cycle time repeated batch operation.

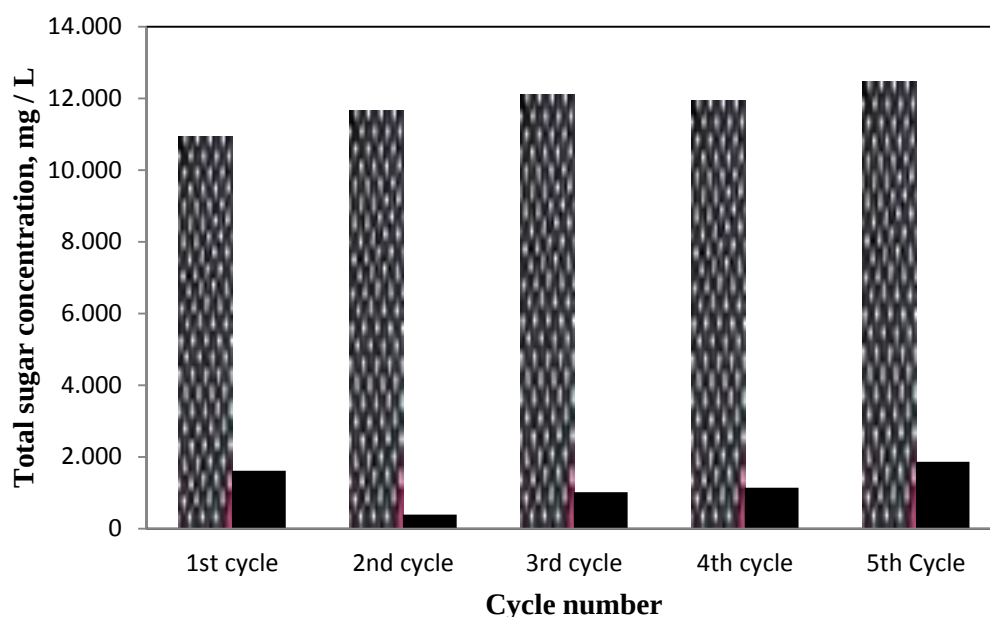


Figure 3.52 Initial and final TS concentrations for different cycles of plastic scouring sponge pad particle at 1 day cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.3.2 Plastic Scouring Sponge Pad with Metal Mesh Particle

The cumulative hydrogen production was 91 ml with this particle for the first cycle (Figure 3.53). Percentage of hydrogen in total produced gas was around 38%. Second and third cycle cumulative hydrogen productions were 137 ml and 158 ml, respectively. At the end of fifth cycle CHV was 184 ml. Variation of hydrogen production yields as ml H₂/g TS and mol/mol glucose were given in Figure 3.54 and Figure 3.55, respectively. Maximum hydrogen production yield was 107.51 ml H₂/g TS (0.78 mol H₂/mol glucose). Total sugar concentrations was shown Figure 3.55 the highest sugar consumption was obtained as 86% at the fifth cycle.

First of all in this experiments hydrogen production volume was considerably high. Hydrogen yield was low in the first three cycles of organisms have shown they could not yet be immobilized on the support material. However, sugar consumption has increased in the last two cycles.

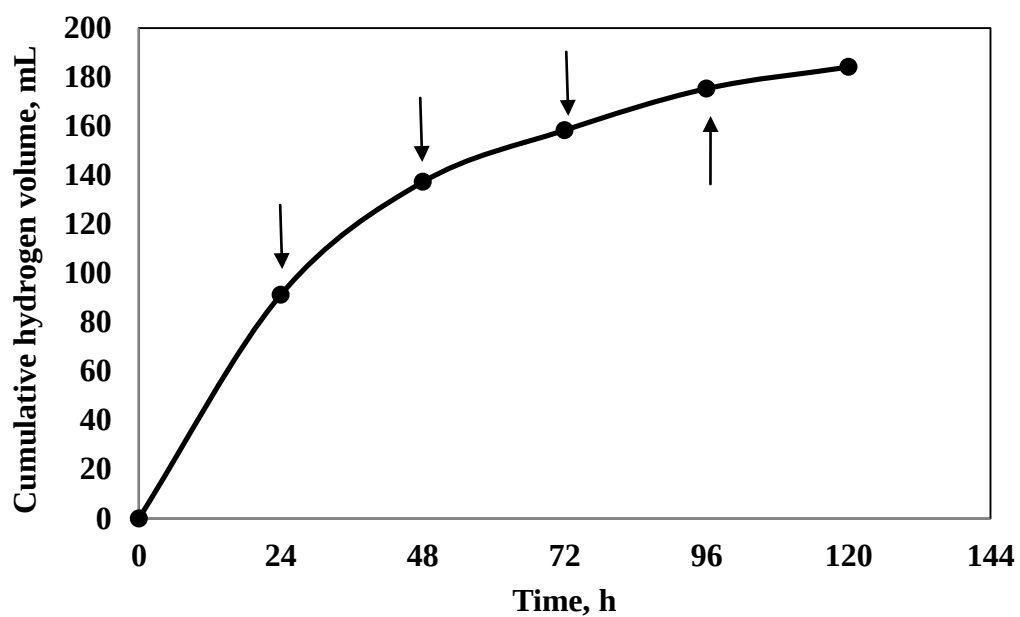


Figure 3.53 Variation of cumulative hydrogen gas volume with time for plastic scouring sponge pad with metal mesh particle at 1 day cycle time repeated batch operation

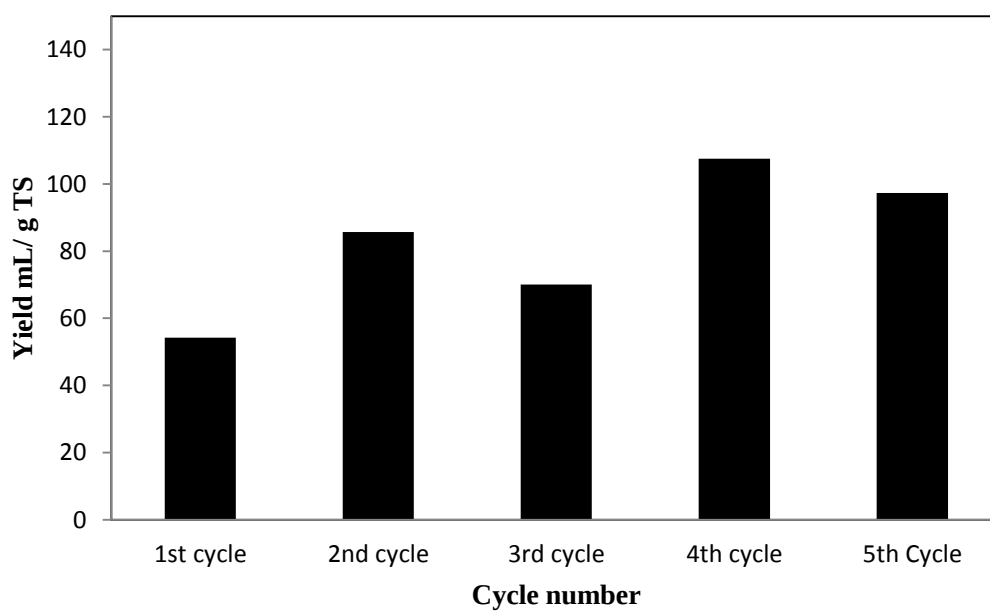


Figure 3.54 Variation of hydrogen production yield ml H_2 / g TS with cycles for plastic scouring sponge pad with metal mesh particle at 1 day cycle time repeated batch operation.

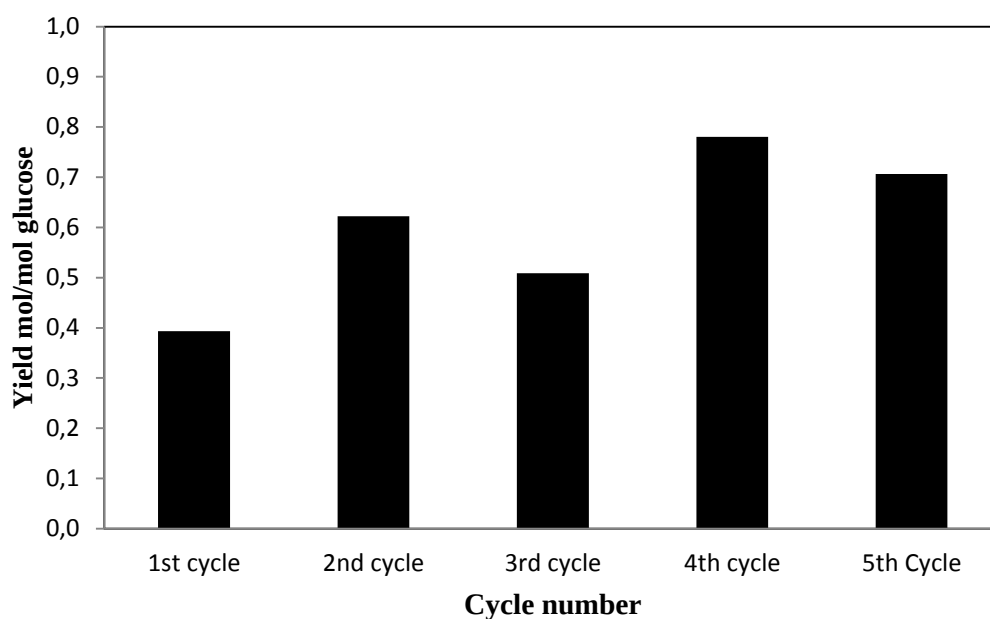


Figure 3.55 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic scouring sponge pad with metal mesh particle at 1 day cycle time repeated batch operation.

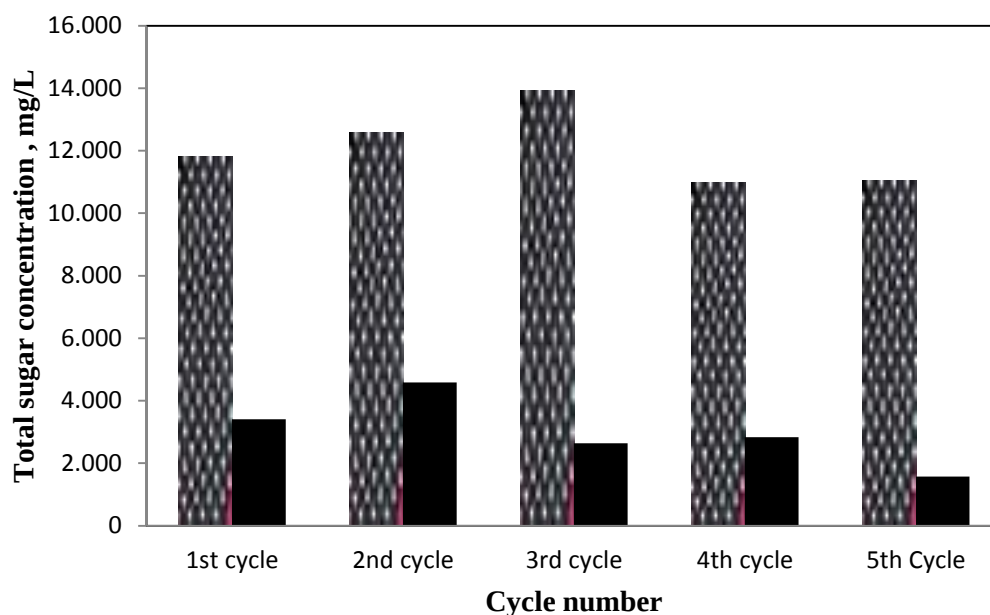


Figure 3.56 Initial and final TS concentrations for different cycles of plastic scouring sponge pad with metal mesh particle at 1 day cycle time repeated batch operation (— ■ —, Final TS concentration, — ■ —, Initial TS concentration).

3.1.3.3 Plastic Nylon Sponge

Cumulative hydrogen gas volume for plastic nylon sponge particle was shown in Figure 3.57 the volume of hydrogen produced at the end of first cycle was 98 ml. Hydrogen percentage in total gas was 35%. The total cumulative hydrogen produced at the end of operation was 245 ml. Figure 3.58 and figure 3.59 show variation of hydrogen production yield ml H₂/ g TS and mol H₂/ mol glucose with cycles for plastic scouring sponge pad at 1 day cycle repeated batch operation. Yields of formation for first and second cycle were 47.82 ml H₂/ g TS, 68.33 ml H₂/ g TS, respectively. In the fifth cycle, hydrogen yield increased significantly to 140 ml H₂/ g TS (1.13 mol H₂/ mol glucose). The total sugar consumption was around 91% at the second cycle (Figure 3.60).

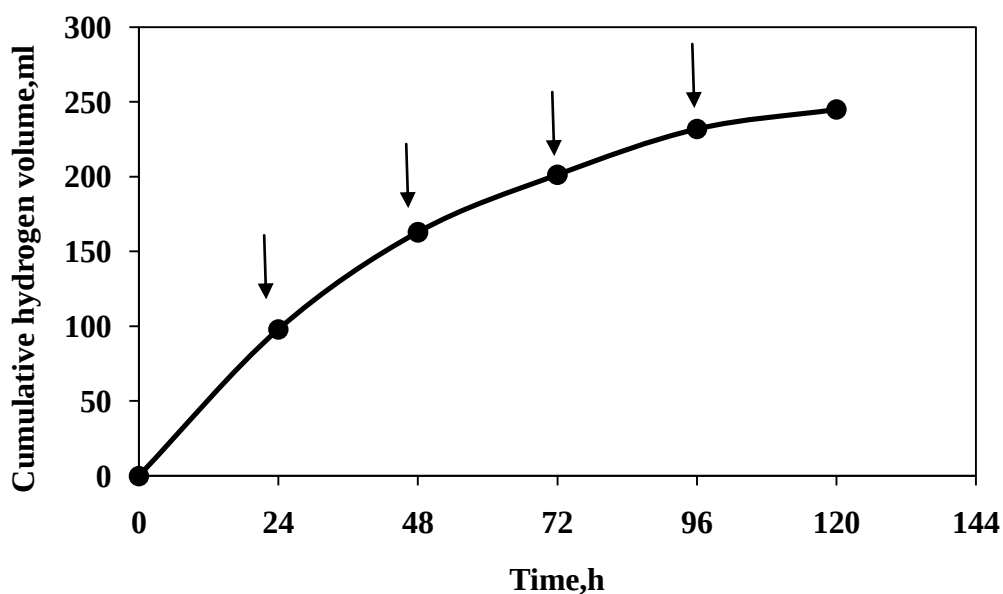


Figure 3.57 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge particle at 1 day cycle time repeated batch operation.

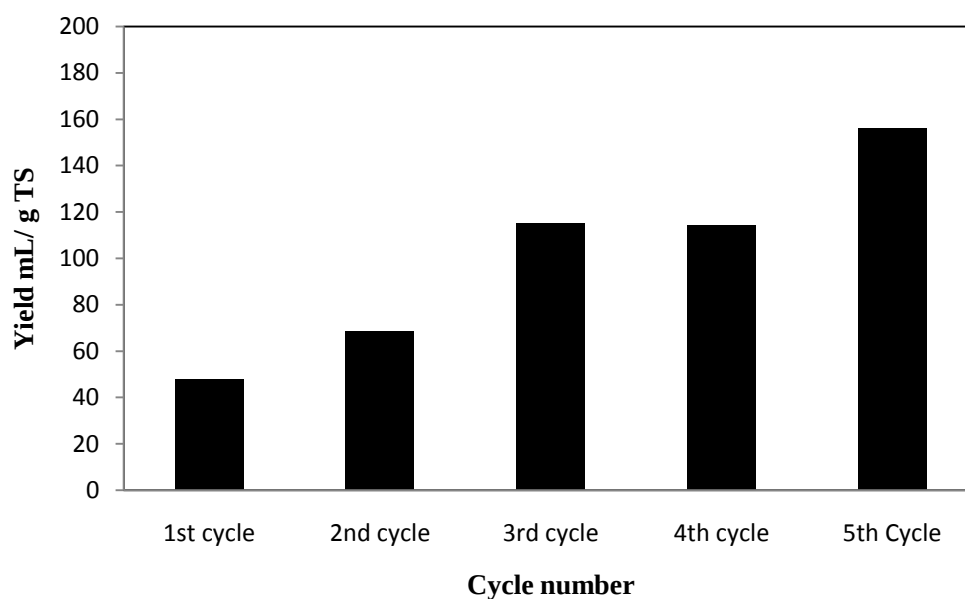


Figure 3.58 Variation of hydrogen production yield ml H₂/ g TS with cycles for plastic nylon sponge particle at 1 day cycle time repeated batch operation.

