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Biyokütleden Biyohidrojen Üretimi

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Özet: Dünya'nın enerji ihtiyacı sürekli artmakta buna karşın fosil kökenli enerji kaynakları hızla tükenmektedir. Bu nedenle yenilenebilir ve sürdürülebilir alternatif enerji kaynakları arayışları artmış bu konulardaki araştırmalar çok önem kazanmıştır. Alternatif enerji taşıyıcısı olarak biyohidrojen, biyokütle gibi yenilenebilir enerji kaynaklarının kullanılmasıyla karanlık fermentasyon veya fotofermentasyon ile üretilebilmektedir. Karanlık fermentasyonda kullanılan glikoz ancak 2-3 karbonlu organik asitlere kadar parçalanabilmektedir. Buna karşın, karanlık fermentasyon ve fotofermentasyon proseslerinin ard arda yürütülmesi ile oluşan iki basamaklı biyoproses ile, karanlık fermentasyonda oluşan organik asitler fotofermentasyon aşamasında kullanılıp karbondioksite kadar parçalanmakta ve böylece tüm süreç maliyet ve enerji açısından daha uygun ve verimli olmaktadır.
Biyokütle olarak şeker pancarı melası kullanılarak, termofilik bakterilerin kullanıldığı karanlık fermentasyon ve devamında fotosentetik bakterilerin kullanıldığı fotofermentasyon ile iki basamaklı hidrojen üretim biyoprosesi geliştirilebilmektedir. Böylece, kolaylıkla ve düşük maliyetle temin edilebilen melas kullanılarak hidrojen üretimi gerçekleştirilebilir, melasın en iyi şekilde değerlendirilmesi sağlanır ve Türkiye'nin enerji ihtiyacının bir kısmı yenilenebilir ve sürdürülebilir bir enerji kaynağından sağlanmış olunur. Bu çalışmada Türkiye'de üretilen şeker pancarı melasından biyohidrojen üretim potansiyeli incelenmiştir. Ayrıca, üretilen hidrojenin Proton Değişim Zarlı Yakıt Pilinde (PEMFC) elektriğe dönüştürülmesiyle oluşabilecek enerji potansiyeli de değerlendirilmiştir. Buna göre Türkiye'de 2010 yılında üretilen melasdan iki basamaklı biyohidrojen üretimi neticesinde 14 362 073 011.98 mol H_2 ve 446 804 091.40 kWh elektrik enerjisi elde edilebilir.
Anahtar Kelimeler; Biyohidrojen, Biyokütle, Fotofermentasyon, Karanlık Fermentasyon, Melas

Biohydrogen Production From Biomass

Abstract: The world's energy demand is continuously increasing but the fossil fuels are being exhausted rapidly. Therefore, the search for renewable and sustainable alternative energy sources has been increased and the research in this subject has gained special importance. Biohydrogen as an energy carrier can be produced by dark fermentation or photofermentation through the use of renewable energy sources such as biomass. The glucose can be oxidized to 2-3 carbon organic acids in dark fermentation but with consecutive running of dark and photofermentation, the 2-3 carbon organic acids are used in the photofermantation making the all process economically and energetically feasible. Two stage hydrogen production bioprocess (dark fermentation followed by photofermentation) could be developed using sugar beet molasses. Thus, hydrogen production could be performed using molasses provided sufficiently, easily and at low cost. In this way, molasses will be utilized in the best way and some of energy demand of Turkey will be provided from renewable and sustainable energy resources. In this study, the potential of biohydrogen production from sugar beet molasses wais investigated. In addition, the electricity resulting from the usage of hydrogen in Proten Exchange Fuel Cell (PEMFC) was evaluated. Accordingly, 14 362 073 011.98 moles of H_2 and 446 804 091.40 kWh of energy could be obtained from the molasses produced in Turkey in 2010.
Key words; Biohydrogen, Biomass, Photofermentation, Dark Fermentation, Molasses

INTRODUCTION

Fossil based energy sources are limited but energy consumption in the world is increasing rapidly (Figure 1) [EIA, International Energy Statistics database, the web site: www.eia.doe.gov/oiaf/IEO/world.html]. In addition, the use of fossil based fuels leads to environmental pollution as they release green house gasses upon burning. So today, in addition to the existing energy sources alternative energy sources must be developed. Being clean and sustainable are

