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Photofermentative Hydrogen Generation in Presence of Waste Water from Food Industry

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1. Introduction

Constantly increasing demand for energy has created extensive consumption of fossil fuels and the thread of their exhaustion has become a serious concern. At the same time it has been an inspiration for search for new, environmental friendly energy sources, out of which hydrogen seems to be one of the most promising. It is easily accessible, harmless, renewable and effective (high heat of combustion) energy carrier (Ball, 2009). Within the numerous methods of hydrogen production, biological methods (so called “green technology”) have gained substantial importance. These methods consist of fermentative decomposition of organic substances, biophotolysis of water by algae and cyanobacteria, decomposition of organic compounds by photosynthetic bacteria and two-stage hybrid systems with fermentative and photosynthetic bacteria (Waligórska, 2006, Koku, 2002, Su, 2009).

Photofermentation represents the process where heterotrophic bacteria in the presence of light decompose organic substances and produce hydrogen and CO₂. It has been already shown that purple non-sulphur bacteria *Rhodobacter sphaeroides* act as efficient biocatalyst in the process of hydrogen production from the wastes coming from breweries and dairy industry. Brewery wastes carry high concentration of organic compounds (COD 0.8-2.5kg/hl of beer) and represent high volumes (1.3-1.8 hl/hl of beer). The amount of waste during beer production is enormous and equals the amount of water applied for production diminished with water present in beer (usually 3-4 hl of waste per 1 hl of beer). A chemical composition of waste strongly depends on the kind of beer produced and fermentation degree. Such waste can contain aminoacids, proteins, organic acids, sugars, alcohols, as well as vitamins of the B group. (Wojnowska-Baryła, 2002, Srikanth, 2009, Cui, 2009) As far as dairy wastes are concerned, they contain an average of 5-50 g O₂ /l. These wastes are mainly composed of remaining of milk, fats and whey. Typical Polish dairy produces 450-600 m³ of wastes per day, half of which goes directly to rivers, lakes and to the ground. These wastes easily undergo fermentation, which causes acidification, intense oxygen consumption, bottom sedimentation and growth of fungi. The organics in both dairy and brewery wastes represent the efficient substrate for *Rhodobacter sphaeroides* and seem to be a promising source for energy production. The efficient use of food wastes in hydrogen generation with

simultaneous degradation of these laborious wastes seems to be a very environmentally friendly solution. The US Department of Energy Hydrogen Program in United States estimates that contribution of hydrogen to total energy market will be 8-10% by 2025 (National Hydrogen Energy Roadmap, 2002). It is predicted that hydrogen will become the main carrier of energy in the near future due to environmental and universal applications reasons. It is clean, highly energetic energy carrier (142.35 kJ/g), with almost tripled gravimetric energy density compared to ordinary hydrocarbons. Although the described method is relatively simple and cheap it still requires optimization due to the obtained unsatisfied yields.

2. Materials and methods

2.1 Inoculum, medium and procedures

Photoheterotrophic bacteria *Rhodobacter sphaeroides* O.U. 001 ATTC 4919 (Fig.1) were cultivated on Van Niel's medium containing: K_2HPO_4 (1.0 g/l), $MgSO_4$ (0.5 g/l), yeast extract (10g/l) and tap water filled up to 1 l and then activated according to the procedure already described (Waligórska, 2006). For hydrogen generation a modified Biebl and Pfennig medium (Biebl, 1981) was applied. This standard medium contained: KH_2PO_4 (0.5 g/l); $MgSO_4 \cdot 7H_2O$ (0.2 g/l); NaCl (0.4 g/l); $CaCl_2 \cdot 2H_2O$ (0.05 g/l), L-malic (2.0 g/l); sodium glutamate (0.36 g/l), ferric tartrate (0.005 g/l); yeast extract (0.17 g/l) and microelements: $ZnCl_2$ (0.07 g/l); $MnCl_2 \cdot 4H_2O$ (0.1 g/l); H_3BO_3 (0.06 g/l); $CoCl_2 \cdot 6H_2O$ (0.2 g/l); $CuCl_2 \cdot 2H_2O$ (0.02 g/l); $NiCl_2 \cdot 6H_2O$ (0.02 g/l); $Na_2MoO_4 \cdot 2H_2O$ (0.04 g/l); HCl 25% (1ml/l).

The untreated food waste were initially filtered through cotton wool, next sterilized at 120°C by autoclaving for 20 min and re-filtered applying paper filter.