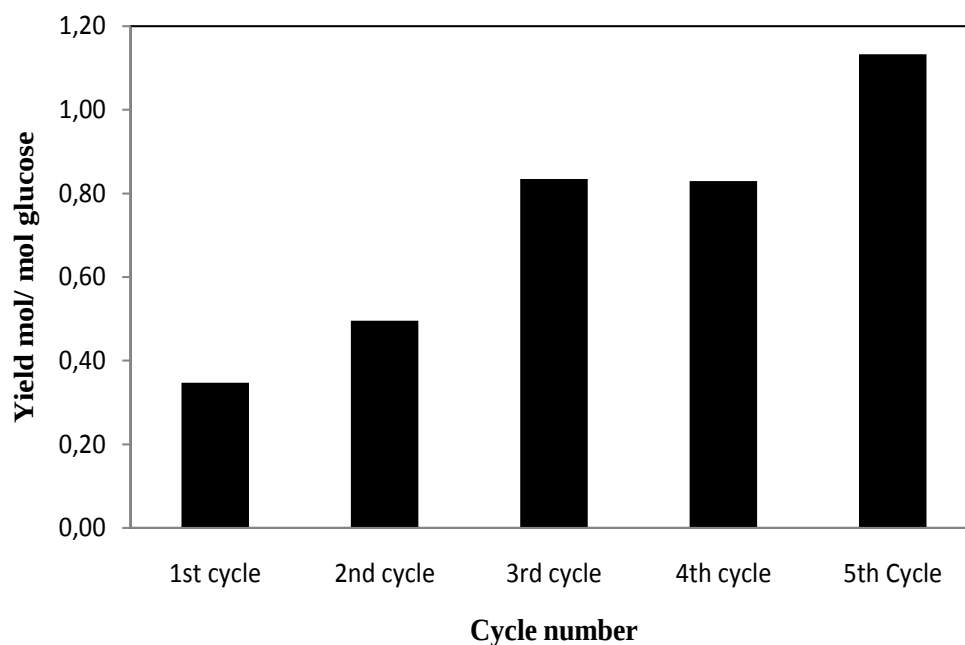


Figure 3.59 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for plastic nylon sponge particle at 1 day cycle time repeated batch operation.

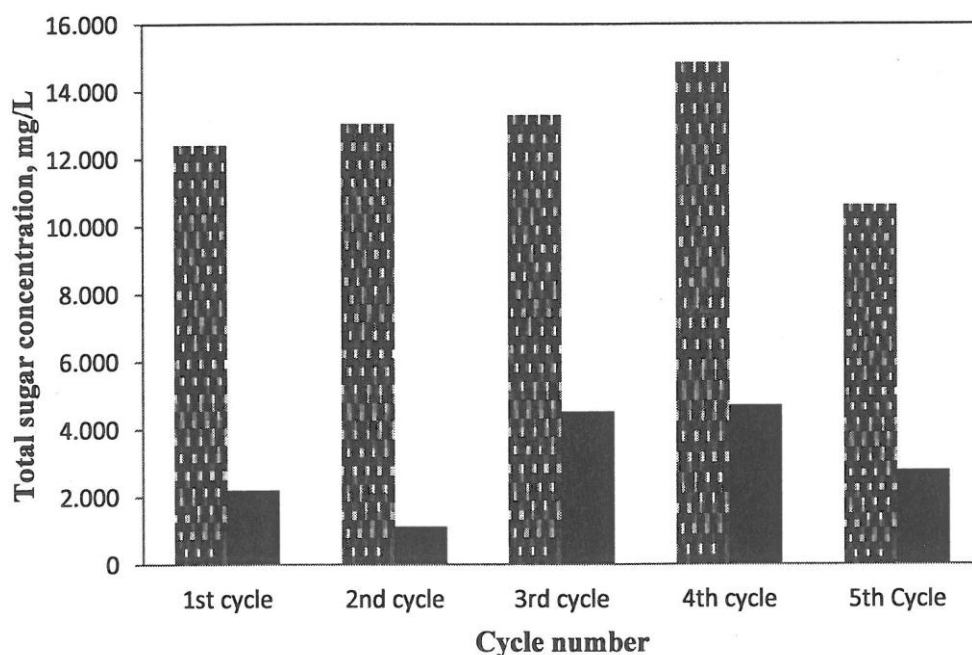


Figure 3.60 Initial and final TS concentrations for different cycles of plastic nylon sponge particle at 1 day cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.3.4 Plastic Nylon Sponge with Metal Mesh

This support particle resulted in the highest hydrogen productions at five days and three days cycles. Similar to previous results the highest production in 1 day cycle was obtained with this particle. Cumulative hydrogen volume was CHV = 170 ml in the first cycle and increased to CHV= 279 ml at the end of the second cycle (Figure 3.61). A steady increase was observed as the system was loaded with fresh media. The final volume reached to CHV = 456. 5 ml. Hydrogen production yield as ml H₂/ g TS is shown in Figure 3.62 The yield of production was 120. 16 ml H₂/ g TS for the first load and the maximum yield was calculated as 285 ml H₂/g TS (2.07 mol H₂/ mol glucose) at the end of 5th cycle. Initial and final TS were shown that Figure 3.64. The highest sugar consumption of 96% was obtained at the end of second cycle.

The hydrogen yield was increased in every cycle successively. As it was stated before the theoretical yield was 4 mol/ mol glucose but in practice 2. 5 mol/mol is

acceptable due to utilization of substrate for growth and also production of non hydrogen generating organic acids or low hydrogen generating organic acids. The result obtained in this stage of study was close to the maximum yield that can be reached in practice. In addition this is the highest yield observed in repeated batch operation with different particles and at different cycle times. Therefore, it can be concluded that plastic nylon sponge with metal mesh is the best particle type among the others and 1 day cycle repeated batch is the best approach to get high hydrogen volumes and yields.

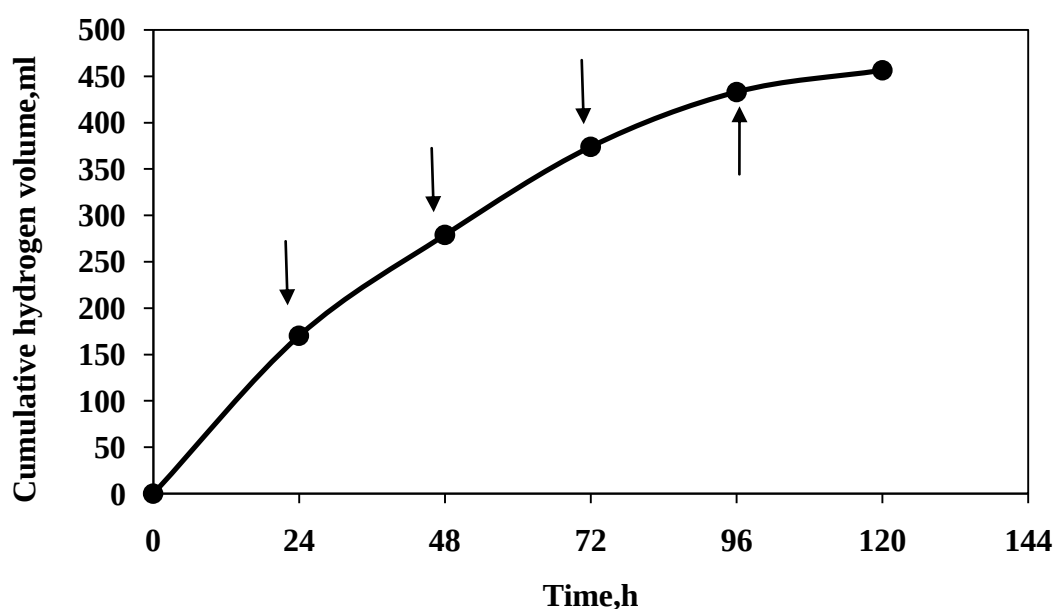


Figure 3.61 Variation of cumulative hydrogen gas volume with time for plastic nylon sponge with metal mesh particle at 1 day cycle time repeated batch operation.

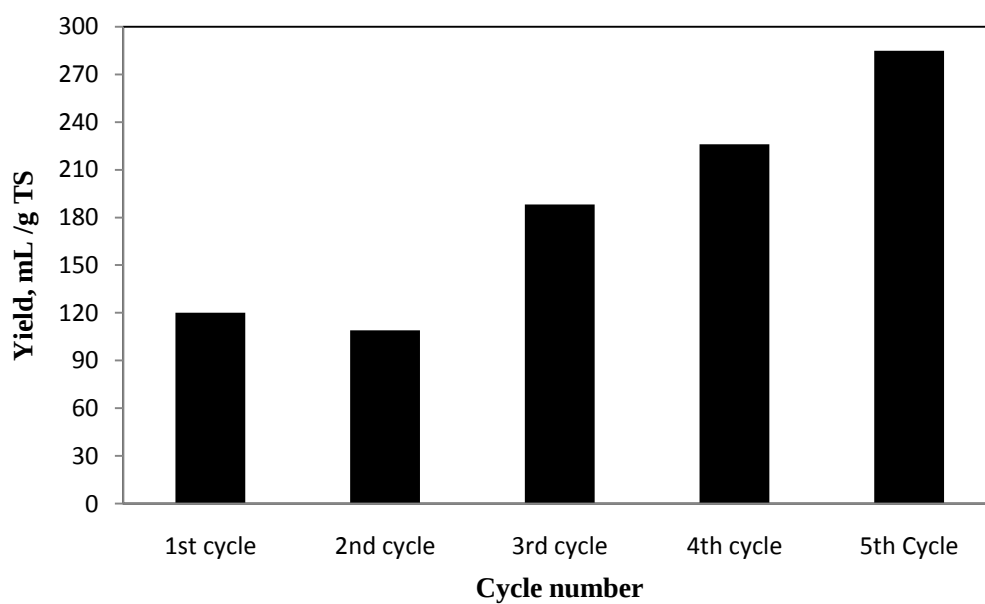


Figure 3.62 Variation of hydrogen production yield ml H_2 / g TS with cycles for plastic nylon sponge with metal mesh particle at 1 day cycle time repeated batch operation.

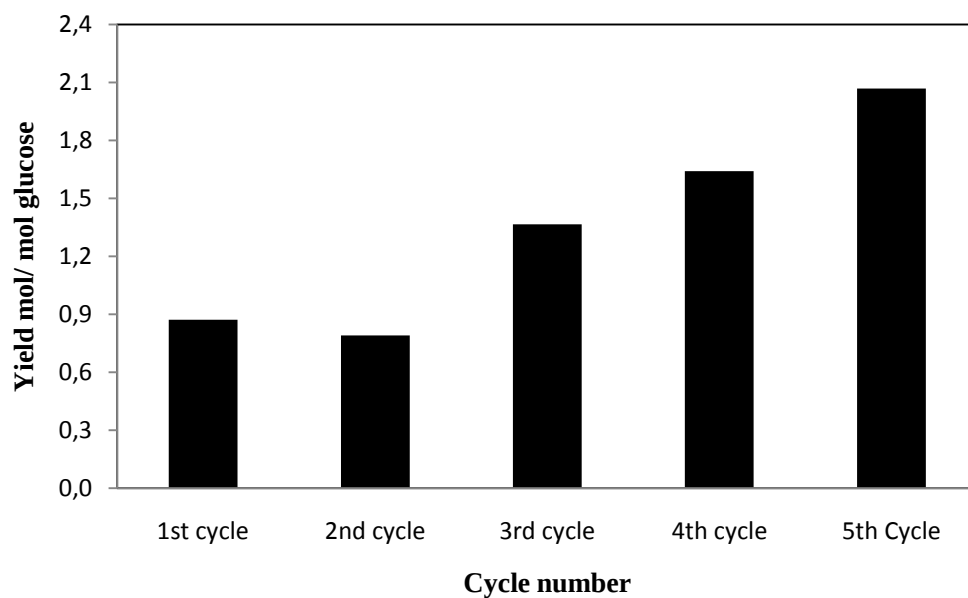


Figure 3.63 Variation of hydrogen production yield mol H_2 / mol glucose with cycles for plastic nylon sponge with metal mesh particle at 1 day cycle time repeated batch operation.

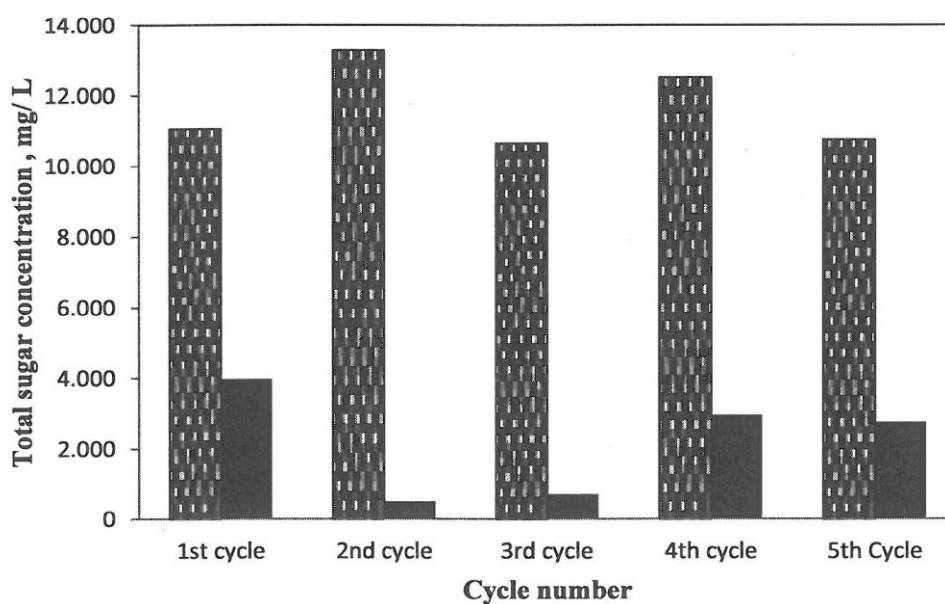


Figure 3.64 Initial and final TS concentrations for different cycles of plastic nylon sponge with metal mesh particle at 1 day cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.3.5 Black Porous Sponge

This particle type was not successful in hydrogen production for 3 days and 5 days cycle operations. Similar results were obtained for 1 day cycle. Cumulative hydrogen gas volume was only 99 ml at the end of operation (Figure 3.65). There was no substantial change in the gas volume after third cycle. System reached to steady state after this stage. The highest percentage of hydrogen was 30% and obtained in the third cycle. Yield of formation was also very low (Figure 3.66 and figure.3.67) Sugar consumption was relatively lower as well (Figure 3.68).

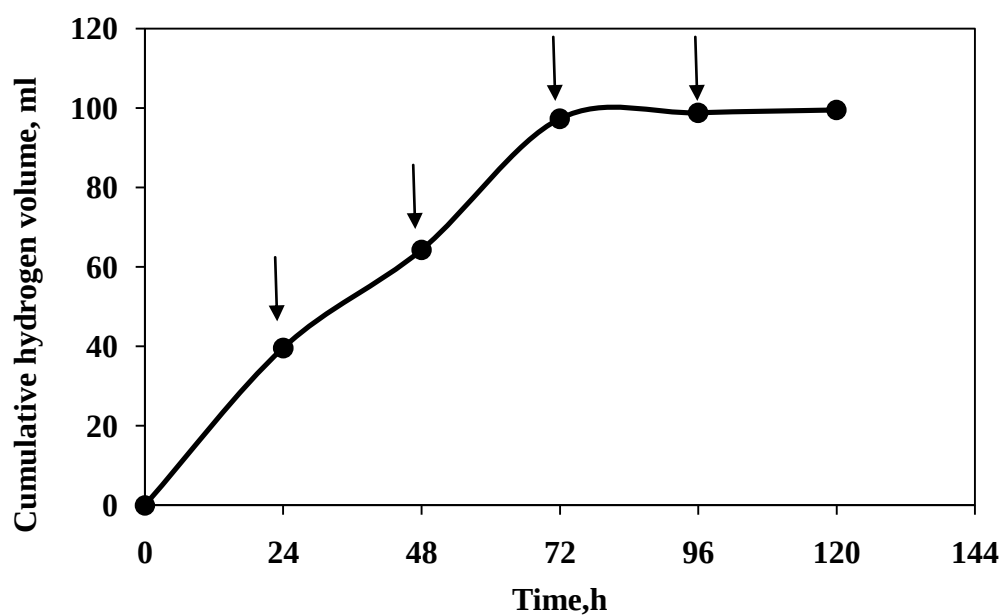


Figure 3.65 Variation of cumulative hydrogen gas volume with time for black prous sponge particle at 1 day cycle time repeated batch operation.