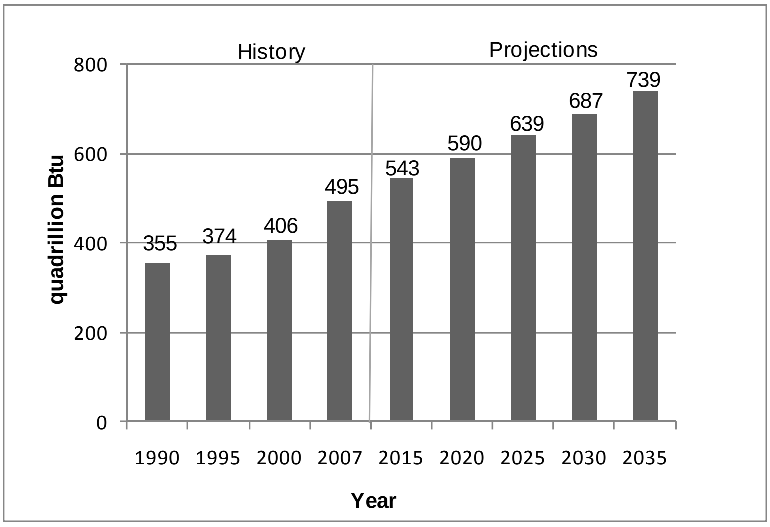

Figure 1. Energy consumption between 1990 and 2035 (EIA, International Energy Statistics database, web site: www.eia.doe.gov/oiaf/ieo/world.html).

extremely important properties for the alternative energy sources. Biohydrogen is a promising energy carrier because of the following features [Elam *et al.*, 2003]:

1. Hydrogen can be produced from renewable energy sources such as biomass, wind and solar energy.
2. Carbon-based gas emissions do not occur during the use of hydrogen.
3. Storage and transportation of hydrogen are possible.
4. Each country will produce its own renewable hydrogen sources, energy diversification and security will be created.
5. Hydrogen has a specific energy density of 120-142 Mj/kg which is higher compared to other energy sources such as ethanol (23.4 – 26.8 Mj/kg) and biodiesel (37.8 Mj/kg) (www.juliantrubin.com /encyclopedia/engineering/fuel_energy.html).

Today, hydrogen gas is produced mostly by non-biological means such as steam reformation which requires high temperature and pressures. However, biological hydrogen production methods are still being investigated and developed. And, hydrogen production at industrial scale has not yet been achieved. Biological hydrogen production processes can be classified as follows (Das and Veziroglu, 2001):

1. Hydrogen production by dark fermentation
2. Photobiological hydrogen production
2.1. Photoautotrophic hydrogen production
2.2. Photoheterotrophic hydrogen production
3. Two-stage hydrogen production

Hydrogen production processes given above are performed by a variety of microorganisms. The responsible enzymes are hydrogenases and/or nitrogenases (Kars and Gündüz, 2010). Thus, hydrogen production efficiencies are limited with the efficiencies of these enzymes. Below, each hydrogen production process is examined in detail.

Hydrogen production by dark fermentation

Many microorganisms living under anaerobic conditions can produce hydrogen. They use organic compounds for their cellular processes but the excess of energy (electrons) are utilized for hydrogen production by their hydrogenases (Akkerman *et al.*, 2003). For a cost-efficient biohydrogen production process, various biomasses and biowastes can be used as substrates. But, those substrates need some enzymatic and mechanic pretreatment to be converted into simple sugars which are readily utilizable by microbes. The composition of dark fermentation effluent depends upon the type of microorganism and bioreactor operating conditions. But, dark fermentation effluent is mainly composed of acetate, lactate, butyrate when mesophilic and thermophilic anaerobes are used (Argun *et al.*, 2009; Uyar, 2008 (PhD Thesis)). Thus, the effluent is valuable and still rich in organic acids. In the process, anaerobic sludge and mixed culture could also be used and considerable amount of hydrogen can be obtained (Argun *et al.*, 2009; Azbar *et al.*, 2009). But, the composition of microorganisms changes upon changing process parameters and by time (Hallenbeck and Ghosh, 2009; Koskinen *et al.*, 2007; Maintinguer *et al.*, 2008; Lin *et al.*, 2008; Lin *et al.*, 2006). Therefore, one can obtain different results in various operations and this abolishes the reproducibility of the results. Moreover, using microbial consortia does not allow genetic improvements in hydrogen production. Theoretically, hydrogen production efficiency for one mole of glucose occurs as follows:

$$C_6H_{12}O_6 + 4H_2O \longrightarrow 2CH_3COO^- + 2HCO_3 + 4H^+ + 4H_2,\ \Delta G^0 = -206\ kJ.mol^{-1} \quad (1)$$

One of the advantages of hydrogen production by dark fermentation is that the process does not require light as an energy source. *Clostridia* species, rumen bacteria, thermophilic bacteria like *Caldicellulosiruptor* and *Thermotoga* and methanogenic archea are hydrogen producing anaerobes. *Caldicellulosiruptor saccharolyticus* has been reported to produce 3.3 mol H_2 / mol hexose at 70 ºC with 83 % efficiency using sucrose (Akkerman *et al.*, 2003). The microorganisms mentioned above have different hydrogen production metabolisms, therefore, the bioreactor need to be selected according to the type of microorganism.

