The microalgae *Scenedesmus* sp. is a potential source for biohydrogen production

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ABSTRACT

Biohydrogen production is among the most promising solutions to energy scarcity as a renewable source to replace fossil fuel. Most of hydrogen nowadays is produced by chemical means. Biohydrogen could be produced from vast arrays of substrates. In this study, the biohydrogen was produced from the Scenedesmus sp. microalgae by dark fermentation. The algal cultures were pretreated before digestion with the seeding material to investigate the best performance for hydrogen production. The seeding material was sludge from wastewater treatment plant. The pretreatments included microwave digestion, ultrasonic, hydrochloric acid pretreatment and a combination between acid pretreatment and microwave digestion. For the control, experiments without pretreatment were performed. Pretreated and not pretreated Scenedesmus sp. cultures were digested in batch experiment under mesophilic conditions with different pH-values to produce hydrogen. The quality and quantity of gas were monitored during the experiment by eudiometer for measuring the quantity of gas produced and infrared gas analyzer to know the composition of produced gas. The quantity and quality of gas volume produced differed between the various pretreatments. The highest H_2 concentration produced at pH 5.0 glucose fermentation of ca. 130 ml_N was attributed to ultrasonic pretreatment while the lowest (< 97 ml_N) was recorded by microwave and hydrochloric acid digestion. At pH 6.0 fermentation by the algal strain, the highest H_2 yield of ca. 118 ml_N was produced by microwave digestion at 70 °C for 15 min. The effect of Scenedesmus sp. pretreatments on organic acid yields as well as diluted organic carbon (DOC) and total nitrogen bound (TNb) as indirect measures for biohydrogen production was discussed

Key words: *Scenedesmus sp. – Pretreatments – Biohydrogen – Renewable energy – Dark fermentation.*

INTRODUCTION

For a sustainable development of the world, solving the problem of the energy dependence on fossil fuels is a primary issue. According to Ni *et al.* (2006) and Quintana *et al.* (2011), over 80% of the energy consumption is coming from fossil fuels. However, many environmental problems have been arisen due to excessive usage of petroleum fuels. This circumstance led to climate changes, greenhouse effect, degradation of environment and health issues, as well as to rapid exhaustion of natural energy sources. Apart from that, the emission of

carbon dioxide, CO_2 had become a major threat to daily life which builds up in the atmosphere. Therefore, sustainable energy production has gained increasing interest in all countries worldwide (Krupp, 2007 and Ni *et al*., 2006)

In contrast to chemical processes, hydrogen as a fuel (energy content of 122 kJ g^{-1}) is regarded as ideal, clean and sustainable energy source (Singh *et al*., 2013). It has higher potential to be considered as the energy carrier of the future. Since mid-1980s, studies had been done on hydrogen production, application as well as analysis. Hydrogen can be produced from renewable or non-renewable energy source (Alalayah *et al*., 2008; Momirlan and Veziroglu, 2002). However, hydrogen production from renewable sources are unlimited and have little impact on the environment compared to non-renewable sources. For example, there is zero carbon dioxide emitted with pure water if hydrogen was generated via electrolysis (Levin, 2004 and Levin and Chahine, 2010).

Despite of achieving sustainable generation of hydrogen as a fuel, a range of technologies is introduced. This includes water electrolysis and thermo-catalytic reformation for hydrogen rich organic compounds. However, the biological conversion of wastes to hydrogen could be alternative method that requires neither high energy nor production cost (Hawkes *et al*., 2007; Levin, 2004 and Levin and Chahine, 2010). Nevertheless, due to technical issues and challenges from hydrogen production in sufficient quantities to decrease cost, increasing transmission and distribution, the potential of hydrogen as a fuel replacer for future has yet to be studied and realized (Dunn, 2002).

Biological processes provide a wide range of approaches to generate hydrogen. Mostly, microorganisms are widely developed in this new generation as they offer higher potential in production of usable hydrogen. Renewable biomass is now the most economical and appropriate resource that is generated from food wastes and wastewater. In this respect, microalgae have been the prior choice in producing biomass as they can be used for food supplement or wastewater treatment (Lin *et al*., 2012 and Quintana *et al*., 2011). Wastewater contains high concentration of nitrogen in the form of ammonia that may be inhibitory for microalgal growth (Rawat *et al*., 2011). Several methods are used to harvest microalgae including centrifugation, flocculation and sedimentation (Mutanda *et al*., 2011). Selection of adequate methods is depending on cost effectiveness and high recovery efficiency.

In addition, hydrogen production via biological process includes bio-photolysis, photo fermentation and dark fermentation as they can be used to affect the global CO_2 levels. The production of hydrogen via dark fermentation have an important characteristic which is sustainability, and was already reported and examined in some projects. High yield of hydrogen production mostly occurred at mesophilic conditions with less energy demand (Brunstermann *et al*., 2010). Dark fermentation is the conversion of organic substrates to bio-hydrogen involving anaerobic conversion and proceeds without the presence of light. The advantages compared to other processes are lower energy requirement, simplified process, hydrogen production in higher rate and utilization of low value waste as raw materials (Levin, 2004; Levin and Chahine, 2010). According to Nandi and Sengupta (1998), the pure cultures of the genera *Clostridium Enterobacter* and *Bacillus* are involved in producing high yield of hydrogen. Clostridia bacteria are reported to be the dominant microorganisms in anaerobic hydrogen fermentation processes (Lin *et al*., 2012). Untill now, no commercial systems are