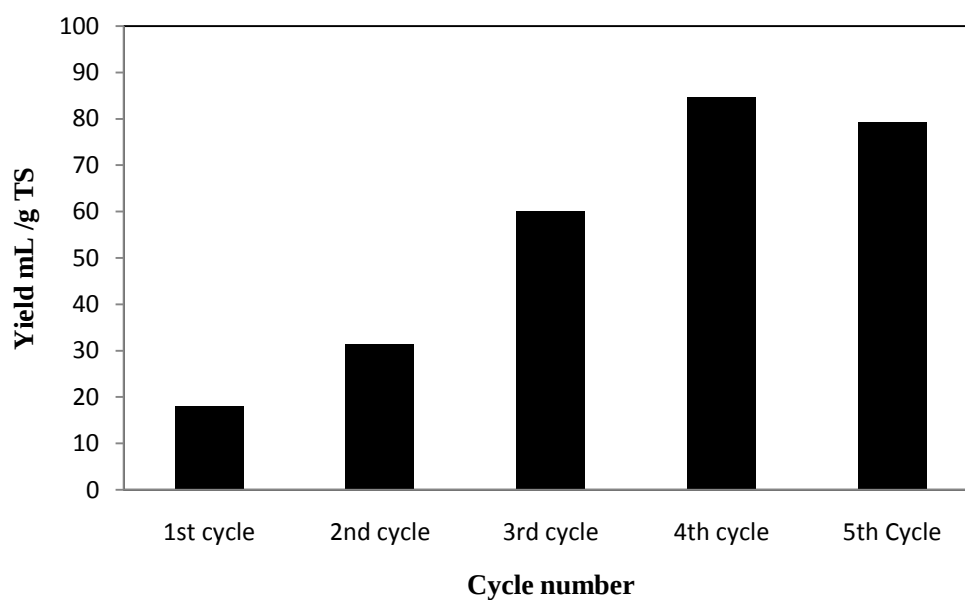


Figure 3.66 Variation of hydrogen production yield ml H₂/ g TS with cycles for black prous sponge particle at 1 day cycle time repeated batch operation.

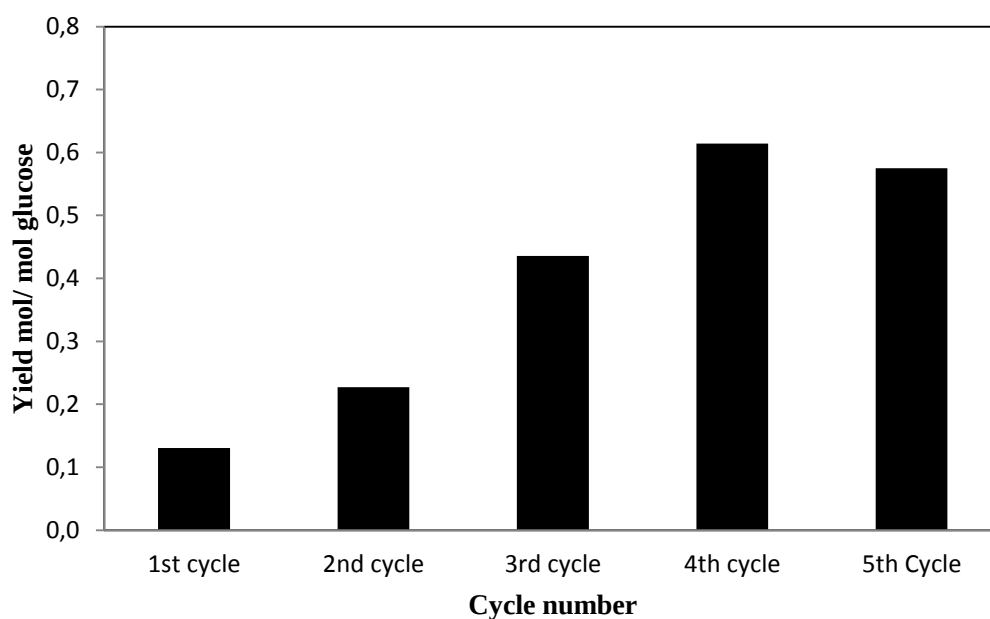


Figure 3.67 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for black prous sponge particle at 1 day cycle time repeated batch operation.

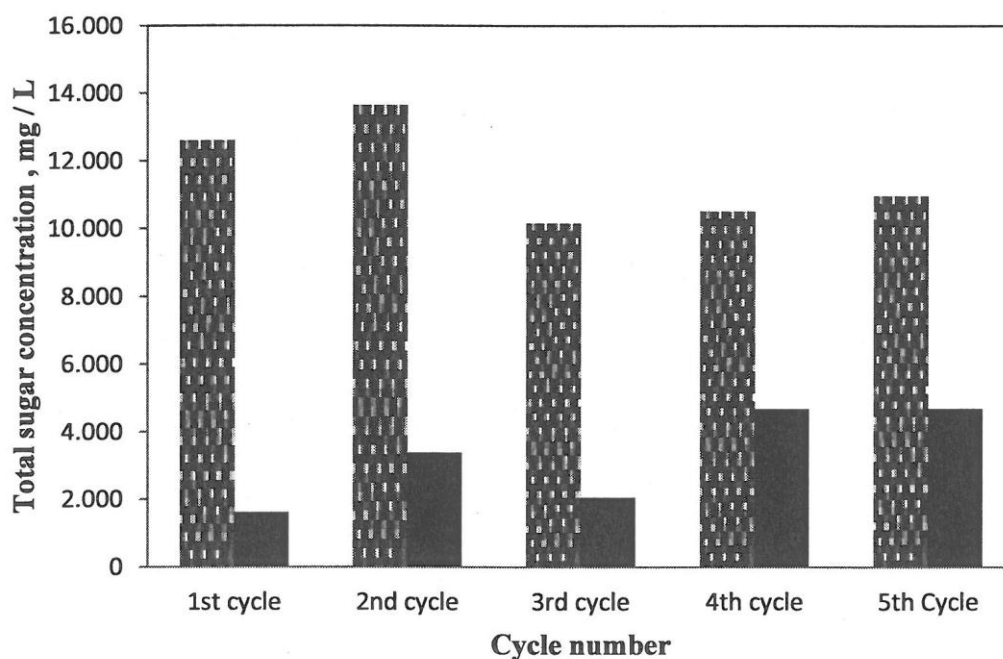


Figure3.68 Initial and final TS concentrations for different cycles of black prous sponge particle at 1 day cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.1.3.6 Control Experiments without Support Material

This experiment without the support material gave the almost the same results obtained in 5 days and 3 days cycles. The cumulative volume of hydrogen is shown in the figure 3.69. Total gas volume produced for five days 650 ml. The total hydrogen gas produced at the end of operation was around 150 ml. About 120 ml of this hydrogen gas production was achieved at the end of 1 st cycle and there was no substantial change afterwards (Figure 3.69). The maximum yield obtained through out the operation was 120 ml H₂/g TS (Figure 3.70) or 1 mol/ mol glucose (Figure 3.71)

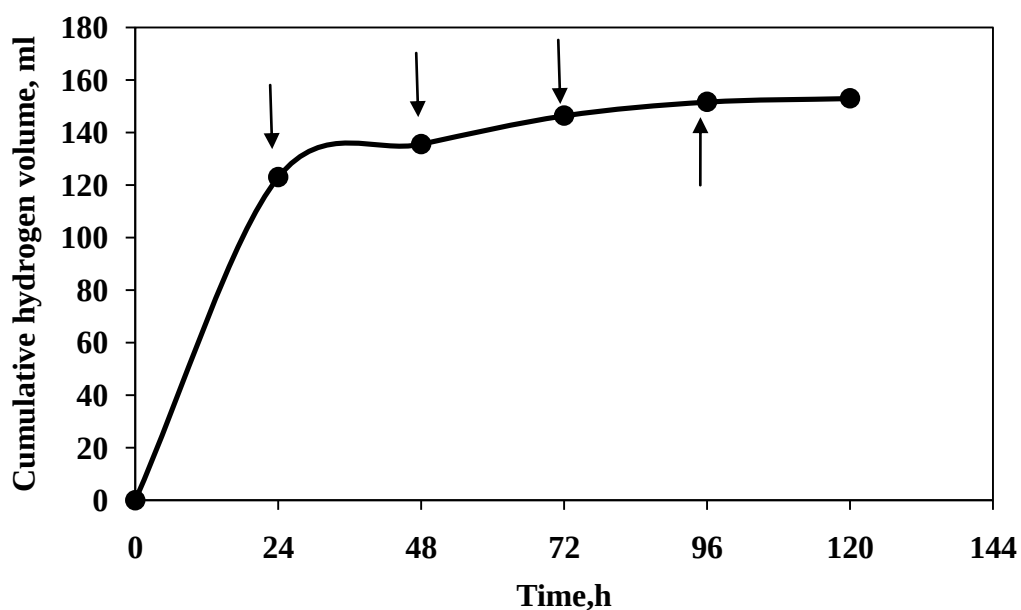


Figure 3.69 Variation of cumulative hydrogen gas volume with time for control experiments without support material at 1 day cycle time repeated batch operation

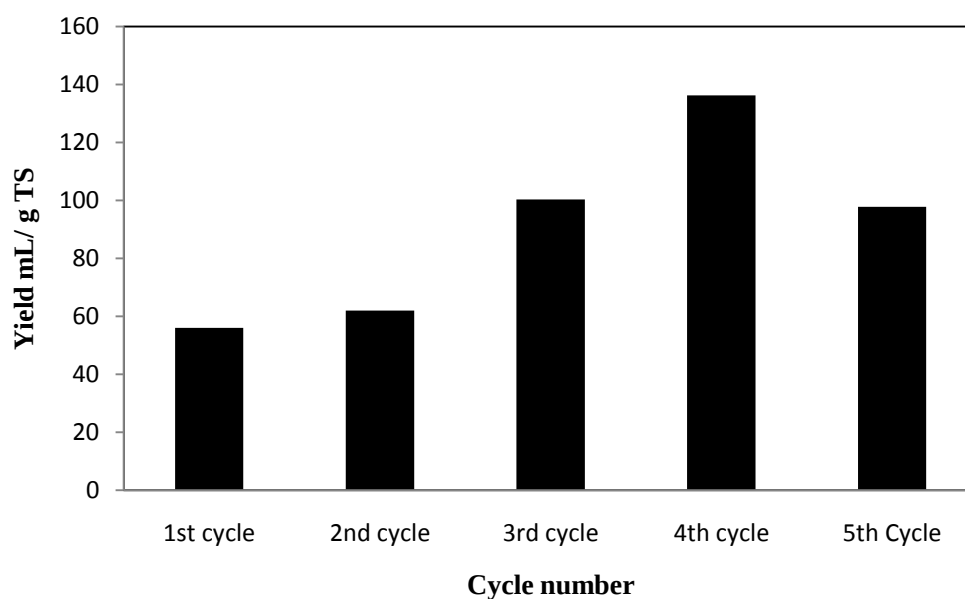


Figure 3.70 Variation of hydrogen production yield ml H₂/ g TS with cycles for control experiments without support material at 1 day cycle time repeated batch operation.

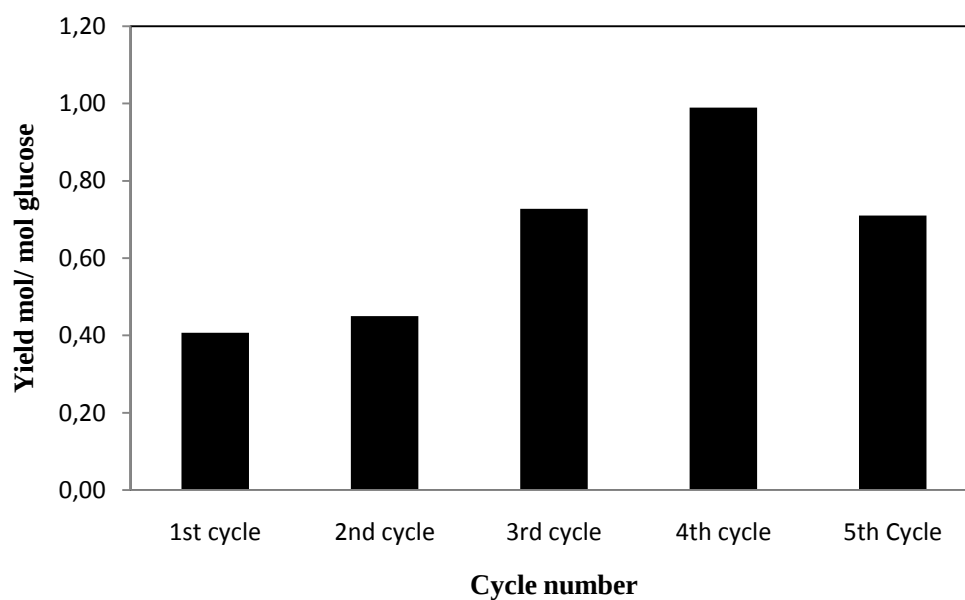


Figure 3.71 Variation of hydrogen production yield mol H₂/ mol glucose with cycles for control experiments without support material at 1 day cycle time repeated batch operation.

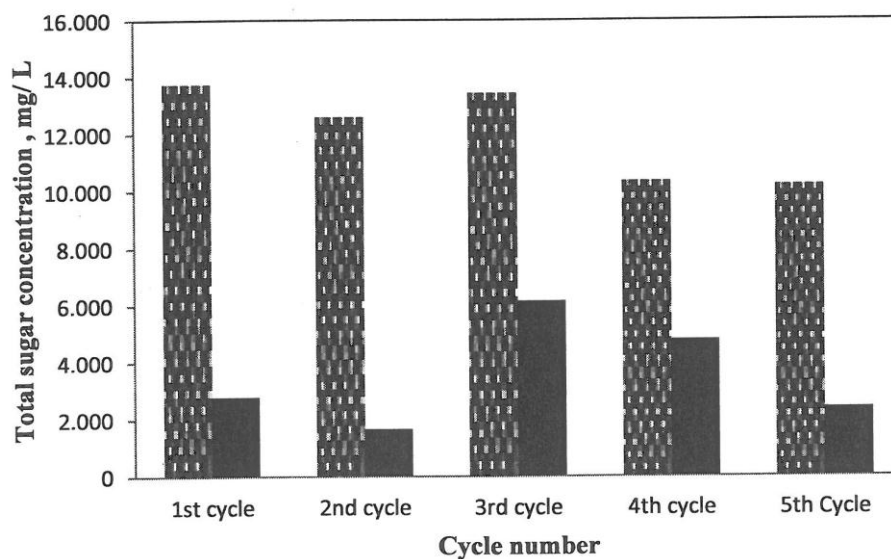


Figure 3.72 Initial and final TS concentrations for different cycles of control experiments without support material at 1 day cycle time repeated batch operation (— ▨ —; Initial TS concentration, — ■ —, Final TS concentration).

3.2 Hydrogen Gas Production in a Continuously Operated Upflow Anaerobic Packed Bed Reactor

The up-flow packed bed reactor was continuously operated at HRT=2 h, 4h, 6h, 10h and 13 h. The microorganism support particle was plastic nylon sponge covered with metal mesh. Total particle number in the reactor was 85. Initial total sugar concentration was $TS_0 = 5000$ mg/L. Reactor was operated continuously until daily hydrogen production volume was almost constant.

The system performance was evaluated in terms of total sugar consumption, volumetric hydrogen production rate, daily hydrogen production volume and daily hydrogen production yields at each hydraulic retention times.

3.2.1 Transition Behavior of the Hydrogen Production at Different HRTs

3.2.1.1 Hydrogen Production at HRT=13 h

Packed bed reactor was first operated at HRT= 13 h. Figure 3.73 depicts variation of hydrogen volume with time at HRT=13 h. The gas volume in the initial phase of operation was around 1200 ml. The reactor was at dynamic state up to $t=96$ hours of operation. Increases and decreases in the volume were observed. But it remained almost constant around 1600 ml hydrogen volume for the operation period between $t=120$ h and $t=192$ h. At least three results were almost equal to each other which indicates that the system reached to steady state with regard to hydrogen production. Hydrogen percentage in the total gas was around 35% at repeated batch operation. The percentage of hydrogen was obtained around 58 % in this set of operation.

Volumetric hydrogen production (VHP) is a comparable value with the other results presented in the literature than hydrogen volume. Because, large reactor volume of a reactor will produce higher volume of hydrogen than smaller volume of the same reactor. Therefore, volumetric production rate was also presented in the thesis. The reactor volume in this study was 2.100 L. By dividing the daily hydrogen production volumes, given in Figure 3.73 to reactor volume $V=2.100$ L, volumetric hydrogen productions at HRT= 13 were calculated and results were depicted in Figure 3.74. The maximum VHP was $0.75 \text{ L H}_2/\text{L/day}$ which is a low production rate compared to the reports. Yield of production and substrate removal efficiency were around $0.6 \text{ mol/mol glucose}$ (Figure 3.75) and 92% (Figure 3.76), respectively.