Wastes with different COD values (46 g O_2 /l for dairy wastes, 220 and 27 g O_2 /l for brewery wastes) after pretreatment were introduced to the medium, which did not contain L-malic acid. The medium was inoculated with bacteria 30% v/v (0.36 g dry wt/l). The process was performed in small vials (25 ml) made from sodium glass and filled with 12.5 ml of inoculated medium. Tightly closed vials were carefully deaerated with argon before starting the illumination. All experiments were carried out at $28 \pm 2^\circ C$ and pH after sterilization and inoculation varied between 7.0 and 7.2. The mercury-tungsten lamp (Ultra-Vitalux -300W from Osram) was applied in all experiments. The intensity of light during hydrogen generation was 9 klx (116 W/m²). The vials with Biebl and Pfennig standard medium was used as reference (Biebl, 1981).

2.2 Analytical methods

The content of H_2 , CO_2 was measured with gas chromatography (Varian GC-3800 equipped with Carboplot P7 capillary column and TCD). The loss of organic substances was monitored with COD measurement (dichromate method) after centrifugation of biomass (Standard methods, 1995). The biomass content was established spectrophotometrically measuring optical density at 660 nm (DU640 UV-VIS spectrophotometer from Beckmann). Cell dry weight was determined using gravimetric method. Six samples from the same kinetic measurement points at respective time intervals were mixed together, 10 ml of cell suspension was centrifuged at 12000 g for 12 min, the pellet was washed twice with

deionized water and dried at 80°C for 4 h. Elemental analysis of the foot wastes (C,H,N,O) was performed in triplicate using an elemental analyser (Vario EL III Elementary). Concentration of Fe, Ca, Mg in purified wastes was measured by ICP OES spectroscopy. The value of pH was measured with glass electrode ERH-11. The intensity of luminance was measured at the external wall of the bottle with a luxometer Lx204 made by Slandi, Poland and a pyranometer CMP3 by Kipp & Zonen (Waligórska, 2006). The light conversion efficiency (η) was calculated based on the following formula (Koku, 2002):

$$\eta(\%) = \frac{33.61 \cdot \rho \cdot V}{I \cdot A \cdot t} \quad (1)$$

where “V” is the volume of produced H₂ in liters, “ ρ ” is the density of the produced hydrogen gas in g/l, “I” is the light intensity in W/m², “A” is the irradiated area in m² and “t” is the duration of hydrogen production in hours.

Substrate efficiency Y_{sub} (l /l waste) was calculated as final hydrogen concentration per l of waste:

$$Y_{\text{sub}} = \frac{H_{\text{max}}}{V_{\text{waste}}} \quad (2)$$

where H_{max} is a final hydrogen concentration in l, V_{waste} is waste concentration in l.

Specific efficiency Y_{sp} (l H₂ / g COD) was calculated based on following equation:

$$Y_{\text{sp}} = \frac{H_{\text{max}}}{\text{COD}_{\text{loss}}} \quad (3)$$

The modified Gompertz (Eq. 4) was applied for calculations of cumulative amounts of hydrogen and carbon dioxide (Mu, 2007, Nath, 2008, Chen, 2006):

$$H = H_{\text{max}} \exp \left\{ -\exp \left[\frac{R_{\text{max}, H_2} e}{H_{\text{max}}} (\lambda - t) + 1 \right] \right\} \quad (4)$$

where: H - cumulative hydrogen (l/l_{medium}), H_{max} - maximum cumulative hydrogen (l/l_{medium}), R_{max, H_2} -maximum rate of hydrogen production (l/l/h), t - fermentation time (h), λ - lag time (h), e - exp = 2.718.

3. Results and discussion

3.1 Pretreatment of wastes

The wastes applied in this series of experiments required high temperature pretreatment (120°C for 20 min), which had significantly increased the efficiency of hydrogen production by removing from the crude waste microorganisms realizing competitive fermentation. The crude wastes were acidic (dairy waste pH 4.2, brewery waste pH 4.7) and contained high concentration of NH₄⁺ (40 mg/l dairy waste and 96 mg/l brewery waste), which can significantly reduce hydrogen production (Waligórska, 2009). High concentration of

N-NH₄⁺ as well as N₂ inhibits hydrogen production by nitrogenase. In the absence of nitrogen in the system the nitrogenase catalyses the reduction of protons to molecular hydrogen (Melis, 2006, Yakunin, 1988, Pawlowski, 2003, Dubbs, 2004).