available, and the practical applications of biohydrogen and its processes not fully researched which make us facing a lot of questions. Among those, can biohydrogen systems be developed as practical and commercial applications or integrated with hydrogen fuel cell technologies to generate electricity at a practical scale? One of the significant constraints to the functional use of biohydrogen frameworks is that researchers who study biohydrogen frameworks don't converse with engineers who creates and develop hydrogen fuel cell technologies and vice versa. Hence, the rates of hydrogen created by biological frameworks are obscure to fuel cell engineers and the amount of hydrogen required for practical applications, for example, fuel cells, are obscure to biohydrogen analysts and researchers. In addition, the rates of hydrogen delivered by different biohydrogen frameworks are expressed in various units, making it hard to survey and compare the rates and measures of hydrogen produced by various biohydrogen technologies (Levin *et al*., 2004). This study was performed to assess the capability of the microalgae *Scenedesmus* sp. to produce biohydrogen by applying dark fermentation process under mesophilic conditions.

MATERIALS AND METHODS

Culture propagation and maintenance

Scenedesmus sp. (Figure 1) culture was kindly provided by Wastewater Treatment Laboratory, Faculty of Engineering, University of Duisburg Essen, Germany. *Scenedesmus* sp. is a single cell eukaryotic microalgae, belonging to kingdom *Plantae*. The culture was propagated on Allen and Arnon medium (Allen and Arnon, 1955) with continuous supply of air and continuous illumination of 3000 lux (Salim *et al*., 2011).

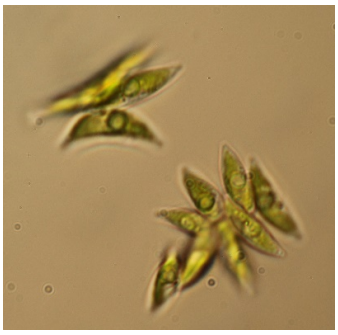

Fig.(1): Light micrograph for Scenedesmus sp. (x1000).

Harvesting and pretreatment of *Scenedesmus* sp. biomass

The sedimentation process was applied for concentrating the biomass of *Scenedesmus* sp. The flasks were left for 2 days to allow biomass to settle down, then the supernatant was removed, and the sedimentary layer was obtained for the dark fermentation process. *Scenedesmus* sp. cultures were pretreated before dark fermentation. Pretreatments adopted were those presented in Table (1). The experiment was carried out according to DIN 38414 S8 (Krupp, 2007), batch test was used to monitor and determine the amount of gas produced. Each substrate was held in triplicates as well as the reference (Glucose) while the inoculum (Sludge) which acts as baseline held in duplicates. Total soluble solids (TSS) were measured to obtain the concentration of the *Scenedesmus* sp. samples.

Seeding material (Inoculum)

Digested sludge was obtained from wastewater treatment plant in Duisburg Kasserfeild, Germany. The sludge was heated to 70 °C for one hour for 3 constitutive days. This process was done to select the spore forming and hydrogen producing clostridia and eliminating methanogens. Without clostridia, the fermentation will lead to propionate or lactate production without hydrogen gas production (De Vrije and Claassen, 2003).

Table(1): Summary of the probes used and the pretreatments done in biohydrogen production batch test for dark fermentation under mesophilic conditions.

Probes	Pretreatment	Specification
Glucose	No treatment	
	No pretreatment (NP)	
	Microwave digestion	70 °C for 10 min heat, 5 min hold (MW70)
		70 °C for 10 min heat, 5 min hold + 1 % HCl (MWA70)
		100 °C for 10 min heat, 5 min hold (MW100)
Scenedesmus sp.		100 °C for 10 min heat, 5 min hold + 1 % HCl (MWA100)
		Pressure of 2 bar for 10 min heat, 5 min hold (MWP)
	Sonication (US)	Power 4 kW, duty cycle 50% for 5 min
	Hydrochloric acid	1 % HCl for 12 hour (HCl 1%)
		5 % HCl for 12 hour (HCl 5%)
Inoculum (Sludge)	Heat pretreatment	70 °C for 1 hour for 3 successive days.

Hydrogen production by microalgae

The experiment held under mesophilic conditions (35 °C ± 2 °C). According to VDI 4630 (Krupp, 2007), it is not necessary to work in a nitrogen atmosphere. In order to ensure anaerobic conditions and preventing negative effect on hydrogen gas yield, the vessel headspace was flushed with nitrogen gas to get rid of air. The inoculum ratio was calculated according to Krupp (2007) as follows: the ratio of inoculum to substrate was 1:1 related to volatile solid (VS), therefore, 1g VS of substrate and 1 g VS of inoculum as heat - treated sludge were used in the experiment and then filled with tap water to 300 g. The pH was measured for each sample and adjusted to desired pH (the experiment was conducted at pH values of 5.0 and 6.0).

During the experiment, the vessels were shacked daily to prevent the formation of a layer of floating substances and allow the hydrogen gas production. The gas volume readings, temperature and ambient pressure were daily recorded. The gas volume was determined by liquid replacement method in eudiometers. The gas collected was tested for the presence of hydrogen gas, carbon dioxide, oxygen, and methane.

Since the gas production starts within the first week of the experiment, gas production was observed after 5 to 8 days. At the end of the experiment, the pH values of the tested samples were determined. Organic carbon forms of the residual including, organic acid, diluted organic carbon (DOC), total nitrogen bound (TNb), dry matter (DM) and volatile solid (VS) were assayed.