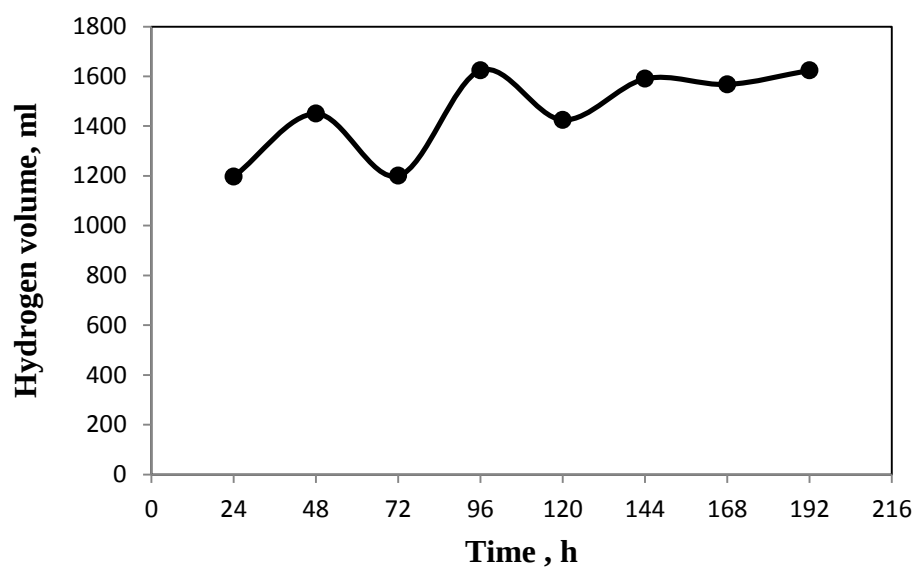


Figure 3.73 Variation of hydrogen volume with time at HRT=13 h

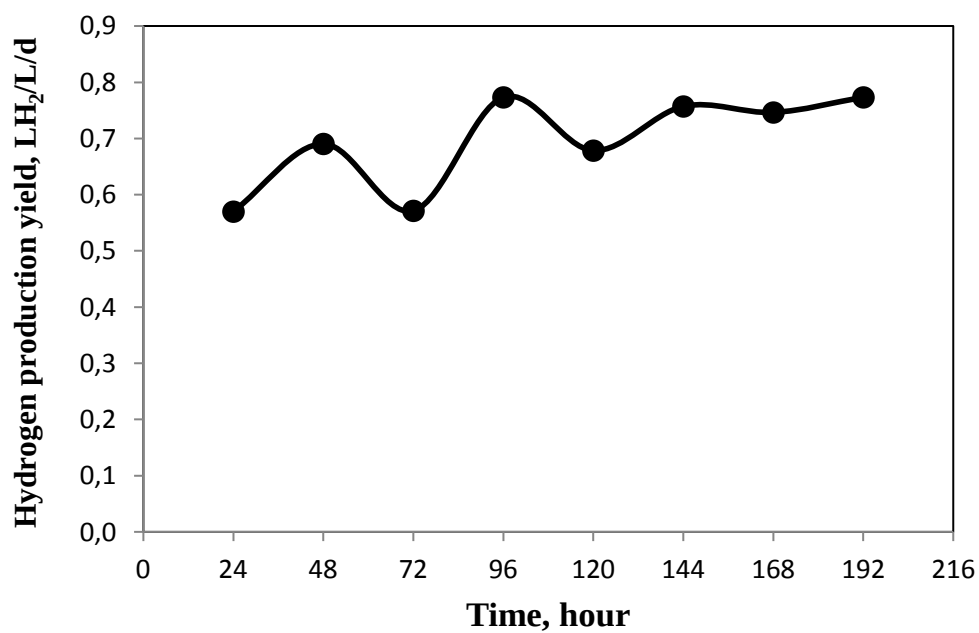


Figure 3.74 Variation of volumetric hydrogen production (L H₂/L/day) with time at HRT =13 h.

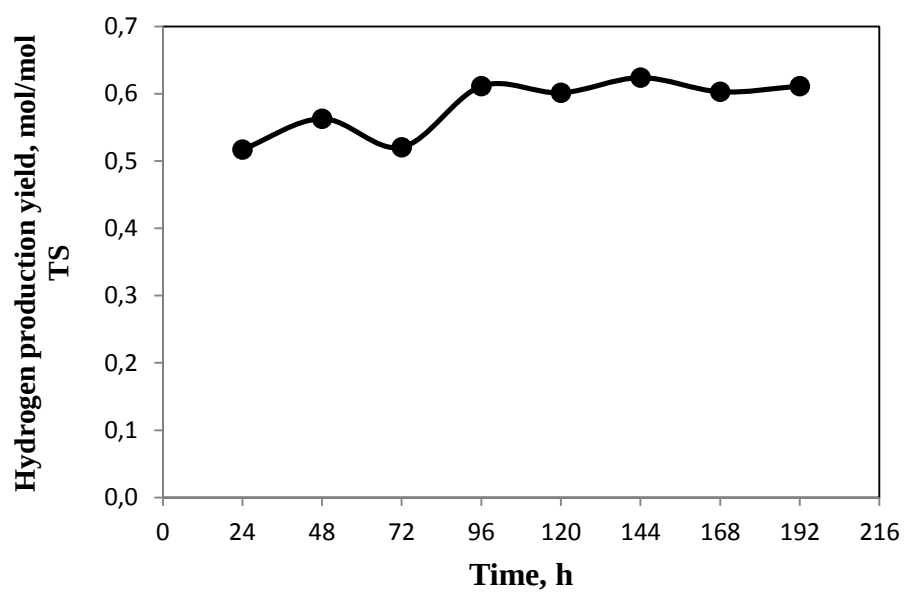


Figure 3.75 Variation of hydrogen production yield (mol/ mol TS) with time at HRT =13 h.

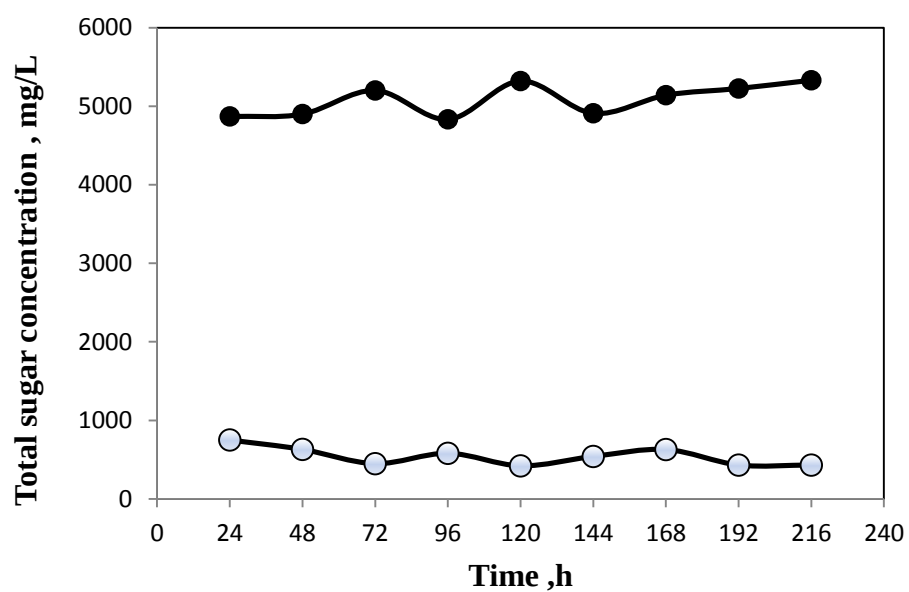


Figure 3.76 Variation of influent and effluent total sugar concentrations with time at HRT=13 h.
(—●—; influent, —○—; effluent)

3.2.1.2 Hydrogen Production at HRT=10 h

Hydrogen production in the first seventy-two hours was around $V_{H_2}=1325$ ml. Volume steadily increased to 2700 ml up to 96 hours of operation and remained almost at this value for the rest of operation. It can be concluded that the system reaches to the steady state in terms of hydrogen production after 4th day. A substantial increase in volume of production was observed with the decrease in HRT from 13 h to 10 h. Percentage of hydrogen in total gas was %63. It was almost the same as hydrogen percentage at HRT=13h. Volumetric hydrogen production reached to VHP = 1300 L H_2 /L/day (Figure 3.78). The production was almost doubled compared to the one obtained at HRT=13 h. The yield of production substantially varied with time (Figure 3.79). Yield of production depends on the amount of hydrogen produced per amount of substrate consumed ($Y = \Delta H_2 / \Delta TS$). System reached to steady state after 4th day of operation. Sugar consumption was about to reach to steady state at the same period (Figure 3.80). However, a drastic increase in the effluent was observed. Depending on this increase, yield of production rised either. As a results, the production yield was around $Y_{H_2}=0.9$ mol/ mol for the first 5 days ($t=120$ hours) and rised to $Y_{H_2}=1.37$ mol/ mol TS at $t=144$ h.

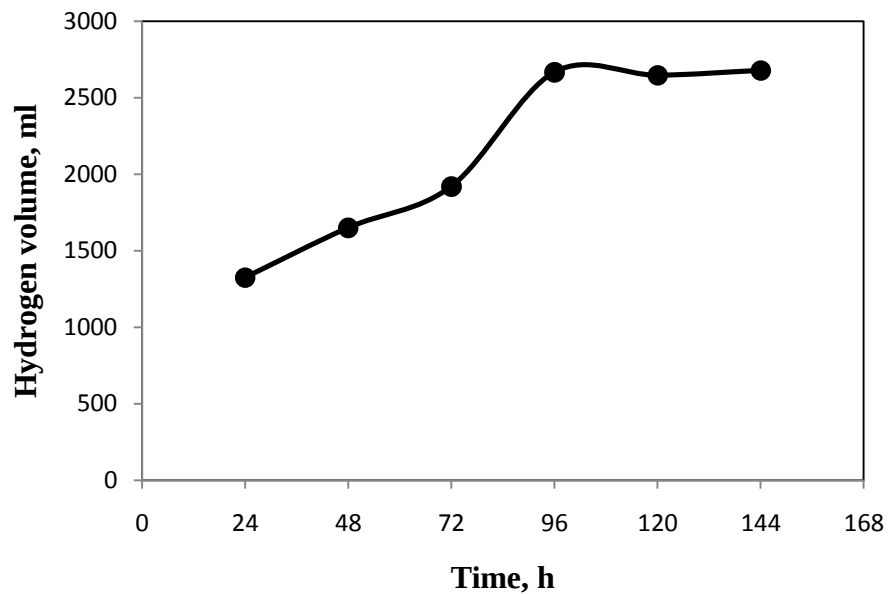


Figure 3.77 Variation of hydrogen volume with time at HRT=10 h

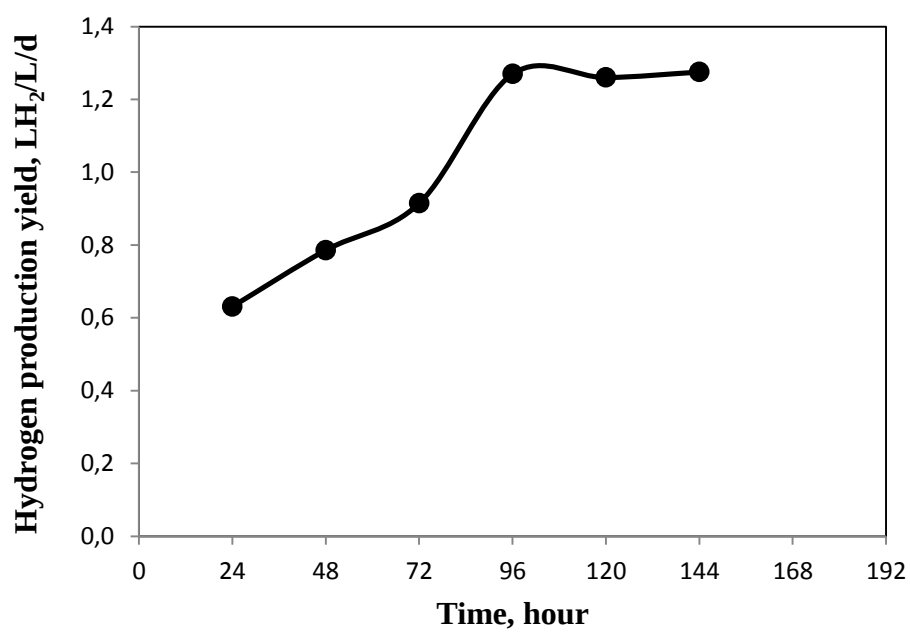


Figure 3.78 Variation of volumetric hydrogen production ($\text{L H}_2/\text{L/day}$) with time at HRT =10 h.

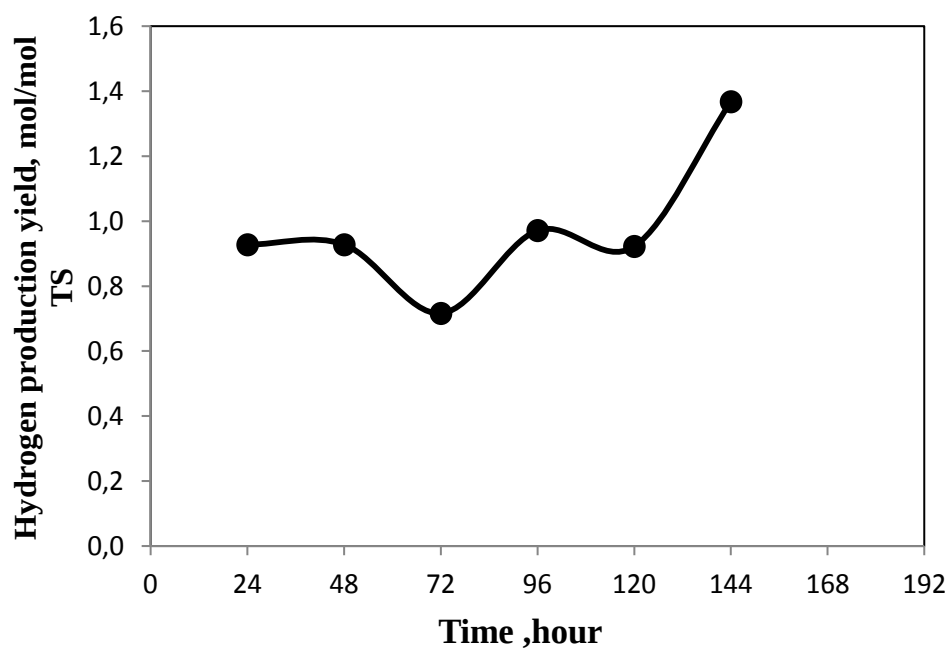


Figure 3.79 Variation of hydrogen production yield (mol/mol TS) with time at HRT =10 h.

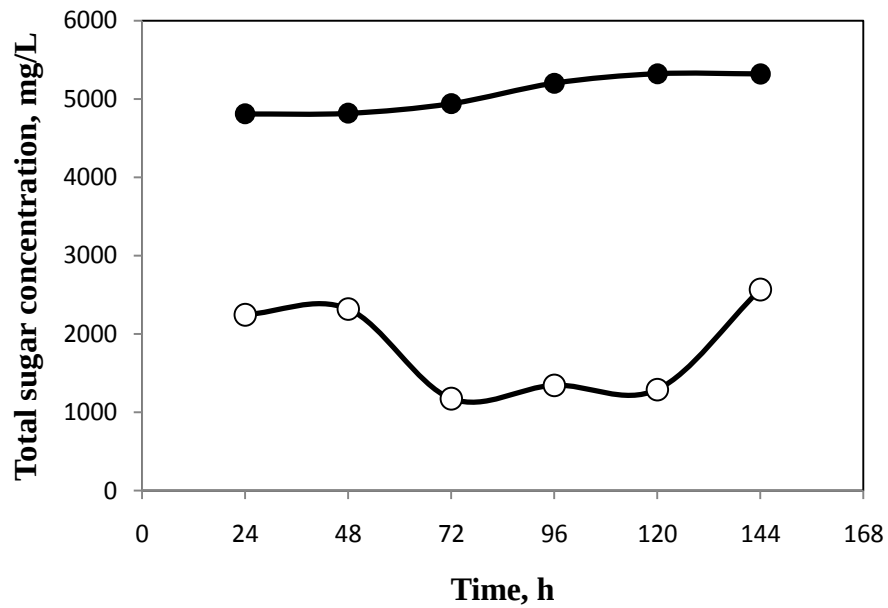


Figure 3.80 Variation of influent and effluent total sugar concentration with time at HRT=10 h.
(—●—; influent, —○—; effluent)

3.2.1.3 Hydrogen Production Volume with Time at HRT=6 h

Figure 3.81 depicts variation of daily hydrogen production at HRT=6 h. Volume of hydrogen was 1250 ml at the beginning of operation. It first rose to 2635 ml for the operation period between 48 h and 120 h. A second rise to 3100 ml was observed after that and no substantial change in the volume was observed for further operation. The percentages of hydrogen in the produced gas were about 68. Volumetric hydrogen production was $VHP = 1255 \text{ L H}_2/\text{L/day}$ (Figure 3.82) at steady state condition. Yield of production did not change substantially with relative to HRT= 10 h. the maximum production yield observed at this HRT was $Y_{H_2} = 1.47 \text{ mol H}_2/\text{mol TS}$ (Figure 3.83). Effluent sugar concentration was 2000 mg/L in during pre-steady state condition but rose to around 3000 mg/L resulting in 33% sugar consumption efficiency.