The characteristics of applied wastes is given in Table 1. In order to establish the influence of the wastes pretreatment conditions on the final production of hydrogen a series of experiments with non-treated and sterilized waste were performed. These measurement were performed with solution containing brewery waste at concentration of 10% v/v inoculated with 10% and 30% v/v of inoculums. The results of these experiments are shown on Fig.2. The experiments with “raw”, undiluted dairy waste failed.

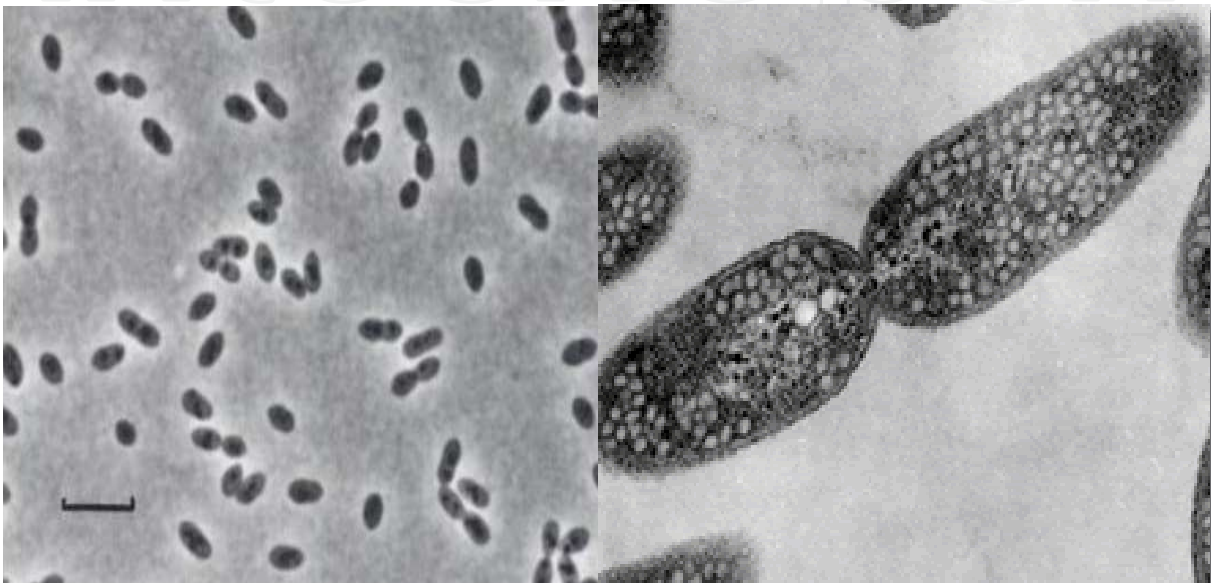


Fig. 1. *Rhodobacter sphaeroides* ATCC 17032. Micrographs performed with electron microscope with phase contrast (PCM). Tab on left micrograph equals 5 μm (Garritty, 2005).

Parameters	pH	COD [g/dm ³]	NH ₄ ⁺ [g/dm ³]	N [%]	C [%]	H [%]	S [%]	Ca [mg/l]	Fe [mg/l]	Mg [mg/l]
Brewery waste I	4.7	220	96	0.7	36.7	7	0.05	37.2	1.04	96
Brewery waste II	8.5	27	12	0.5	13.3	3	0.01	88.4	0.8	58.6
Dairy waste	4.2	46	40	1.05	35.5	6.3	0.08	1043	0.54	80

Table 1. Characteristics of the food wastes.

Application of the sterilized brewery waste with concentration of inoculum 10% v/v resulted in double amount of produced hydrogen. Triplication was observed at higher concentration of inoculums (30% v/v). Many laboratories apply similar pretreatment conditions. Thermal treatment at 95°C for 45 min (Yetis, 2000), filtration or sedimentation (Salih,1989) as well as dilution leads towards removal of fermentation bacteria and solid sediments from medium. Moreover, were applied: illumination with UV radiation, termal treatment at 50°C in presence of 1vol. % of hydrogen peroxide. It was found that only thermal sterilization was successful method.

Many food waste, for example dairy waste, contained significant amounts of whey particles. It was interesting to check whether microorganisms utilize only organic compounds from solution or may be originate from consumption of solid particles as well? The results indicated that at higher concentration of waste the amount of generated hydrogen increased about 40 – 60% when we non-filtered waste (Fig 3). The only exception can be observed in waste with lower concentration 5 % v/v. Here, large differences in hydrogen production are not observed.