Experimental setup

The experiments were carried out using the method DIN 38414 S8 (Krupp, 2007). The anaerobic batch test took place in 0.5 L fermentation vessels at mesophilic conditions (35 °C). Substrate tests and reference (glucose) were conducted in triplicate, while inoculum (base line) was tested in duplicate. Quality and quantity of hydrogen gas produced were examined. In addition, parameters of the residuals including DOC, TN_b, and pH were determined.

RESULTS AND DISCUSSION

Hydrogen production from *Scenedesmus* sp. at pH 5.00.

The morphological features of the microalgae *Scenedesmus* sp. after the various pretreatments are illustrated in Fig. (2). Results in Fig. (3) indicate that glucose sample, at pH 5.00, which acted as reference, had the highest accumulated volume of gas produced (384.87 ml_N) at day 2 throughout the dark fermentation process then tended to decrease after that. The batch test was stopped on day 4 where the sample reached maximum yield with no more gas produced. Sludge inoculum, which acted as base line for the experiment, produced the lowest carbon dioxide and water levels. The accumulated volume of gas produced in sludge treatment was 47.96 ml_N gas on day 6.

Under dark fermentation conditions, microalgae samples and its pretreatments showed that they had almost the same volume of gas (Figure, 4). The gas production increased gradually with time as the biomass started to be degraded. The highest accumulated volume of gas was around 129.03 ml_N for ultrasonic pretreatment (US), while the lowest quantity of 96.65 ml_N was produced in microwave digestion (MWA70) treatment. Regarding H_2 production (Figures 3 and 4), it was found that the reference sample (glucose) produced the highest H_2 concentration as 51.61% of total gases produced. While the remainder samples containing microalgae and its pretreatments produced the lowest H_2 concentration compared to reference sample. The concentrations of H_2 produced from microalgae without pretreatment (NP) were 16.2 and 14.49 % for microwave digestion at 100 °C /15 minutes + HCl 1% (MWA100), respectively, while the lowest (7.79 %) was produced for ultrasonic pretreatment (US). In this respect, Levin *et al*., (2004) showed that dark fermentation systems seem to have the considerable potential to be created and developed as practical biohydrogen frameworks. Substantial enhancements, can be made through fast gas removal and separation, bioreactor design, and genetic modification of the microorganisms.