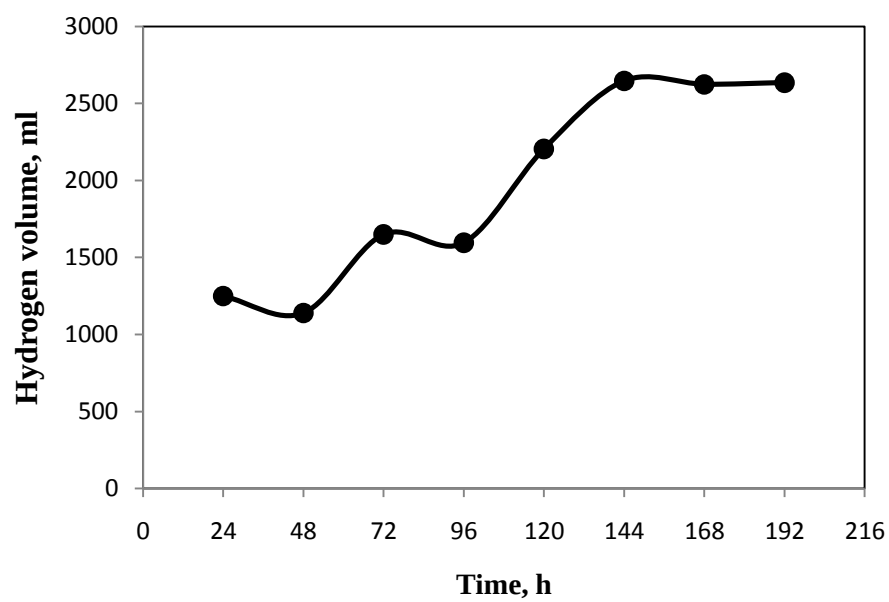


Figure 3.81 Variation of hydrogen volume with time at HRT=6 h

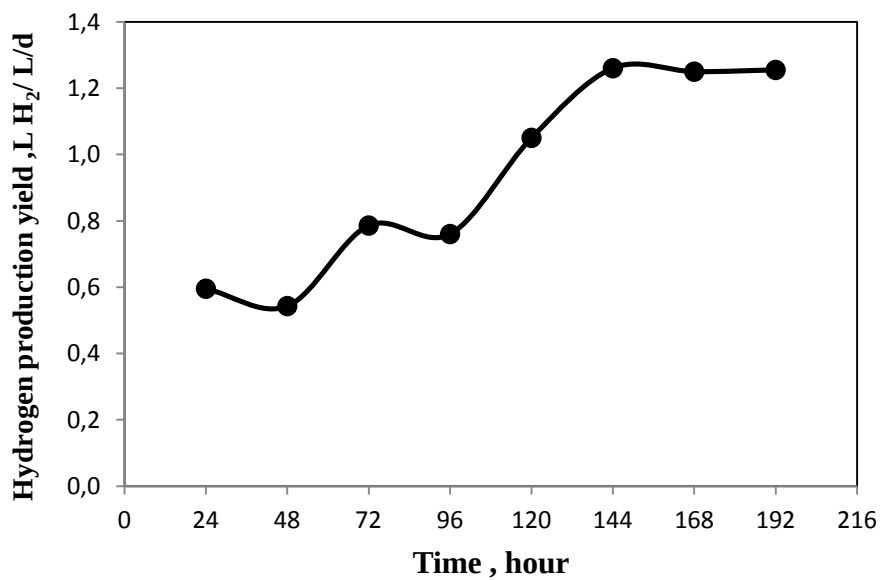


Figure 3.82 Variation of volumetric hydrogen production (L H₂/L/day) with time at HRT =6 h.

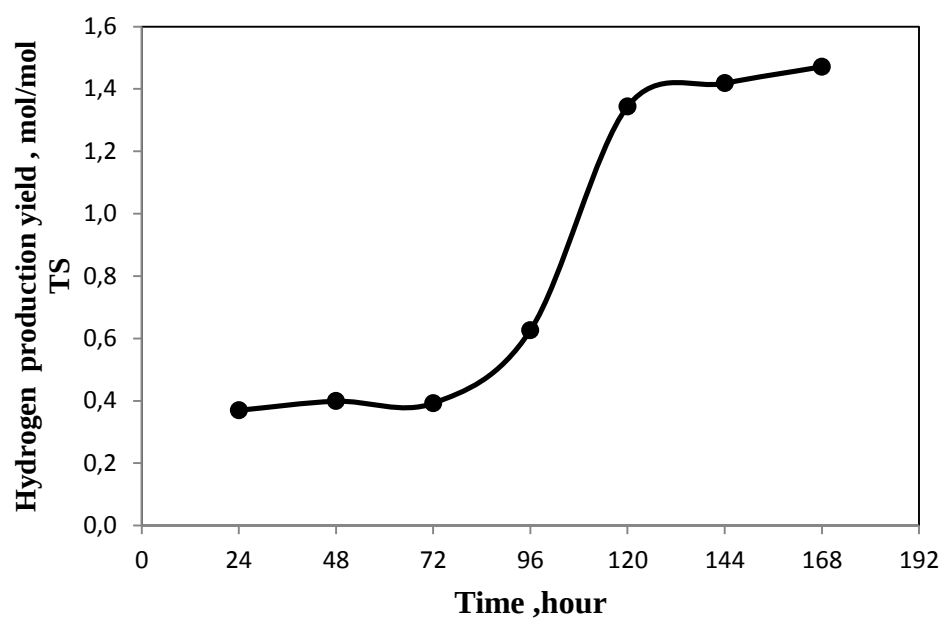


Figure 3.83 Variation of hydrogen production yield (mol/ mol TS) with time at HRT=6 h.

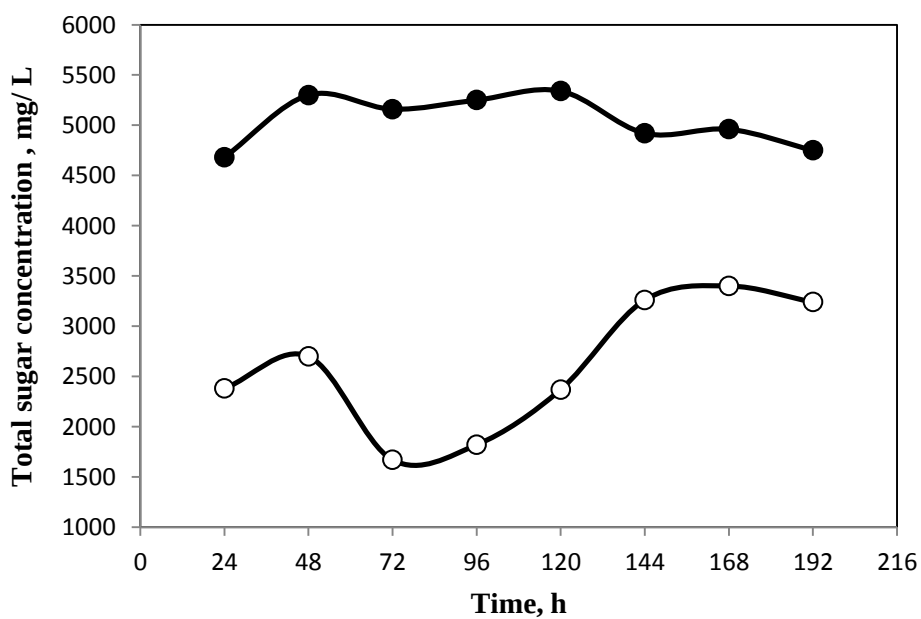


Figure 3.84 Variation of influent and effluent total sugar concentration with time at HRT=6 h.
(—●—; influent, —○—; effluent)

3.2.1.4 Hydrogen Production at HRT=4 h

Figure 3.85 shows variation of hydrogen production volume with time at HRT=4 h. The volume of hydrogen produced was monitored daily. Hydrogen gas volume produced after the first 24 hours of continues operation was around $V_{H_2}=1200$ ml. The gas production increased to $V_{H_2} = 3082$ ml after 8 days of operation and then daily hydrogen production volume remained almost constant indicating that system reached to steady state condition. Volumetric hydrogen production was $VHP= 1.4$ L H_2 / L/ d (Figure 3.86). Yield of production was around $Y_{H_2}=0.5$ mol H_2 / mol glucose for the first 4 days of operation (Figure 3.86). Then an increasing trend in the yield was observed and it reached to $Y_{H_2}=1.5$ mol H_2 /mol glucose for the time period between $t=5$ days and $t=8$ days which corresponds to steady state condition with regard to hydrogen volume. Similarly, effluent total sugar concentration remained almost constant around 2300 ± 200 mg/L for the first 5 days with average 45% removal efficiency (Figure 3.88). However, effluent sugar concentration increases to 3600 mg/L at the steady state conditions. The resulting substrate removal efficiency was 24%.

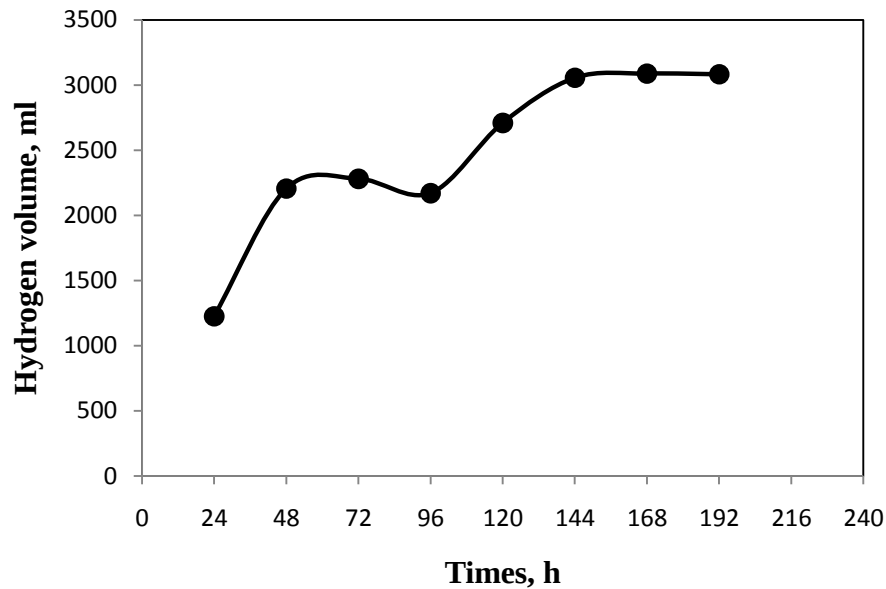


Figure 3.85 Variation of hydrogen volume with time at HRT=4 h.

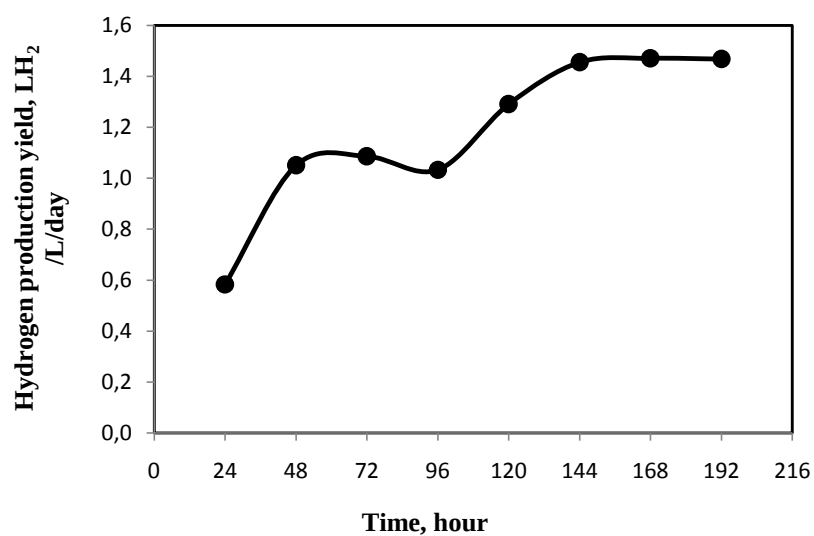


Figure 3.86 Variation of volumetric hydrogen production (LH_2 /L/day) with time at HRT =4 h.

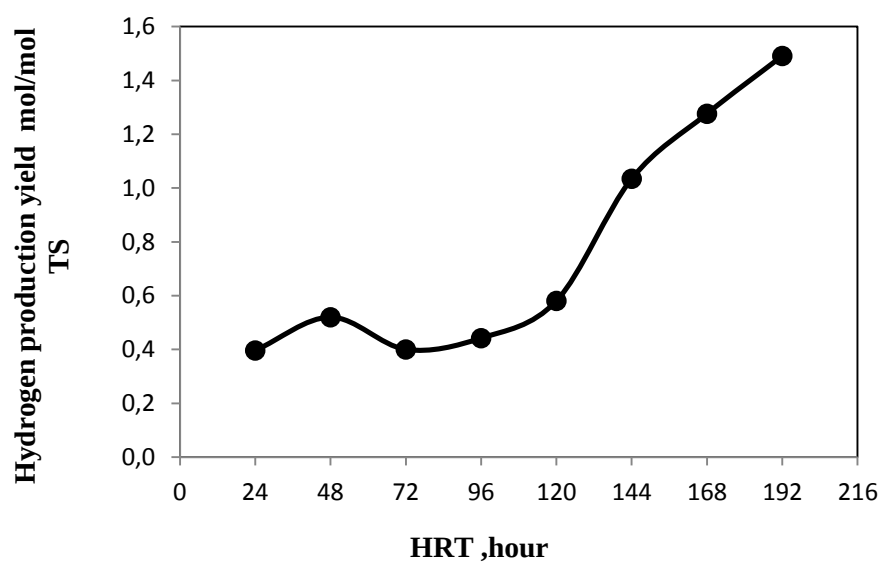


Figure 3.87 Variation of hydrogen production yield (mol/ mol TS) with time at HRT =4 h.

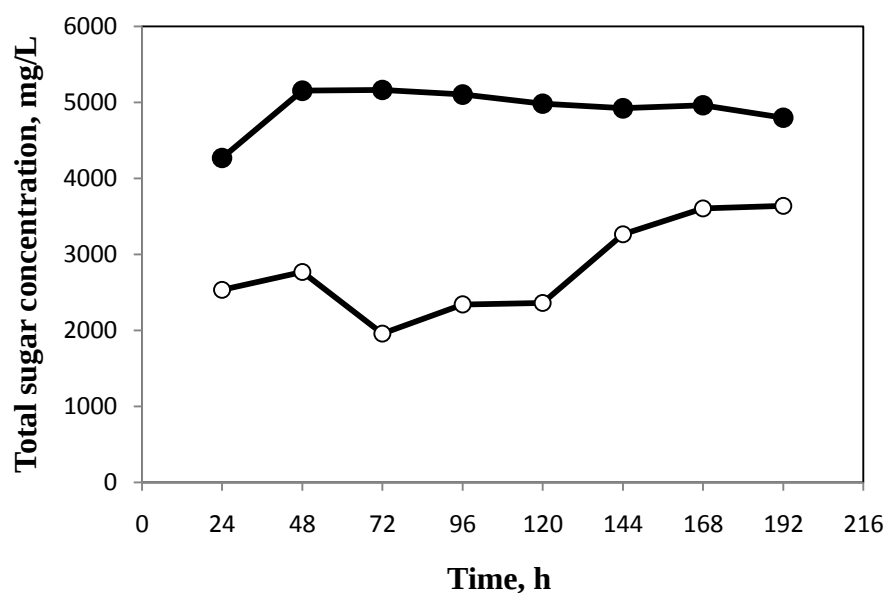


Figure 3.88 Variation of influent and effluent total sugar concentration with time at HRT=4 h.
(—●—; influent, —○—; effluent)

3.2.1.5 Hydrogen Production at HRT=2 h

The final HRT examined in the thesis was HRT= 2 h. Effect of HRT=2 h on hydrogen production volume is given in Figure 3.89. Daily production was 1500 ml at the beginning of operation (t=24). It reached to V= 2400 ml for the periods between t= 48 h and t=96 h. Then a steady increase in the volume to 3680 ml was observed up to t=168 h. Finally, it remained constant there until t = 192 h. The volume produced at this HRT was the highest one among the obtained volume at other HRTs. All operated upflow anaerobic packed bed reactor experiments among the highest hydrogen yield, were also seen at HRT= 2h. Volumetric hydrogen production reached to VHP= 1.75 L H₂ /L /d (Figure 3.90). Yield of production was 1.6 mol H₂ / mol TS which was the highest obtained in continuous operation. However, sugar consumption was very low. Effluent sugar concentration was around 2500 mg/L and steadily increased up to around 4000 mg/L at the end of operation (Figure 3.91). The main reason for this low sugar consumption was short HRT at which there was not enough time to consume all the sugar. But there was efficient conversion of substrate

to organic acid. High rate of loading the system increased the rate of hydrogen production.