Photobiological hydrogen production

Photobiological hydrogen production is performed by photoheterotrophic and photoautotrophic microorganisms. Microalgae and cyanobacteria can perform photoautotrophic hydrogen production using carbondioxide and light as carbon and energy sources respectively (Kars, 2008 (PhD Thesis)). Photoheterotrophic hydrogen production occurs if there are available organic substrates as carbon and energy source under light. Below both processes are investigated in detail.

Photoautotrophic hydrogen production

Microalgae and cyanobacteria can synthesize organic compounds $[C_n(H_2O)_n]$ using sun light and CO_2. Under anaerobic conditions, these microorganisms can produce hydrogen by water photolysis using light. This reaction given below is catalysed by oxygen sensitive hydrogenase.

$$4H_2O + light \longrightarrow 2O_2 + 4H_2,\ \Delta G^0 = +1498\ kJ \quad (2)$$

In this process, the evolved hydrogen is mixed with oxygen since water is split by light. Therefore, hydrogen should be purified in another process. The process in which both hydrogen and oxygen are simultaneously produced is called direct photolysis. In addition, the process in which the hydrogen production is separated temporally and spatially is called as indirect photolysis. In *Chlamydomonas reinhardtii*, green algae, the stage of oxygen and organic compound accumulation is separated from the stage of hydrogen production (Melis *et al.*, 2000; Melis, 2002). In this way, since hydrogen and oxygen production productions are separated temporally, there is no need for additional step for hydrogen purification. In heterocyst cyanobacteria, these stages are separated spatially. There are improvements in hydrogen production using green algae with direct photolysis (Hallenbeck and Benemann, 2002).

Photoheterotrophic hydrogen production

Photoheterotrophic hydrogen production can be performed by anoxygenic photosynthetic bacteria like *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirullum rubrum* and *Rhodopseudomonas palustris* [Kars and Gündüz, 2010]. These bacteria can produce hydrogen by their nitrogenases using organic acids like acetate, lactate and malate under anaerobic conditions. The primary function of nitrogenase is fixing molecular nitrogen into ammonia. But, in the case of anaerobic conditions, all the electrons received from ferrodoxin are used to produce hydrogen at the expense of ATP. Since hydrogen evolution is catalysed by nitrogenase enzymes, the effciciency of the reaction depends on type, amount and activity of the enzyme (Hallenbeck and Benemann, 2002).

While microalgae and plants have two photosystems (PSI and PSII), these photosynthetic bacteria have only one. This photosystem is not powerful enough to split water and therefore the hydrogen evolved is free from oxygen (Akkerman *et al.*, 2003). In the ammonia free environment, two electrons and four ATPs are required to produce one mole of hydrogen and the reaction is as follows:

$$2H^+ + 2e^- + 4ATP \longrightarrow H_2 + 4ADP + 4P_i \quad (3)$$

The properties of hydrogen production process by purple non sulfur (PNS) bacteria are summarized as follows:

1. Since they perform anoxygenic photosynthesis, the evolved hydrogen is free from oxygen.
2. Hydrogenase enzyme is negatively affected in the presence of hydrogen gas, however, nitrogenase enzyme can produce hydrogen under 100 % hydrogen atmosphere.
3. PNS bacteria can utilize a wide range of substrates.
4. PNS bacteria have a wide range of metabolic activities. Therefore, they can cope with different physiological conditions.
5. Many genomes of PNS bacteria have been sequenced and genetic manipulation techniques have been optimized. Therefore, they could be genetically improved for hydrogen production (Kars and Gündüz, 2010).

Two-stage hydrogen production

Glucose is not completely decomposed into CO_2 in dark fermentation. Depending on the type of bacteria and substrate, dark fermentation effluent is composed of organic acids like acetic acid, lactic acid and butyric acid (Uyar, 2008 (PhD Thesis)). Therefore, using dark fermentation effluent in photofermentation as a substrate makes it possible to have a cost-efficient process. In this way, hydrogen production will occur in both processes and therefore hydrogen production efficiency will increase. And, the cost of the operations will be decreased. The reactions in dark and photofermentation are given below.

Dark fermentation:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CO_2 + 2CH_3COOH + 4H_2, \ \Delta G^{o\prime} = -206 \text{ kJ/mole} \quad (4)$$

Photofermentation:

$$CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 4H_2, \ \Delta G^{o\prime} = +104 \text{ kJ/mole} \quad (5)$$

Biomass types used for hydrogen production

A wide variety of biomasses have been used for biohydrogen production (Kapdan and Kargı, 2006; Şentürk and Büyükgüngör, 2010). It is possible to classify these substrates as follows:

1. Energy plants like switch grass and miscanthus cultivated for biofuel production.
2. Industrial and municipal wastes like sugar and olive oil refinery.
3. Agricultural and animal wastes.