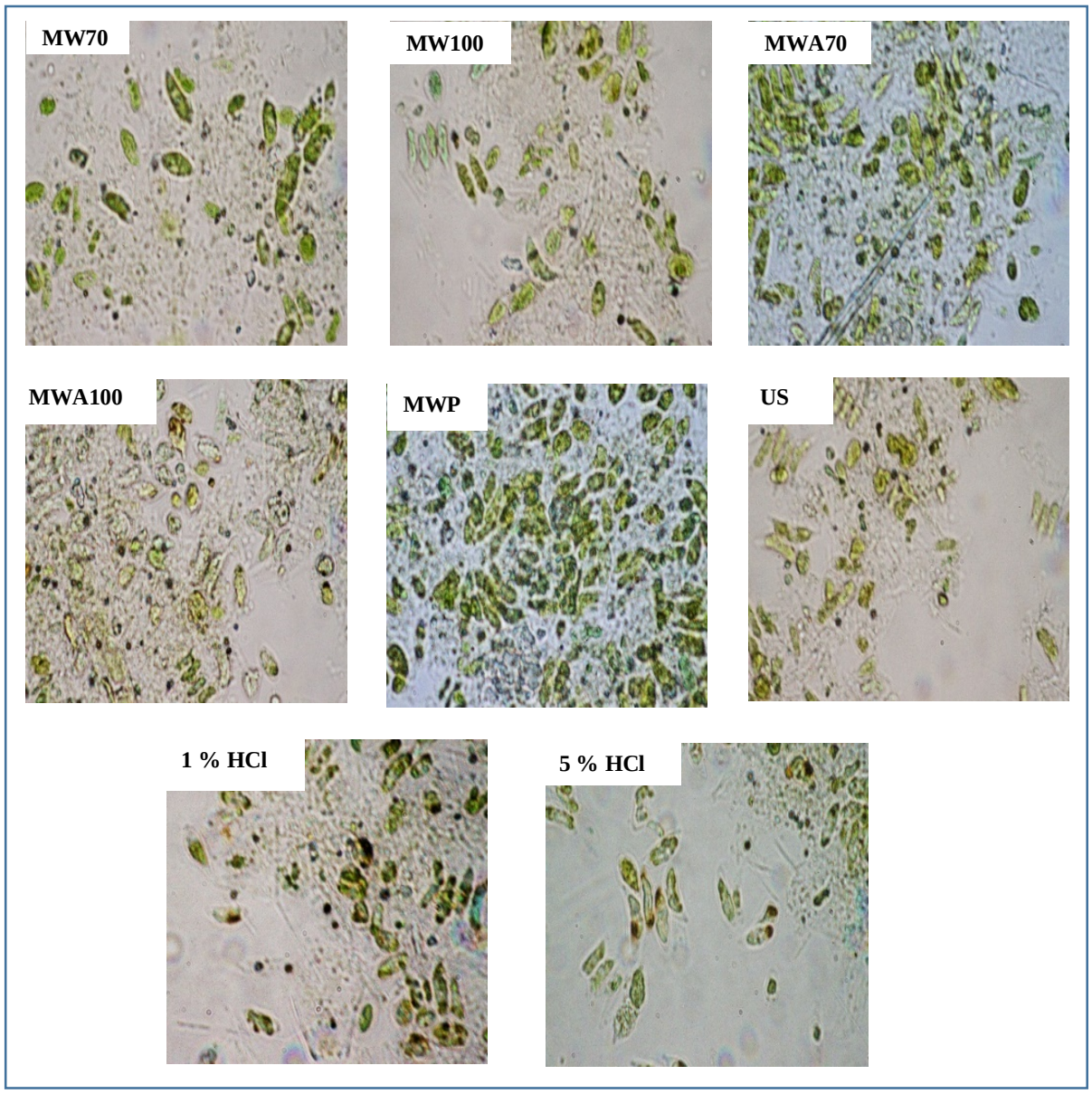

Fig. (2): Light micrographs for pretreatments done on Scenedesmus sp.MW70, Microwave digestion at 70 °C for 10 min. heat, 5 min. hold, MW100, Microwave digestion at 100 °C for 10 min.heat, min. hold, MWA70 Microwave digestion at 70 °C for 10 min. heat, 5 min. hold + 1 % HCl,MWA100 icrowave digestion at 100 °C for 10 min. heat, 5 min. hold + 1 % HCl, MWP microwave digestion pressure 2 bar for 10 min. heat, 5 min. hold,US Sonication by power 4 kW, duty cycle of 50% for 5 min., 1 % HCl acid treatment by 1% HCl for 12 hour, and 5% HCl acid treatment by 5 % HCl for 12 hour.

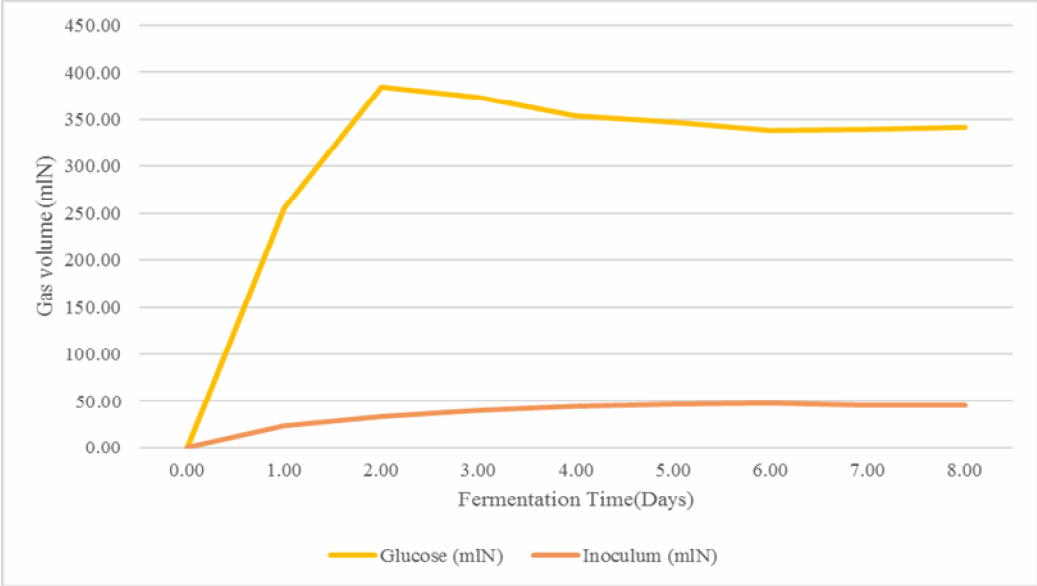

Fig. (3): Gas volume (ml_N / g VS) produced at pH 5.0 fermentation for glucose and sludge samples.

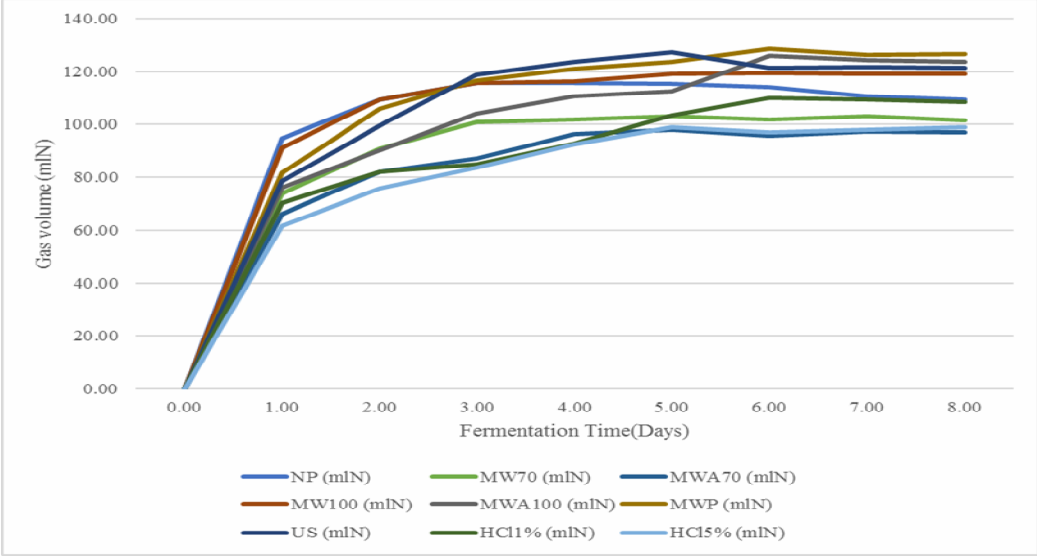

Fig. (4): Gas volume (ml_N / g VS) produced at pH 5.0 fermentation for all tested samples.

DOC, TNb, organic acids and pH.