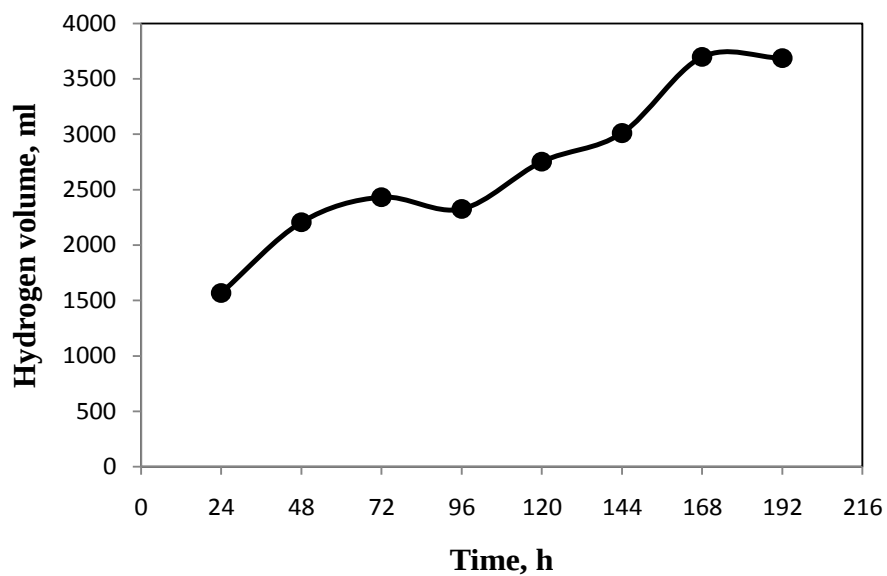


Figure 3.89 Variation of hydrogen volume with time at HRT=2 h

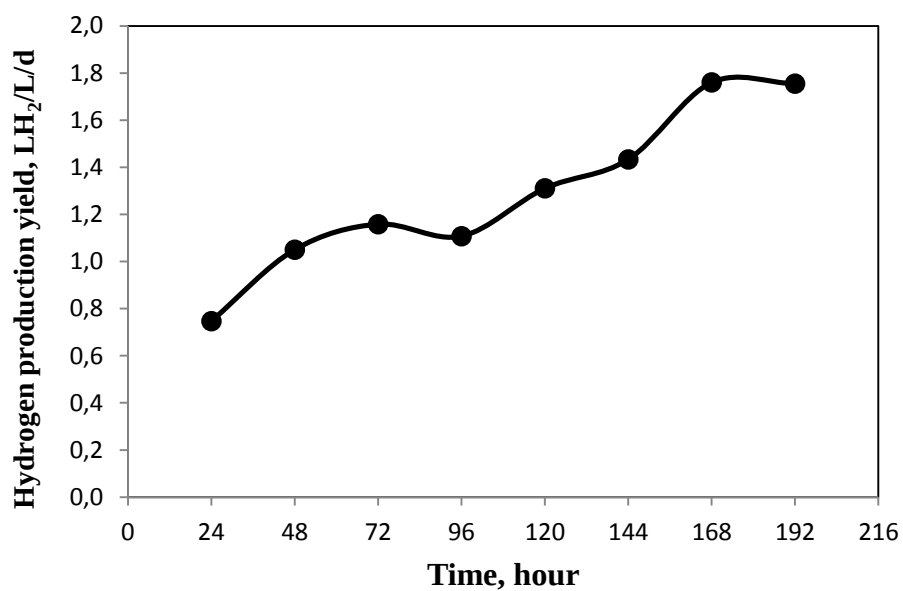


Figure 3.90 Variation of volumetric hydrogen production (L H₂/L/day) with time at HRT =2 h.

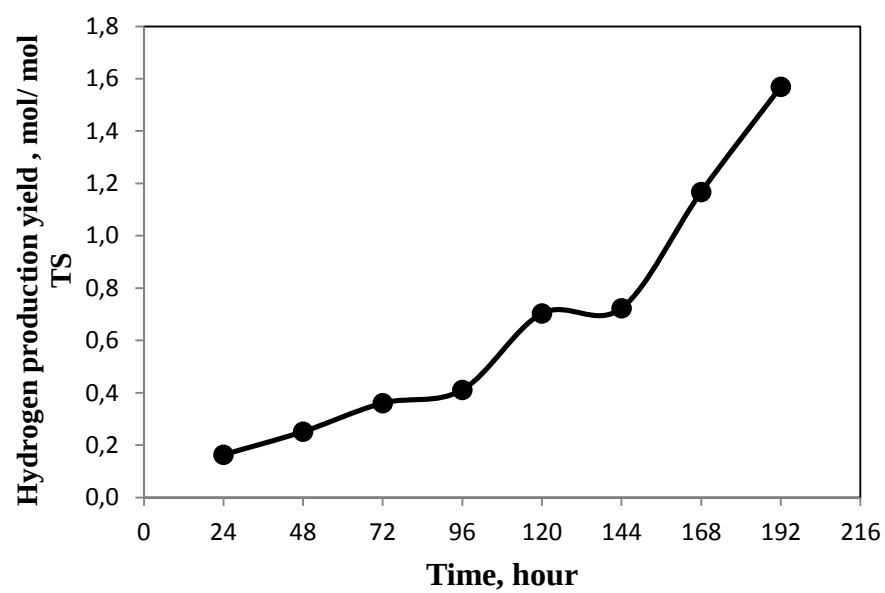


Figure 3.91 Variation of hydrogen production yield (mol/ mol TS) with time at HRT =2 h.

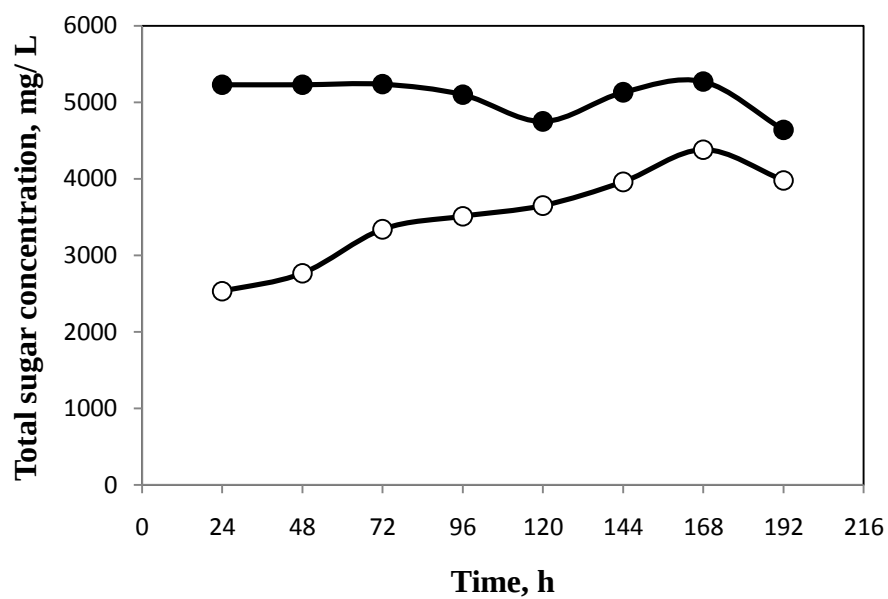


Figure 3.92 Variation of influent and effluent total sugar concentration with time at HRT=2 h
(—●—; influent, —○—; effluent)

CHAPTER FOUR

CONCLUSIONS

Hydrogen is a renewable and a clean gas with high energy value. It is considered as an alternative energy source. However, the technology for hydrogen gas production must be improved to make hydrogen competitive with other renewable energy sources. Biological methods, recently, received a significant attention among the other available physical and chemical methods. Biohydrogen gas production from carbohydrate rich waste materials is one of the major concerns.

Dark and photofermentation are the two biological hydrogen production methods. Dark fermentation is preferred due to high hydrogen production rates and yields, requirement of simple operation conditions and controls. Wide varieties of studies have been conducted about effect of substrate, microbial culture type and environmental condition on hydrogen gas production by batch dark fermentation. Recently, the studies devoted to evaluation of dark fermentative hydrogen production by continuous operation. The studies indicated that immobilized systems are superior over suspended growth systems for hydrogen production.

The main research subjects about hydrogen production in immobilized growth systems are selection of support material type and determination of optimum operating conditions. Hydraulic retention time (HRT) is one of the parameters in continuously operated system. However, its effect on hydrogen production yield has not been clearly stated yet. High hydrogen yields were obtained at low HRTs in some studies and relatively longer HRTs in other ones. The relationship between support particle characteristic and HRT is one of the reasons of this variation. Therefore, the conditions in continuously operated immobilized systems must be determined specific to the support particle type.

By considering this fact, the thesis aimed to investigate hydrogen production in immobilized systems by dark fermentation. The studies conducted for this purpose

are support particle selection for immobilization of microorganisms, hydrogen production in repeated batch operation with immobilized bacterial culture and continuous operation of up-flow packed bed reactor containing selected support particle as immobilization surface at different HRTs.

Repeated batch operation studies indicated that the yield and volume of production increases with the decreasing cycle time from 5 days to 1 day. The cycle time represent the contact time of the reaction volume with the microorganisms. Short reaction times could enhance the microbial activity for the product formation. In addition, accumulation of organic acids in the liquid phase inhibits hydrogen product. Exposure of these produced organic acids was longer at 5 days contact time compared to 1 day. Therefore, inhibition effect of organic acids at long cycle times was more substantial. As a result, low productions were observed for all particle types. Yield of production with metal mesh covered plastic nylon sponge increased from 0.55 mol/ mol glucose to 2.07 mol/mol glucose when cycle time was decreased from 5 days to 1 day. In conclusion, short batch periods are suggested for high hydrogen productions.

Durability of support particle under extreme conditions is another factor in selection of immobilization surface material. Particles were exposed to extreme pH values as $\text{pH} = 5 \pm 0.5$, temperature, $T = 37^\circ\text{C}$, and a corrosive gas of hydrogen. Black sponge was a proper particle in terms of pore size and density. However, it was easily destructed under these environmental conditions. The other particles were firm and no destructions were observed.

Hydrogen productions obtained from suspended culture experiments (control experiments) revealed that immobilization substantially improves production. The maximum yield obtained from suspended cultures was less than one obtained from most of the support particles for all cycle times. For example, it was 0.99 mol/ mol glucose at 1 day cycle time with control although it was over 2 mol/ mol glucose for plastic nylon sponge with metal mesh.

Repeated batch operation was conducted with all particle types. Plastic nylon sponge with metal mesh was superior over other articles used. Pore size of plastic nylon sponge was too large which caused insufficient biofilm formation. Covering this particle with metal mesh reduced pore size and hence provided better environment for immobilization or biofilm formation. High concentration of microorganisms with this biofilm formation resulted in high hydrogen production. Pore size of the particle also affects the gas release from inside of particles. Large pore size will be more effective in this respect compared to small ones. However, it must be optimum for biofilm formation and gas release. Covering particle with metal mesh provided ideal pore size for both functions. This particle was selected for continuous reactor studies.

Packed bed reactor was operated at HRT= 2- 13 h. Hydrogen production volume and yield increased with decreasing HRT. Yields and rates at HRT= 2 were almost two times of that at HRT= 13 h. The maximum daily hydrogen production volume reached up to 3500 ml at HRT= 2. Similarly, volumetric production rate raised to 1.7 L H₂/L. d. However, substrate consumption decreased which means that some of the substrate was not used and they were wasted.

Continuous operation resulted in higher hydrogen productions compared to repeated batch operations. The maximum gas volume reached to 3500 ml although it was around 600 ml in repeated batch experiments. Similarly, hydrogen purity in total gas reached up to 67% by continuous operation while it was only 37% in repeated batch.

In summary, dark fermentative hydrogen production is a promoting technology for biological renewable gas generation. Immobilized systems should be the first choice to get high volume of production. However, process needs further improvements for higher productions in order to make it compatible with the other chemical or physical ones. The followings are the recommendations for this purpose.

- The process can be operated at higher initial substrate concentrations.

- Process performance can be evaluated under thermophilic conditions.
- Lower particle sizes can be used to increase the surface area of immobilization.
- Two stage dark fermentation can be applied to be able use the waste sugar from the first reactor.

REFERENCES

- Argun, H., Kargi, F., & Kapdan, İ.K. (2008). Light fermentation of dark fermentation effluent for bio-hydrogen production by different *Rhodobacter* species at different initial volatile fatty acid (VFA) concentrations. *International Journal of Hydrogen Energy*, 33, 7405–7412.
- Argun, H., Kargi, F., Kapdan, İ.K., & Oztekin, R. (2008). Biohydrogen production by dark fermentation of wheat powder solution: Effects of C/N and C/P ratio on hydrogen yield and formation rate. *International Journal of Hydrogen Energy*, 33, 1813 – 1819.
- Argun, H., Kargi, F., & Kapdan, I.K. (2009). Effects of the substrate and cell concentration on bio-hydrogen production from ground wheat by combined dark and photo-fermentation. *International Journal of Hydrogen Energy*, 34 (15), 6181–6188.
- Argun, H., & Kargi, F. (2011). Bio-hydrogen production by different operational modes of dark and photo-fermentation: An overview. *International Journal of Hydrogen Energy*, 36 (13), 7443–7459.
- Arriaga, S., Rosas, I., Alatrliste-Mondrago'n, F., & Razo-Flores, E. (2011). Continuous production of hydrogen from oat straw hydrolysate in a biotrickling filter. *International Journal of Hydrogen Energy*, 36, 3442-3449.
- Azbar, N., & Kapdan, İ.K. (2012). Use of Immobilized Cell Systems in Biohydrogen Production. N. Azbar, & D. Levin, (Ed.), *State of the Art and Progress in Production of Biohydrogen* (227-249).
- Barbosa, M.J., Rocha, J.M.S., Tramper, J., & Wijffels, R.H. (2001). Acetate as a carbon source for hydrogen production by photosynthetic bacteria. *Journal Biotechnol*, 85, 25–33.
- Barros, A.R., Amorim, E.L.C., Reis, C.M., Shida, G.M., & Silva, E.L. (2010). Biohydrogen production in anaerobic fluidized bed reactors: Effect of support

- material and hydraulic retention time. *International Journal of Hydrogen Energy*, 35, 3379–3388.
- Bartacek, J., Zabranska, J., & Lens, P.N.L. (2007). Developments and constraints in fermentative hydrogen production. *Biofuels, Bioproducts & Biorefining*, 1, 201-214.
- Benemann, J.R., & Hallenbeck, P.C. (2002). Biological hydrogen production; fundamentals and limiting processes. *International Journal Hydrogen Energy*, 27, 1185–1193
- Carrillo-Reyes, J., Celis, L.B., Alatrliste-Mondrago'n, F., & Razo-Flores, E. (2012). Different start-up strategies to enhance biohydrogen production from cheese whey in UASB reactors. *International Journal of Hydrogen Energy*, 37, 5591-5601.
- Chang, H.N. (1987). Membrane bioreactors: Engineering aspects. *Biotechnology Advances*, 5, 129-145.
- Chang, J.S., Lee, K.S., & Lin, P.J. (2002). Biohydrogen production with fixed-bed bioreactors. *International Journal of Hydrogen Energy*, 27 (11-12), 1167-1174.
- Chang, F.Y., & Lin, C.Y. (2004). Biohydrogen production using an up-flow anaerobic sludge blanket reactor. *International Journal of Hydrogen Energy*, 29 (1), 33-39.
- Chong, M.L., Sabaratnam, V., Shirai, Y., & Hassan, M.A. (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. *International Journal of Hydrogen Energy*, 34 (8), 3277–3287.
- Chojnacka, A., Błaszczuk, M.K., Szczęsny, P., Nowak, K., Sumińska, M., Tomczyk-Żak, K., et al. (2011). Comparative analysis of hydrogen-producing bacterial biofilms and granular sludge formed in continuous cultures of fermentative bacteria. *Bioresource Technology*, 102, 10057–10064.

- Collet, C., Adler, N., Schwitzguébel, J.P., & Péringer, P., (2004). Hydrogen production by *Clostridium thermolacticum* during continuous fermentation of lactose, *International Journal of Hydrogen Energy*, 29, 1479-1485.
- Das, D., & Veziroğlu, T.N. (2001). Hydrogen production by biological processes: A survey of literature. *International Journal of Hydrogen Energy*, 26, 13-28.
- Das, D. (2009). Advances in biohydrogen production processes: An approach towards commercialization. *International Journal of Hydrogen Energy*, 34, 7349-7357.
- Davila-Vazquez, G., Arriaga, S., Alatrisme-Mondragón, F., de León-Rodríguez, A., Rosales-Colunga, L.M., & Razo-Flores, E. (2008). Fermentative biohydrogen production: trends and perspectives. *Environmental Science Biotechnology*, 7, 27-45
- Davila-Vazquez, G., Cota-Navarro, C.B., Rosales-Colunga, L.M., de Leon-Rodriguez, A., & Flores, E.R. (2009). Continuous biohydrogen production using cheese whey: Improving the hydrogen production rate, *International Journal of Hydrogen Energy*, 34, 4296-4304.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analatic Chemistry*, 8, 350-366.
- Gavala, H.N, Skiadas, I.V., & Ahring, B.K. (2006). Biological hydrogen production in suspended and attached growth anaerobic reactor systems. *International Journal of Hydrogen Energy*, 31 (9), 1164-1175.
- Genç, N. (2009). Biyolojik hidrojen üretim prosesleri, *Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 11, (2), 17-36.
- Guo, X.M., Trably, E., Latrille, E., Carrère, H., & Steyer, J.P. (2010). Hydrogen production from agricultural waste by dark fermentation: A review. *International*

Journal of Hydrogen Energy, Indo-French Workshop on Biohydrogen: from Basic Concepts to Technology, 35 (19), 10660–10673

Hallenbeck, P.C. (2009). Fermentative hydrogen production: Principles, progress, and prognosis. *International Journal of Hydrogen Energy*, 34, 7379-7389.