Some examples of substrates used for hydrogen production are; sugar beet, sweat sorghum or sugar cane molasses, potato steam peels, olive mill waste water etc. In Turkey, there are significant biomass potentials such as sugar beet molasses and olive mill waste water. In Table 1, various types of substrates, microorganisms and operational modes are listed.

RESULTS

After giving brief information about biological hydrogen production processes and types of available biomasses, the potential of Turkey for biohydrogen production from sugar beet molasses and conversion of produced hydrogen into electricity by fuel cell will be investigated in the following sections.

The potential of Turkey for biohydrogen production from sugar beet molasses

Molasses is the final syrup/liquor obtained in the sugar production process and contains about 50 % (w/w) sucrose. As there are also vitamins, organic and inorganic compounds in molasses, it is a rich mixture. Therefore, molasses is one of the valuable substrates and used as a substrates in many processes like yeast, ethanol and animal food production (www.konyaseker.com).

Below, the amount of hydrogen that could potentially be produced from molasses in Turkey will be calculated. According to the data obtained from Turkish Statistical Institute (TurkStat), 17 942 108 tons of sugar beet have been produced in 2010 in Turkey (www.tuik.gov.tr). Considering the fact that processed sugar beet produces molasses by nearly 4 % (w/w) as by product, 717 684 tons of molasses are expected to be produced. And, if it is assumed that sucrose constitutes 50 % (w/w) of molasses, 358 842.16 tons of sucrose are obtained. This makes 1 048 326 497.22 moles of sucrose (sucrose: 342.30 g/mol).

Özgür *et al.,* have found that 13.7 mol H_2/mol sucrose could be produced from a two stage hydrogen production process in which first the extreme thermophilic bacterium *Caldicellulosiruptor*

1 **Table 1. Hydrogen production using various substrates and microorganisms in different operations**

Substrate	Microorganism	H_2 efficiency	Bioprocess	Reference
Sugar beet molasses	Mixed culture	1.7 mol H_2/mol hexose	Batch dark fermentation	(Hussy *et al*, 2005)
Starch	*C. pasteurium*	106 mL/g starch	Batch dark fermentation	(Liu and Shen, 2004)
Molasses	*Enterobacter aerogenes*	0.52 mol H_2/mol hexose	Batch dark fermentation	(Tanisho *et al*, 1998)
Potato starch	Mixed culture	0.59 mol/mol starch	Batch dark fermentation	(Logan *et al*, 2002)
Wheat starch	Mixed culture	0.83 mol/mol starch	Continuous dark fermentation	(Hussy *et al*, 2000)
Olive mill waste water	*R. sphaeroides* OU 001	4 mL/L culture h	Batch photofermentation	(Eroğlu *et al*, 2004)
Tofu waste water	*R. sphaeroides*	0.393 mL/mg DWh	photofermentation	(Zhu *et al*, 2002)

saccharolyticus (in dark fermentation) and then a PNS bacterium *Rhodobacter capsulatus* (in photofermentation) are used (Özgür *et al.*, 2010). So, taking this fact into account, 14 362 073 011.98 moles of H_2 can be produced from 1 048 326 497.22 moles of sucrose.

The electricity production with proton exchange membrane (PEM) fuel cell using produced hydrogen

In this section, the amount of electricity that could theoretically be produced using proton exchange membrane (PEM) fuel cell will be calculated. For this, all of the hydrogen which was found in the previous section will be fed to PEM fuel cell and the amount of electricity will be estimated. At 0 °C and 1 atm pressure, 3.5 Wh energy can be obtained from 0.045 mol of H_2 using a PEM fuel cell (www.siei.org/storagetank). If the PEM fuel cell is assumed to work with 40 % efficiency, 31.11 Wh is obtained for one mol of H_2. Since 14 362 073 011.98 moles of H_2 could possibly be produced from molasses in a two stage hydrogen production process, 446 804 091.40 kWh energy can be achieved using PEM fuel cell.

An average monthly energy demand of a house is about 180 kWh in Turkey. 2 482 245 houses' monthly energy demand can be satisfied with those produced electricity. Or, it makes 206 854 houses' annual energy demand.

CONCLUSIONS

Using low cost substrates, genetic and biochemical improvements of microorganisms, efficient bioreactor designs and improvements in fuel cell technology will help to develop industrial scale hydrogen production processes. The wide range utilization of hydrogen as a fuel will provide many benefits. The environmental problems will lessen due to decreased CO_2 emissions; each country will provide its own renewable sources and standard of living will be increased. Moreover, although molasses is being used in some areas like animal feed and alcohol production, the utilization of molasses for the production of such a high value added product will help to develop cost efficient bioprocesses.

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