Data in Table (2) and Fig. (5) revealed that, glucose led to the highest concentration of organic acid, with an average of 1420 mg/l. In addition, the reference sample produced high volume of H_2 with an average of 154.83 ml_N/g VS. In this sample, there was no further process after production of organic acids and the end product was only H_2 and organic acids as residual. In addition, high concentration of organic acids led to high production of DOC. Glucose treatment exhibited the highest concentration of organic acids and DOC being 1021.9 mg/l. Other samples showed an average production of 324 mg/l. The lower concentration of organic acids means the lower the concentration of DOC (Brunstermann *et al.*, 2010).

On the other hand, results in Fig. (5) revealed that samples containing the microalgae and its pretreatments had higher concentrations of TN_b of 360 mg/l as average compared to sludge and glucose at concentrations were 294.6 and 299.8 mg/l, respectively.

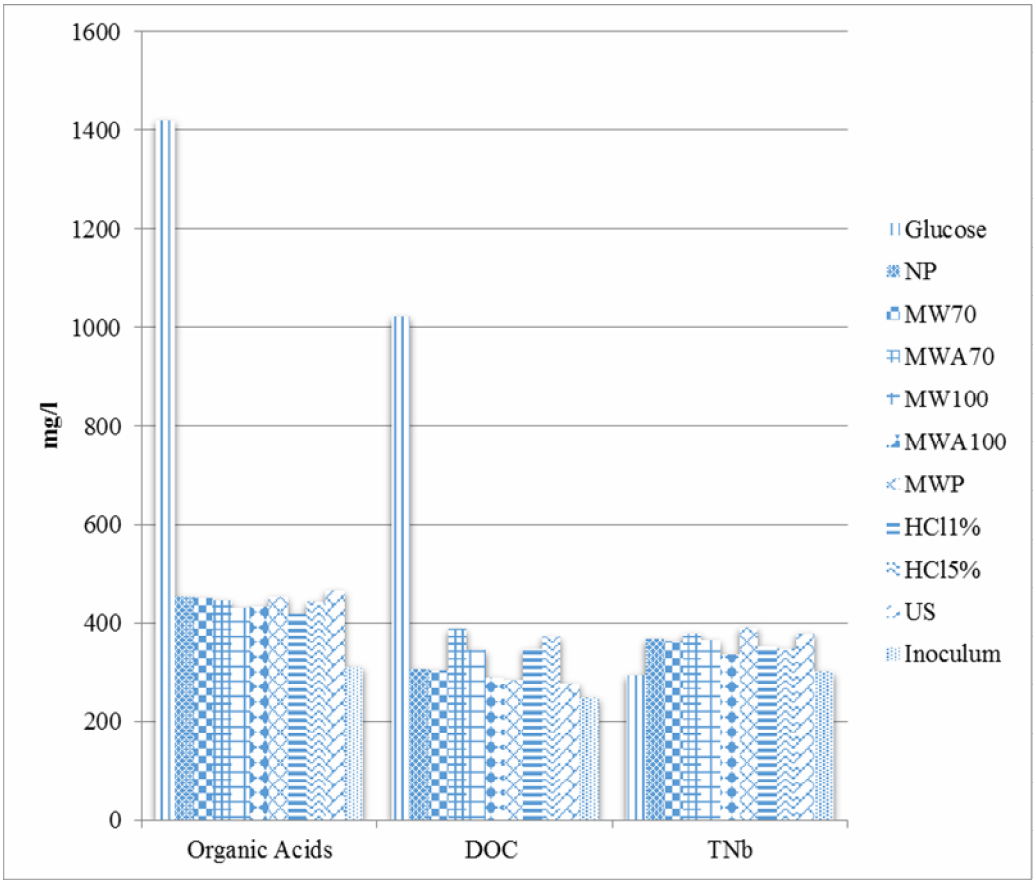

Fig. (5): Organic acids, DOC and TNb values (mg/l) for all treatments at pH 5.00.

Hydrogen production from *Scenedesmus* sp. at pH 6.00.

Concerning the efficiency of *Scenedesmus* sp. to produce H_2 at pH 6.00, results in Fig. (6) indicated that glucose sample had the highest accumulated volume of gas produced (305.57 ml_N) on day 6. Gas production started to decrease after day 2. This suggests that batch test should be stopped on day 4 since the sample reached its maximum yield with no more gas produced. The inoculum which acted as base line for the experiment, produced the lowest gas volume. The accumulated volume of gas mixture was 24.06 ml_N after 3 days. It is well established that, environmental and ecological conditions are the significant parameters to be controlled for hydrogen generation and production. For example, medium's pH has a great influence on hydrogen production yield, biogas composition, specific hydrogen production rate and type of organic acids produced at the end of fermentation. The reported pH range for maximum hydrogen yield is between pH 5.0 and 6.0 (Chen *et al.*, 2001; Fang and Liu, 2002; Khanal *et al*., 2004; Lay *et al*., 1999 and Lay, 2000, 2001). However, a few specialists reported the ideal pH range was between 6.8 and 8.0(Collet *et al*., 2004; Kanai *et al*., 2005; Lay, 2001 and Liu and Shen, 2004 and Zhang *et al*., 2003) and around pH 4.5 for the thermophilic culture (Shin et al., 2004). Previous studies indicated that final pH in anaerobic hydrogen production is around 4.0–4.8 regardless of initial pH (Lay, 2001; Liu and Shen, 2004; Liu *et al.*, 2003; Morimoto *et al*., 2004; Yokoi *et al.*, 2001 and Zhang *et al*., 2003). The decreasing in pH is because production of organic acids which affects the buffering capacity of the medium results in low pH at the end of fermentation (Khanal *et al.*, 2004). Gradual decrease in pH inhibits hydrogen production since pH affects the activity of iron containing hydrogenase enzyme (Dabrock *et al.*, 1992). Hence, control of pH at the ideal level is required, hence initial pH additionally affects the extent of lag phase in batch hydrogen production (Kapdan and Kargi, 2006). Respecting microalgae pretreatments, results showed that, they exhibited almost the same volume of gas mixture produced (Figure, 7). The gas mixture production gradually increased with time as the biomass started to be degraded by the bacteria. The accumulated volume of gas was 117.50 ml_N for microwave digestion at 70 ℃ /15 minutes (MW70) being the highest, and 79.32 ml_N for ultrasonic pretreatment (US) being the lowest. At the same time, results in Fig. (4) revealed that the reference sample produced the highest H_2 concentration of 38.07%. While the rest of samples containing microalgae and its pretreatments produced low H_2 concentration compared to reference sample. This phenomenon may be caused by anaerobic digestion of bacteria, the produced hydrogen in the beginning might be consumed by bacteria in the sample and CO_2 was generated as end product (Brunstermann *et al*., 2010).