Hallenbeck, P.C., Ghosh, D., Skonieczny, M.T., & Yargeau, V. (2009). Microbiological and engineering aspects of biohydrogen production. *Indian Journal Microbial*, 49, 48-59.

Jo, J.H., Lee, D.S., Park, D., & Park, J.M. (2008). Biological hydrogen production by immobilized cells of *Clostridium tyrobutyricum* JM1 isolated from a food waste treatment process. *Bioresource Technology*, 99, 6666–6672.

Kapdan, İ.K., & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*, 38, 569–582.

Kapdan, İ.K., Kargi, F., Oztekin, R., & Argun, H. (2009). Bio-hydrogen production from acid hydrolyzed wheat starch by photo-fermentation using different *Rhodobacter* sp, *International Journal of Hydrogen Energy*, 34, 2201–2207.

Keskin, T., Aksöyek, E., & Azbar, N. (2011). Comparative analysis of thermophilic immobilized biohydrogen production using packed materials of ceramic ring and pumice stone. *International Journal of Hydrogen Energy*, 36, 15160-15167.

Kumar, N., & Das, D. (2001). Continuous hydrogen production by immobilized *Enterobacter cloacae* IIT-BT 08 using lignocellulosic materials as solid matrices. *Enzyme and Microbial Technology*, 29 (4-5), 280-287.

Leung, D., Leung, M., Sumathy, K., & Ni, D. (2006). An overview of hydrogen production from biomass. *Fuel Processing Technology*, 87, 451–472.

Lima, D.M.F., Moreira, W.K., & Zaiat, M. (2013). Comparison of the use of sucrose and glucose as a substrate for hydrogen production in an upflow anaerobic fixed-bed reactor. *International Journal of Hydrogen Energy*, 38 (35), 15074–15083.

- Lina, C.N., Wua, S.Y., & Change, J.S. (2006). Fermentative hydrogen production with a draft tube fluidized bed reactor containing silicone-gel-immobilized anaerobic sludge. *International Journal of Hydrogen Energy*, 31, 2200–2210.
- Marques dos Reis, C., & Silva, E.L. (2011). Effect of upflow velocity and hydraulic retention time in anaerobic fluidized-bed reactors used for hydrogen production. *Chemical Engineering Journal*, 172 (1), 28–36.
- Mitchell, R.J., Kim, J.S., Jeon, B.S., & Sang, B.I. (2009). Continuous hydrogen and butyric acid fermentation by immobilized *Clostridium tyrobutyricum* ATCC 25755: Effects of the glucose concentration and hydraulic retention time. *Bioresource Technology*, 100 (21), 5352-5355.
- Mohan, S.V., Mohanakrishna, G., Reddy, S.S., Raju, B.D., Rao, K.S.R., & Sarma, P.N. (2008). Self-immobilization of acidogenic mixed consortia on mesoporous material (SBA-15) and activated carbon to enhance fermentative hydrogen production. *International Journal of Hydrogen Energy*, 33 (21), 6133-6142.
- Nath, K., & Das, D. (2004). Improvement of fermentative hydrogen production: various approaches. *Appllication Microbial Biotechnology*, 65, 520-529.
- Nedovic, V., & Willaert, R. (1996). *Fundamentals of cell immobilization biotechnology*. USA: Kluwer cademic Publisher.
- Oh, S.E., Iyer, P., Bruns, M.A., & Logan, B.E. (2004). Biological Hydrogen Production Using a Membrane Bioreactor. *Bioechnology and Bioengineering*, 87 (1), 119-127.
- Oh, Y.K., Scol, E.H., Kim, M.S., & Park S. (2004). Photoproduction of hydrogen from acetate by a chemoheterotrophic bacterium *Rhodopseudomonas palustris* P4. *International Journal of Hydrogen Energy*, 29, 1115–1121.
- Pan, C.M., Fan,Y.T., Xing,Y. H., Hou,W., & Zhang, M.L. (2008). Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* sp. *Bioresource Technology*, 99 (8), 3146–3154.

- Peixoto, G., Saavedra, N.K., Varesche, M.B.A., & Zaiat, M. (2011). Hydrogen production from soft-drink wastewater in an upflow anaerobic packed-bed reactor. *International Journal of Hydrogen Energy*, 36, 8953-8966.
- Rupp, R.G. (1985). Use of cellular microencapsulation of yeasts for use in the tower fermenter. *Biotechnology*, 4, 621-626.
- Shida, G.M., Barros, A.R., dos Reis, C.M., Cavalcante de Amorim, E.L., Damianovic, M.H.R.Z., & Silva, E.L. (2009). Long-term stability of hydrogen and organic acids production in an anaerobic fluidized-bed reactor using heat treated anaerobic sludge inoculum. *International Journal of Hydrogen Energy*, 34 (9), 3679-3688.
- Shi, X.Y., & Yu, H.Q. (2004). Hydrogen production from propionate by *Rhodopseudomonas capsulate*. *Applications Biochemistry Biotechnology*, 117 (33), 143–154.
- Tao, Y.Z., He, Y.L., Wu, Y.Q., Liu, F.H., Li, X.F., Zong, W.M., et al. (2008). Characteristics of a new photosynthetic bacterial strain for hydrogen production and its application in wastewater treatment. *International Journal of Hydrogen Energy*, 33 (3), 963–973.
- Vega, J.L., Clausen, E.C., & Gaddy, J.L. (1988). Biofilm reactors for ethanol production. *Enzyme Microbial Technology*, 10 (7), 390-402.
- Wang, J., & Wan, W. (2009). Factors influencing fermentative hydrogen production: A review. *International Journal of Hydrogen Energy*, 34 (2), 799-811.
- Webb, C., & Dervakos, G.A. (1996). *Studies in viable cell immobilization*. Austin, Texas, USA: R.G. Landes Company, Academic Press Inc.
- Wu, S.Y., Lin, C.N., Chang, J.S., & Chang, J.S. (2005). Biohydrogen production with anaerobic sludge immobilized by ethylene-vinyl acetate copolymer. *International Journal of Hydrogen Energy*, 30 (13-14), 1375-1381.

Zhang, H., Bruns, M.A., & Logan, B.E. (2006). Biological hydrogen production by *Clostridium acetobutylicum* in an unsaturated flow reactor. *Water Research*, 40 (4), 728-734.

Zhang, Z.P., Tay, J.H., Show, K.Y., Yan, R., Liang, D.T., Lee, D.J., et al. (2007). Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor. . *International Journal of Hydrogen Energy*, 32 (2), 185-191.

APPENDIX

RAW EXPERIMENTAL DATA

ABBREVIATIONS

EDTA	Etilendiamin tetraasetik asit
CHV	Cumulative hydrogen volume
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DTFBR	Draft tube fluidized bed reactor
GC	Gas chromatograph
HRT	Hydrolic retantion time
MBR	Membrane bioreactor
ORP	Oxidation reduction potential
TS	Total sugar
UASB	Up-flow anaerobic sludge bed bioreactor
VFA	Volatile fatty acid

A.1 Raw Data for Batch Dark Fermentation Experiments

A.1.1 Repeated batch operation with 5 days cycle time

Table A.1 Raw data for plastic scouring sponge pad particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\sum Vt (ml)$	<i>% H</i>	<i>VH₂</i> (mL/day)	$\sum VH_2$ (ml)	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
3.4.2012	24	270	270	27	72,9	72,9	0	6,12		
4.4.2012	48	0	270	14,5	0	72,9	-275	6,25	11.000	
5.4.2012	72	5	275	12	0,6	73,5	-268	6,45		
6.4.2012	96	2	277	7,4	0,148	73,648	-119,8	6,43		
7.4.2012	120	287	564	19	54,53	128,178	-163,5	5,52		
8.4.2012	146	30	594	8	2,4	130,578	-64,7	5,85		240,3
9.4.2012	170	11	605	4,5	0,495	131,073	-75	5,65	9.613	
10.4.2012	194	2,5	607,5	4,2	0,105	131,178	-43	5,34		
11.4.2012	218	100	707,5	31	31	162,178	-132	5,1		
12.4.2012	242	23	730,5	12	2,76	164,938	45	2,54		192,5
13.4.2012	268	12,5	743	3,4	0,425	165,363	-197	4,26	8.632	

14.4.2012	291	10,3	753,3	3	0,309	165,672	-75	2,81		
15.4.2012	317	4,8	758,1	2	0,096	165,768	26	2,78		
16.4.2012	341	72	830,1	24	17,28	183,048	-76	3,8		
17.4.2012	361	30	860,1	5,2	1,56	184,608	-65	3,78		1.357
18.4.2012	387	20	880,1	4	0,8	185,408	-21	3,61	10.495	2.574

Table A.2 Raw data for plastic scouring sponge pad with metal mesh particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt (ml)$	<i>% H</i>	<i>VH₂ (mL/day)</i>	$\Sigma VH_2 (ml)$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
3.4.2012	24	255	255	13,8	35,19	35,19	0	6,15	11.000	
4.4.2012	48	150	405	24	36	71,19	-384	6,14		
5.4.2012	72	12	417	24,7	2,964	74,154	-268	6,71		
6.4.2012	96	5	422	10,6	0,53	74,684	-197,8	6,7		413
7.4.2012	120	258	680	14,5	37,41	112,094	-165,4	5,72	9.500	
8.4.2012	146	53	733	16	8,48	120,574	-67,4	5,98		
9.4.2012	170	32	765	18	5,76	126,334	-186	5,94		
10.4.2012	194	6	771	4	0,24	126,574	-124	5,91		229

11.4.2012	218	138	909	45	62,1	188,674	-164	5,66	10.500	
12.4.2012	242	65	974	39	25,35	214,024	-138	5,5		
13.4.2012	268	94	1068	39	36,66	250,684	-165	5,41		
14.4.2012	291	56	1124	23	12,88	263,564	-147	5,34		
15.4.2012	317	3	1127	8,3	0,249	263,813	-116	5,27		221
16.4.2012	341	169	1296	35	59,15	322,963	-180	5,89	10.960	
17.4.2012	361	96	1392	21	20,16	343,123	-187	5,87		
18.4.2012	387	45	1437	12	5,4	348,523	-173	5,37		2.659

Table A.3 Raw data for plastic nylon sponge

Date	Time	Vt (ml)	$\sum Vt$ (ml)	% H	VH₂ (mL/day)	$\sum VH_2$ (ml)	ORP	pH	So(mg/L)	Se(mg/L)
3.4.2012	24	220	220	31,7	69,74	69,74	0	6,23		
4.4.2012	48	73	293	13	9,49	79,23	-185	6,11		
5.4.2012	72	18	311	13,3	2,394	81,624	-234	6,4		

6.4.2012	96	6	317	5	0,3	81,924	-210,6	6,52		263,2
7.4.2012	120	90	407	26	23,4	105,324	-170,8	5,16	8.656	
8.4.2012	146	34	441	3,8	1,292	106,616	-50,2	5,6		
9.4.2012	170	8	449	1,6	0,128	106,744	-65	5,40		
10.4.2012	194	3	452	1,3	0,039	106,783	-24	4,78		292,2
11.4.2012	218	85	537	19	16,15	122,933	-135	3,99	9.810	
12.4.2012	242	45	582	26	11,7	134,633	-102	3,5		
13.4.2012	268	34	616	33	11,22	145,853	-89	2,8		
14.4.2012	291	24	640	19	4,56	150,413	-24	2,36		
15.4.2012	317	4	644	1,7	0,068	150,481	-76	2,38		236
16.4.2012	341	52	696	10	5,2	155,681	-106	3,73	10.960	
17.4.2012	361	28	724	4	1,12	156,801	-143	3,9		
18.4.2012	387	8	732	3,4	0,272	157,073	-123	3,79		2.456

Table A.4 Raw data for plastic nylon sponge with metal mesh

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	<i>ΣVt (ml)</i>	<i>% H</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂ (ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
3.4.2012	24	254	254	36	91,44	91,44	0	6,11	11.000	
4.4.2012	48	125	379	20	25	116,44	-357	6,13		
5.4.2012	72	13	392	14	1,82	118,26	-157	6,58		
6.4.2012	96	2	394	6	0,12	118,38	-154	6,64		327
7.4.2012	120	294	688	34	99,96	218,34	-133,1	5,56	9.225	
8.4.2012	146	134	822	24	32,16	250,5	-98	5,83		
9.4.2012	170	16	838	4,5	0,72	251,22	-47	5,24		
10.4.2012	194	2	840	4,2	0,084	251,304	-56	5,1		475
11.4.2012	218	134	974	31	41,54	292,844	-187	4,86	9.529	
12.4.2012	242	100	1074	35	35	327,844	-180	4,41		
13.4.2012	268	65	1139	26	16,9	344,744	-64	4,15		
14.4.2012	291	61	1200	10	6,1	350,844	-98	4,16		
15.4.2012	317	55	1255	6,4	3,52	354,364	-105	4,21		1.675
16.4.2012	341	60	1315	34	20,4	374,764	-152,4	4,39	10.083	
17.4.2012	361	28	1343	49	13,72	388,484	-72	4,55		

18.4.2012	387	44	1387	6,7	2,948	391,432	-46	4,47		2.439
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Table A .5 Raw data for black prours sponge

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂ (mL/day)</i>	$\Sigma VH_2 \text{ (ml)}$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
3.4.2012	24	230	230	35	80,5	80,5	0	6,19	11.000	
4.4.2012	48	80	310	21	16,8	97,3	-344	6,28		
5.4.2012	72	22	332	10	2,2	99,5	-267	6,62		
6.4.2012	96	10	342	5	0,5	100	-196,7	6,55		403
7.4.2012	120	176	518	32	56,32	156,32	-160,2	5,49	10.235	
8.4.2012	146	67	585	7,5	5,025	161,345	-187	5,55		
9.4.2012	170	10	595	4,8	0,48	161,825	-155	5,14		
10.4.2012	194	8	603	6	0,48	162,305	-103	5,2		212,5
11.4.2012	218	180	783	18	32,4	194,705	-138	4,3	9.464	
12.4.2012	242	75	858	12	9	203,705	-25	2,4		
13.4.2012	268	23	881	33	7,59	211,295	-122	5,17		
14.4.2012	291	11	892	30	3,3	214,595	-73	2,37		

15.4.2012	317	3	895	3,3	0,099	214,694	-45	2,41		236
16.4.2012	341	45	940	12	5,4	220,094	-148	4,2	10.800	
17.4.2012	361	12	952	4,5	0,54	220,634	-167	4,35		
18.4.2012	387	12	964	3,3	0,396	221,03	-156	4,13		3.260

Table A.6 Raw data for control

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	<i>ΣVt (ml)</i>	<i>% H</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂ (ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
12.4.2012	24	176	176	25	44	44	-331,1	6,30	10.660	
13.4.2012	48	85	261	33	28,05	72,05	-316,8	6,12		
14.4.2012	72	15	276	14	2,1	74,15	-216	6,15		
15.4.2012	96	6	282	5	0,3	74,45	-459	6,35		142,8
16.4.2012	120	102	384	20	20,4	94,85	-80	5,77	10.674	
17.4.2012	146	76	460	13	9,88	104,73	-102,7	5,86		

18.4.2012	170	34	494	12	4,08	108,81	-226,5	5,84		
19.4.2012	194	17	511	5	0,85	109,66	-220,7	5,8		109,6
20.4.2012	218	10	521	3,6	0,36	110,02	-252,9	5,79	10.134	
21.4.2012	242	75	596	17	12,75	122,77	-280,7	5,42		
22.4.2012	268	43	639	15	6,45	129,22	-238	5,2		
23.4.2012	291	25	664	4,5	1,125	130,345	-165,8	4,99		
24.4.2012	317	21	685	4	0,84	131,185	-200,4	4,85		195,7
25.4.2012	341	13	698	3,5	0,455	131,64	-268,8	5,04	9.940	
26.4.2012	361	7	705	13	0,91	132,55	-79,8	4,3		
27.4.2012	387	9	714	12	1,08	133,63	-67,7	4,2		123,6

A.1.2 Repeated batch operation with 3 day cycle time

Table A.7 Raw data for plastic scouring sponge pad particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂</i> <i>(mL/day)</i>	$\Sigma VH_2 \text{ (ml)}$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
31.5.2012	20	260	260	27	70,2	70,2	-220	6,38	11.860	
1.6.2012	43	160	420	25	40	110,2	-224	6,25		552,52
2.6.2012	67	240	660	18	43,2	153,4	-140	5,56	10.877	
3.6.2012	91	120	780	4	4,8	158,2	-145	5,54		
4.6.2012	110	90	870	20	18	176,2	-301	5,63		1.430
5.6.2012	136	197	1067	16	31,52	207,72	-207	4,8	11.328	
6.6.2012	170	87	1154	12	10,44	218,16	-75	4,40		
7.6.2012	194	6	1160	23	1,38	219,54	-43	4,2		369
8.6.2012	220	220	1380	4,5	9,9	229,44	-132	4,8	9.735	
9.6.2012	242	145	1525	12	17,4	246,84	-98	4,75		
10.6.2012	266	12,5	1537,5	4	0,5	247,34	-102	4,72		
11.6.2012	291	4	1541,5	10	0,4	247,74	-20	4,77		3.197
12.6.2012	317	186	1727,5	15	27,9	275,64	-89	4,71	11.859	
13.6.2012	341	56	1783,5	15	8,4	284,04	-76	4,58		