The concentration of H_2 produced from microalgae could be arranged in the following descending order: microalgae without pretreatment (NP) 15.57 % >13.41 % for microwave digestion at 70 ℃ /15 minutes (MW70) > 12.05 % for microwave digestion at 70 ℃ /15 minutes + 1% HCl (MWA70) > 11.55% for ultrasonic pretreatment (US) > 10.71 % for acid treatment by 5% HCl (HCl5%) > 9.73 for acid treatment by 1% HCl (HCl1%) > 8.94 % for microwave digestion 100 ℃ /15 minutes + 1% HCl (MWA100) > 5.26% for microwave treatment by 2 bar pressure for 15 minutes (MWP). Table, (3) shows the produced gas volumes as ml_N per gram volatile solids.

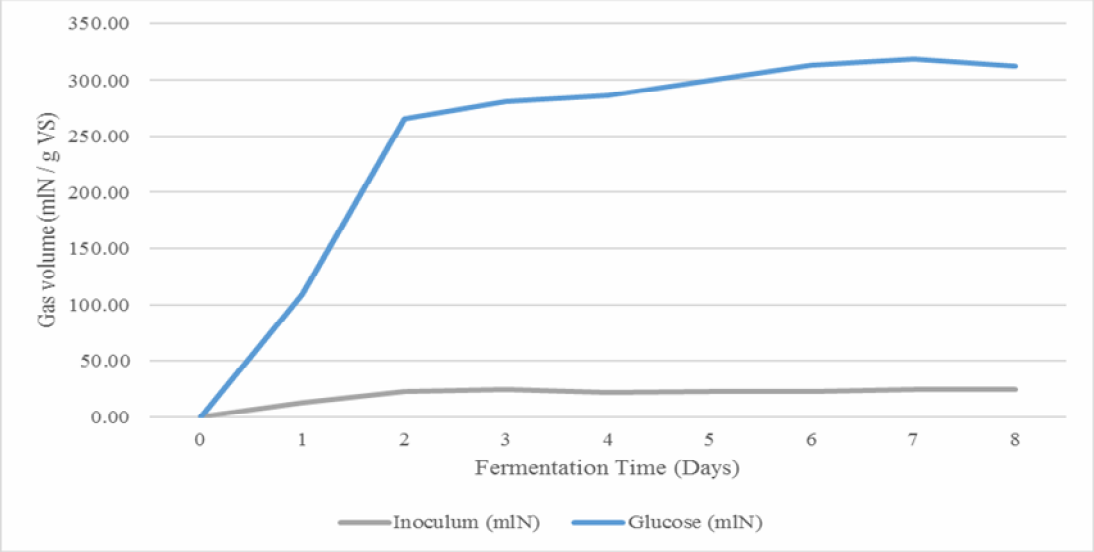

Fig. (6): Gas mixture volume (ml_N) produced at pH 6.0 fermentation conditions for glucose and sludge samples.

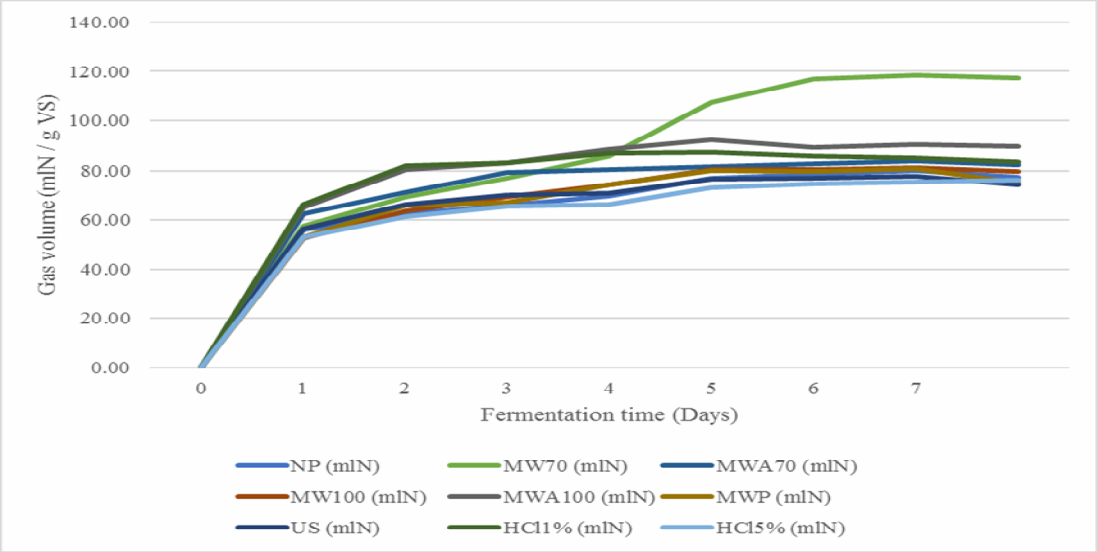

Fig. (7): Gas mixture volume (ml_N) produced at pH 6.0 fermentation for all samples.

Table (2): Production of different gases, for the tested samples at pH 5.00 .

Sample	H_2 (ml_N/gVS)	CO_2 (ml_N/gVS)	CH_4 (ml_N/gVS)
Glucose	154.83	143.36	1.80
NP	16.20	80.99	2.81
MW 70	10.92	86.46	2.62
MWA 70	10.67	76.97	2.36
MW 100	10.41	86.73	2.86
MWA 100	16.44	91.57	2.00
MWP	8.92	99.10	1.98
US	8.57	99.94	1.50
HCl 1%	9.57	87.83	2.61
HCl 5%	8.67	78.76	2.57

Table (3): Gas concentrations produced from the tested samples at pH 6.00.

Sample	H_2 (ml_N / g VS)	CO_2 (ml_N / g VS)	CH_4 (ml_N / g VS)
Glucose	110.39	176.83	2.78
NP	10.90	56.50	2.60
MW 70	13.41	82.01	4.58
MWA 70	8.43	58.12	3.45
MW 100	6.26	59.45	4.29
MWA 100	5.40	71.42	3.18
MWP	4.21	72.70	3.09
US	8.08	59.96	1.95
HCl 1%	7.78	70.03	2.19
HCl 5%	7.49	59.21	3.30

DOC, TNb, organic acids and pH

In respect to the influence of different treatments on DOC, TNb, organic acids and pH, results in Fig. (5) show that glucose produced the highest concentrations, of organic acids, with an average of 1320 mg/l compared to other samples. This indicates that the reference sample produced the highest concentration of H_2 *i.e.* 110.39 ml_N/g VS (Table 3). In this sample, there was no further process after production of organic acids and the end product was only H_2 and organic acids as residual. Kapdan and Kargi (2006), concluded that utilization of simple sugars like glucose is a biodegradable carbon source, present a lot in the industrial effluents and can be obtained from agriculture wastes easily. Theoretically, 12 moles of hydrogen gas is produced from bioconversion of 1 mol of glucose. According to reaction stoichiometry, 4 mol H_2/mol glucose are produced from bioconversion of 1 mole of glucose into acetate, while when the butyrate is the end product, only 2 mol H_2/mol glucose are formed. 2.0–2.4 mol/mol is the highest hydrogen yield produced from glucose (Fang

and Liu, 2002; Morimoto et al., 2004 and Ueno et al., 2001). Generation of butyrate as opposed to acetate might be one reason for deviations from the theoretical yield. Fang and Liu (2002) suggested that partial biodegradation of glucose could be another explanation for lower hydrogen yield (Fang and Liu, 2002).

However, the yield could be under 1.7 mol H_2/mol glucose even when more than 95% of glucose was degraded (Lin and Chang, 2004). In this manner, usage of substrate as an energy source for bacterial development and growth is the principle purpose for acquiring the yields lower than theoretical estimations (Kapdan and Kargi, 2006).

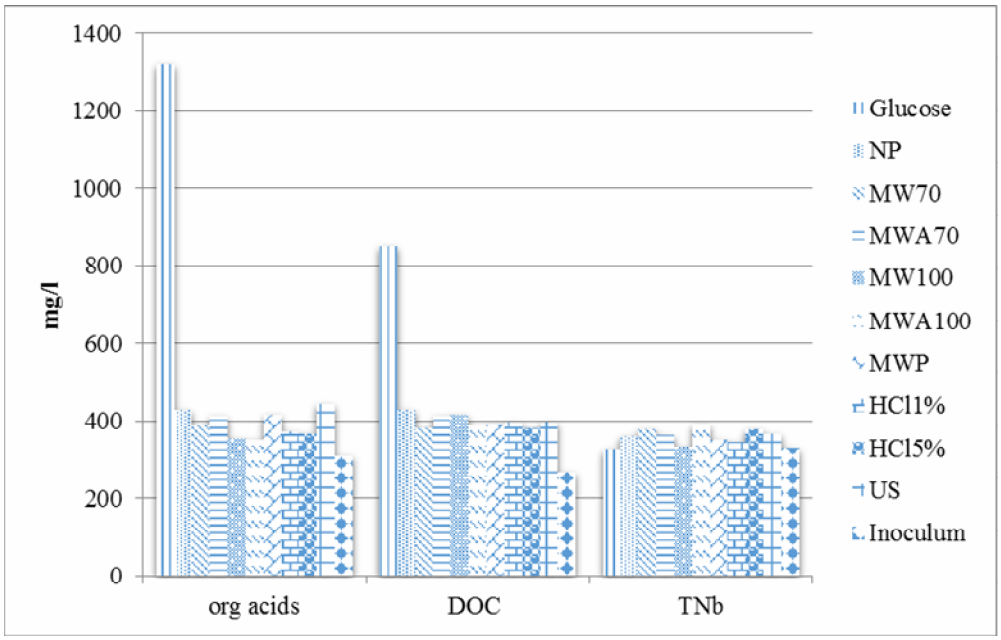

Fig. (8): Organic acids, DOC and TNb values for all treatments at pH 6.00.