14.6.2012	361	34	1817,5	8	2,72	286,76	-79	4,12		2.845
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Table A.8 Raw data for plastic scouring sponge pad with metal mesh particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	<i>ΣVt (ml)</i>	<i>% H</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂(ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
31.5.2012	20	280	280	38	106,4	106,4	-210	6,39		
1.6.2012	43	210	210	24	50,4	156,8	-200	6,06		602,34
2.6.2012	67	260	470	24	62,4	219,2	-312	5,72	12.046	
3.6.2012	91	120	590	19	22,8	242	-316	5,43		
4.6.2012	110	160	750	11	17,6	259,6	-300	5,59		1.167
5.6.2012	136	120	870	26	31,2	290,8	-98	5,12	13.215	
6.6.2012	170	40	910	18	7,2	298	-186	5,16		
7.6.2012	194	15	925	26	3,9	301,9	-124	4,98		4.991
8.6.2012	220	48	973	9	4,32	306,22	-164	4,47	11.183	
9.6.2012	242	23	996	4	0,92	307,14	-138	5,5		
10.6.2012	266	12	1008	3,5	0,42	307,56	-165	5,41		

11.6.2012	291	9	1017	5	0,45	308,01	-147	5,34		2.197
12.6.2012	317	58	1075	5	2,9	310,91	-75	4,46	12.174	
13.6.2012	341	49	1124	4	1,96	312,87	-58	4,03		
14.6.2012	361	20	1144	7	1,4	314,27	-102	4		1.657

Table A.9 Raw data for plastic nylon sponge

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂</i> <i>(mL/day)</i>	$\Sigma VH_2 \text{ (ml)}$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
31.5.2012	20	340	340	41	139,4	139,4	-198	6,10	14.410	
1.6.2012	43	265	605	20	53	192,4	-185	6,11		519
2.6.2012	67	150	755	28	42	234,4	-230	5,68	11.781	
3.6.2012	91	128	883	15	19,2	253,6	-210	5,36		
4.6.2012	110	120	1003	18	21,6	275,2	-349	6,1		274
5.6.2012	136	146	1149	32	46,72	321,92	-105	5,03	11.727	
6.6.2012	170	18	1167	7,5	1,35	323,27	-129	5,13		
7.6.2012	194	4	1171	7	0,28	323,55	-98	4,23		4.811

8.6.2012	220	120	1291	24	28,8	352,35	-135	4,67	12.425	
9.6.2012	242	56	1347	4	2,24	354,59	-102	4,56		
10.6.2012	266	13	1360	2,5	0,325	354,915	-95	5,12		
11.6.2012	291	4	1364	3,5	0,14	355,055	-87	4,76		5.197
12.6.2012	317	36	1400	6	2,16	357,215	-80	4,58	10.502	
13.6.2012	341	12	1412	4	0,48	357,695	-106	3,73		2.860

Table A.10 Raw data for plastic nylon sponge with metal mesh

Date	Time	Vt (ml)	$\sum Vt$ (ml)	% H	VH₂ (mL/day)	$\sum VH_2$ (ml)	ORP	pH	So(mg/L)	Se(mg/L)
31.5.2012	20	340	340	42	142,8	142,8	-196	6,28	12.896	
1.6.2012	43	240	240	30	72	214,8	-194	6,13		542,56
2.6.2012	67	300	300	34	102	316,8	-157	6,58	11.222	
3.6.2012	91	120	420	28	33,6	350,4	-154	6,64		
4.6.2012	110	75	495	23	17,25	367,65	-133,1	5,56		406
5.6.2012	136	235	730	38	89,3	456,95	-98	5,83	12.031	
6.6.2012	170	75	805	18	13,5	470,45	-47	5,24		

7.6.2012	194	2	807	4,2	0,084	470,534	-56	5,1		5.091
8.6.2012	220	120	927	41	49,2	519,734	-187	4,86	12.683	
9.6.2012	242	100	1027	14	14	533,734	-180	4,41		
10.6.2012	266	65	1092	67	43,55	577,284	-64	4,15		
11.6.2012	291	61	1153	12	7,32	584,604	-98	4,16		5.077
12.6.2012	317	60	1213	35	21	605,604	-105	4,21	11.410	
13.6.2012	341	20	1233	37	7,4	613,004	-152,4	4,39		4.567

Table A.11 Raw data for black prous sponge

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	<i>ΣVt (ml)</i>	<i>% H</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂ (ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
31.5.2012	20	209	209	38	79,42	79,42	-220	6,24	12.596	
1.6.2012	43	153	362	21	32,13	111,55	-224	6,14		552,53
2.6.2012	67	200	562	35	105	216,55	-220	5,91	13.799	
3.6.2012	91	123	685	23	28,29	244,84	-198	5,34		
4.6.2012	110	135	820	21	28,35	273,19	-190	4,1		107
5.6.2012	136	75	895	26	19,5	292,69	-104	4,72	11.589	
6.6.2012	170	10	905	7	0,7	293,39	-155	5,14		
7.6.2012	194	8	913	6	0,48	293,87	-103	4,13		4.991
8.6.2012	220	180	1093	18	32,4	326,27	-138	4,4	12.842	
9.6.2012	242	75	1168	12	9	335,27	-112	4,15		
10.6.2012	266	23	1191	33	7,59	342,86	-110	4		

11.6.2012	291	17	1208	5	0,85	343,71	-73	4,75		5.197
12.6.2012	317	20	1228	9	1,8	345,51	-80	4,36	10.490	
13.6.2012	341	12	1240	3	0,36	345,87	-96	4,2		6.576

Table A.12 Raw data for control

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	ΣVt (ml)	<i>% H</i>	<i>VH₂</i> (mL/day)	ΣVH_2 (ml)	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
31.5.2012	20	131	131	25	32,75	32,75	-200	6,08	12.045	
1.6.2012	43	165	296	10	16,5	49,25	-316,8	6,12		1.057
2.6.2012	67	96	392	7,8	7,488	56,738	-185	6,31	11.533	
3.6.2012	91	60	452	12	7,2	63,938	-129	6,12		
4.6.2012	110	45	497	7	3,15	67,088	-258	5,59		2.757
5.6.2012	136	20	517	5	1	68,088	-102	4,45	12.625	
6.6.2012	170	0	517	8	0	68,088	-102	4,35		
7.6.2012	194	6	523	3,5	0,21	68,298	-98	4,18		5.197

8.6.2012	220	24	547	8	1,92	70,218	-83	4,42	10.927	
9.6.2012	242	70	617	3,5	2,45	72,668	-280,7	5,42		
10.6.2012	266	51	668	3	1,53	74,198	-110	5,23		
11.6.2012	291	8	676	2,5	0,2	74,398	-165,8	4,99		3.240
12.6.2012	317	8	684	5	0,4	74,798	-72	4,07	10.260	
13.6.2012	341	11	695	3	0,33	75,128	-47	3,89		6.356

A.1.3 Repeated batch operation with 1 day cycle time

Table A.13 Raw data for plastic scouring sponge pad particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	<i>ΣVt (ml)</i>	<i>% H</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂ (ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
28.6.2012	24	300	300	43	129	129	-241	5,95	10.925	1.615
29.6.2012	48	320	620	34	108,8	237,8	-230	5,85	11.652	393,1
30.6.2012	72	180	800	36	64,8	302,6	-220	4,72	12.110	1.014
1.7.2012	96	160	960	26	41,6	344,2	-415	4,3	11.925	1.143
2.7.2012	120	210	1170	20	42	386,2	-174	4,65	12.478	1.860

Table A.14 Raw data for plastic scouring sponge pad particle

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂</i> <i>(mL/day)</i>	$\Sigma VH_2 \text{ (ml)}$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
29.6.2012	24	240	240	38	91,2	91,2	-222	5,91	11.819	3.409
30.6.2012	48	230	470	20	46	137,2	-234	5,14	12.589	4.583
1.7.2012	72	176	646	12	21,12	158,32	-280	4,34	13.934	2.635
2.7.2012	96	160	806	10,6	16,96	175,28	-165	4,38	10.987	2.835
3.7.2012	120	80	886	11	8,8	184,08	-165,4	5,72	11.033	1.578

Table A.15 Raw data for plastic nylon sponge

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂</i> <i>(mL/day)</i>	$\Sigma VH_2 \text{ (ml)}$	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
28.6.2012	24	280	280	35	98	98	-285	5,82	12.439	2.193
29.6.2012	48	260	540	25	65	163	-260	5,64	13.050	1.123
30.6.2012	72	160	700	24	38,4	201,4	-238	4,73	13.296	4.539
1.7.2012	96	180	880	17	30,6	232	-195	4,54	14.864	4.715
2.7.2012	120	145	1025	9	13,05	245,05	-136	4,21	10.642	2.791

Table A.16 Raw data for plastic nylon sponge with metal mesh

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	ΣVt (ml)	<i>% H</i>	<i>VH₂</i> (mL/day)	ΣVH_2 (ml)	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
28.6.2012	24	460	460	37	170,2	170,2	-274	6,16	11.056	3.974
29.6.2012	48	340	800	32	108,8	279	-204	5,95	13.296	499,3
30.6.2012	72	380	1180	25	95	374	-236	4,92	10.652	715,265
1.7.2012	96	257	1437	23	59,11	433,11	-480	4,34	12.519	2.943
2.7.2012	120	130	1567	18	23,4	456,51	-159	4,29	10.754	2.743

Table A.17 Raw data for black prous sponge

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	ΣVt (ml)	<i>% H</i>	<i>VH₂</i> (mL/day)	ΣVH_2 (ml)	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
28.6.2012	24	220	220	18	39,6	39,6	-256	5,79	12.608	1.615
29.6.2012	48	95	315	26	24,7	64,3	-230	5,18	13.652	3.380

30.6.2012	72	110	425	30	33	97,3	-245	4,56	10.164	2.057
1.7.2012	96	50	475	3	1,5	98,8	-433	4,3	10.519	4.679
2.7.2012	120	20	495	3,5	0,7	99,5	-130	4,04	10.967	4.687

Table A.18 Raw data for control

<i>Date</i>	<i>Time</i>	<i>Vt (ml)</i>	$\Sigma Vt \text{ (ml)}$	<i>% H</i>	<i>VH₂</i> <i>(mL/day)</i>	ΣVH_2 <i>(ml)</i>	<i>ORP</i>	<i>pH</i>	<i>So(mg/L)</i>	<i>Se(mg/L)</i>
28.6.2012	24	410	410	30	123	123	-314	5,72	13.767	2.797
29.6.2012	48	70	480	18	12,6	135,6	-181	5,81	12.617	1.678
30.6.2012	72	90	570	12	10,8	146,4	-174	4,92	13.456	6.157
1.7.2012	96	50	620	10,5	5,25	151,65	-431	4,35	10.359	4.795
2.7.2012	120	30	650	4,5	1,35	153	-146	4,07	10.231	2.410

A.2 Raw Data for Continuous Fermentation Experiments

A.2.1. Continuous Dark Fermentation

Table A.19 Raw data for HRT = 13 hours

<i>Date</i>	<i>Q (ml)</i>	<i>L/day</i>	<i>QH(h)</i>	<i>So(mg/l)</i>	<i>Se(mg/l)</i>	<i>pH</i>	<i>T(°C)</i>	<i>% H</i>	<i>Total Gas (ml) (Vt)</i>	<i>ΣVt (ml)</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂</i>
3.4.2013	160	4	13	4900	630	4,4	37	57	2100	2100	1197	0,000
4.4.2013	160	4	13	5200	448	4,3	37	58	2500	4600	1450	1197
5.4.2013	160	4	13	4833	580	4,3	37	48	2500	7100	1200	2647
6.4.2013	160	4	13	5320	420	4,3	37	58	2800	9900	1624	3847
7.4.2013	160	4	13	4910	540	4,2	37	57	2500	12400	1425	5471
8.4.2013	160	4	13	5140	628	4,3	37	53	3000	15400	1590	6896
9.4.2013	160	4	13	5225	430	4,2	37	57	2750	18150	1568	8486
10.4.2013	160	4	12	5330	430	4,2	37	58	2800	20950	1624	10054
												11678

Table A.20 Raw data for HRT = 10 hours

<i>Date</i>	<i>Q(ml)</i>	<i>L/day</i>	<i>QH(h)</i>	<i>So(mg/l)</i>	<i>Se(mg/l)</i>	<i>pH</i>	<i>T(°C)</i>	<i>% H</i>	<i>Total Gas (Vt) (ml)</i>	<i>Σ Vt (ml)</i>	<i>VH₂ (mL/day)</i>	<i>ΣVH₂</i>
20.3.2013	210	5	10	4809	2245	4,4	37	53	2500	2500	1325	

21.3.2013	210	5	10	4817	2318	4,3	37	55	3000	5500	1650	1325
22.3.2013	210	5	10	4940	1174	4,4	37	60	3200	8700	1920	2975
23.3.2013	210	5	10	5200	1344	4,4	37	62	4300	13000	2666	4895
24.3.2013	210	5	10	5320	1290	4,3	37	63	4200	17200	2646	2666
25.3.2013	210	5	10	5318	2568	4,6	37	63	4250	21450	2678	5312
												7990

Table A.21 Raw data for HRT = 6 hours

<i>Date</i>	<i>Q(ml)</i>	<i>L/day</i>	<i>QH(h)</i>	<i>So(mg/l)</i>	<i>Se(mg/l)</i>	<i>pH</i>	<i>T(°C)</i>	<i>% H</i>	<i>Total Gas (Vt) (ml)</i>	$\sum Vt$ (ml)	<i>VH₂</i> (mL/day)	$\sum VH_2$
24.04.2013	350	8	6	4680	2380	4,3	37	50	2500	2500	1250	
25.04.2013	350	8	6	5300	2700	4,2	37	57	2000	4500	1140	1250
26.04.2013	350	8	6	5160	1670	4,4	37	55	3000	7500	1650	2390
27.04.2013	350	8	6	5250	1820	4,5	37	57	2800	10300	1596	4040
28.04.2013	350	8	6	5340	2370	4,6	37	63	3500	13800	2205	5636
29.04.2013	350	8	6	4920	3260	4,5	37	63	4200	18000	2646	7841
30.04.2013	350	8	6	4960	3400	4,7	37	64	4100	22100	2624	10487

5.01.2013	350	8	6	4750	3240	4,7	37	62	4250	26350	2635	13111
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Table A.22 Raw data for HRT = 4 hours

<i>Date</i>	<i>Q(ml)</i>	<i>L/day</i>	<i>QH(h)</i>	<i>So(mg/l)</i>	<i>Se(mg/l)</i>	<i>pH</i>	<i>T(°C)</i>	<i>% H</i>	<i>Total Gas (Vt) (ml)</i>	ΣVt (ml)	<i>VH₂</i> (mL/day)	ΣVH_2
17.06.2013	525	13	4	4268	2530	5,5	37	49	2500	2500	1225	
18.06.2013	525	13	4	5150	2765	4,8	37	63	3500	6000	2205	1225
19.06.2013	525	13	4	5160	1956	4,5	37	60	3800	9800	2280	3430
20.06.2013	525	13	4	5100	2340	4,4	37	62	3500	13300	2170	5710
21.06.2013	525	13	4	4980	2357	4,3	37	63	4300	17600	2709	7880
22.06.2013	525	13	4	4920	3260	4,4	37	67	4560	22160	3055	10589
23.06.2013	525	13	4	4960	3600	4,8	37	65	4750	26910	3088	13644
24.06.2013	525	13	4	4797	3635	4,7	37	67	4600	31510	3082	16732
												19814

Table A.23 Raw data for HRT = 2 hours

date	Q(ml)	L/day	QH(h)	So(mg/l)	Se(mg/l)	pH	T(°C)	% H	Total Gas (Vt) (ml)	Σ Vt (ml)	VH ₂ (mL/day)	ΣVH ₂
13.7.2013	1050	25	2	5230	2530	5,3	37	56	2800	2800	1568	
14.7.2013	1050	25	2	5230	2765	4,8	37	63	3500	6300	2205	1568
15.7.2013	1050	25	2	5237	3340	4,4	37	64	3800	10100	2432	3773
16.7.2013	1050	25	2	5100	3510	4,4	37	62	3750	13850	2325	6205
17.7.2013	1050	25	2	4750	3650	4,3	37	64	4300	18150	2752	8530
18.7.2013	1050	25	2	5130	3960	4,4	37	66	4560	22710	3010	11282
19.7.2013	1050	25	2	5270	4380	4,2	37	66	5600	28310	3696	14292
20.7.2013	1050	25	2	4640	3980	4,3	37	67	5500	33810	3685	17988
												21673