Regarding *Scenedesmus* sp., samples and its pretreatments, it seem that the bacteria used H_2 and organic acids in the sample to produce CO_2 in the dark fermentation process. For instance, there was high concentration of CO_2 in the gas sample (Table3) and low concentration of organic acids left as end products in alga samples (Figure8). On the other hand, results in Fig. 8 indicate that high concentration of organic acid led to high concentration of DOC, while glucose sample had the highest concentration of organic acids and DOC. 850 mg/l, and the rest of samples had an average of 324 mg/l. It was suggested, therefore, that the lower concentrations of organic acids may lead to the lower concentration of DOC (Brunstermann *et al.*, 2010). In addition, samples containing microalgae and its pretreatments had higher concentration of TN_b, *i.e.* 364 mg/l compared to base line and reference samples, being 329.4 and 326.8 mg/l, respectively (Fig.8).

CONCLUSION

Hydrogen could be considered as the 'energy for future'. Because unlike fossil fuels, it is not promptly accessible in nature. Therefore, new procedures and processes should be created and developed for cost-effective production of hydrogen gas as alternative source of energy. Chemical methods, for example, steam reforming of hydrocarbons and partial oxidation of fossil fuels works at high temperatures, and in this manner are energy escalated and costly ineffective. Also, biological techniques offer distinct preferences for hydrogen creation, for example, operation under mild conditions and particular conversions. In any case, crude and raw materials cost is one of the significant impediments for bio-hydrogen production. Usage of some carbohydrates for example starch or cellulose containing solid wastes and/or some food industrial wastewaters is an appealing methodology for bio-hydrogen production. Strategies used for bio-hydrogen generation and production include (a) water splitting by photosynthetic algae, (b) dark fermentation of carbohydrate-rich wastes and (c) photofermentation of organic acid-rich wastewaters. Algal generation of hydrogen is somewhat slow process, which requires sunlight and is restrained by oxygen. Hydrogen is evolved as a by-product during acidogenic phase of anaerobic digestion of organic wastes, which is known as dark fermentation process. The hydrogen production yield by dark fermentation is low and slow. Organic acids produced during the dark fermentation of carbohydrate-rich wastes might be converted to hydrogen and carbon dioxide gases by photoheterotrophic microorganisms. The procedure requires special organisms, light and strict control of the ecological conditions. Sequential or combined bio-processes of dark and photo-fermentation appear to be the most alluring methodology bringing high hydrogen yields from carbohydrate-rich wastes. Therefore, it is proposed that considerable research, innovative development studies are expected to enhance the 'state of the art' in biohydrogen production.

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الملخص العربي

طحلب الـ *Scenedesmus* sp. مصدر جيد لإنتاج غاز الهيدروجين الحيوى

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يعتبر الهيدروجين الحيوى احد اهم الحلول المستخدمة لحل مشكلة ندرة الطاقة وذلك لتميزه بالتجدد و إستخدامه كبديل للطاقة التقليدية (الوقود الحفرى) بالإضافة لأنه صديق للبيئة. حاليا معظم إنتاج الهيدروجين يتم بواسطة الطرق الكيميائية. تطرقت الدراسة فى هذا البحث لإنتاج الهيدروجين الحيوى من الطحالب عن طريق التخمر تحت الظروف المظلمة. وتم اختيار الطحلب وذلك لقدرتها على التغذية الذاتية وسهولة نموها بدون حدوث تنافس. وتم معالجة مزراع طحلب الـ *Scenedesmus* sp. قبل هضمها مع الحمأه بعدة طرق وهى اشعة الميكروويف و الموجات فوق الصوتية والحامض ومزيج بين معامله الحامض و اشعة الميكروويف و بعد ذلك تم خلطها بالحمأه و بدايه عملية التخمر. المزارع المعالجة و غير المعالجة تم تخميرها على دفعات تحت ظروف متوسطة الحرارة و على درجات مختلفة من درجات الحموضة .تم تكرار التجربة على درجتين مختلفتين من درجات الحموضة ٥ و ٦ و تم قياس حجم و جودة الغاز الناتج من عملية التخمير بإستخدام عملية إحلال الغاز محل سائل لقياس الحجم اما بالنسبة لتركيب الغاز تم عن طريق جهاز تحليل غازات يعمل بالأشعة تحت الحمراء. كان اعلى تركيز للهيدروجين المنتج على درجة حموضة ٥ لتخمير الجلوكوز، ١٣٠ مل، راجعا إلى المعاملات الأولية للموجات فوق الصوتية فى حين أقل تركيز للهيدروجين (< ٩٧ مل) انتج بمعاملة الهضم بأشعة الميكروويف + حمض الهيدروكلوريك. عند درجة حموضة ٦ كان اعلى تركيز منتج من الهيدروجين، ١١٨ مل، نتيجة المعاملة بالهضم بأشعة الميكروويف على ٧٠ درجة مئوية لمدة ١٥ دقيقة. أما عن تأثير المعاملات المسبقة لطحلب الـ *Scenedesmus* sp. على نسبة إنتاج الأحماض العضوية و نسبة الكربون و النيتروجين الحيوى كمقياس غير مباشر لإنتاج الهيدروجين الحيوى فقد تم مناقشتها.