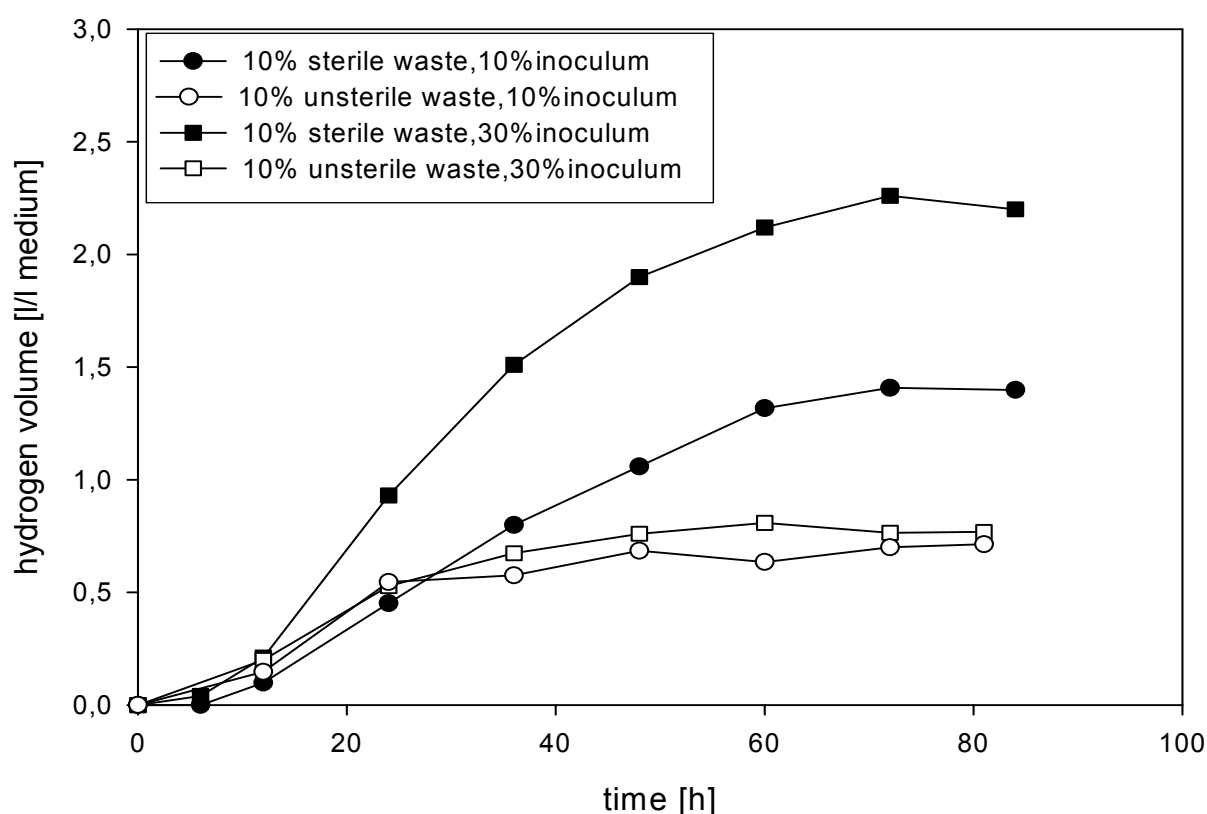


Fig. 2. Influence of sterilization of brewery wastewaters on kinetics of hydrogen generation. (Seifert, 2010)

The whey suspension contains 5 wt.% of lactose, proteins, fats and lactic acid. However, all these components can be an excellent source of organic carbon for *R. sphaeroides* during hydrogen generation (Koku, 2002) due to relatively good solubility. Obeid et al.(2009) used lactic acid as a source of organic carbon in hydrogen generation applying *Rhodobacter capsulatus* and obtained relatively high yield of H_2 (5.5 l H_2 /l) but the acclimatization time was long and lasted 24 h. Sugars, proteins as well as fatty acids were already applied as substrates in hydrogen photogeneration (Eroglu, 2004; Yokoi, 2002; Zhu, 1999)

3.2 Light intensity effect

For these series of experiments the medium containing 40% v/v of dairy waste and 10% v/v of brewery waste were applied. The media were inoculated with *Rhodobacter sphaeroides* O.U.001 in concentration 30% v/v 0,36 g dry wt/l. The effect of the light intensity was checked out for 5, 9 and 13 klx. (Fig.4). The highest volumes of hydrogen (3.2 l H_2 /l medium

for dairy wastes, and 2.3 l H₂/l medium for brewery wastes) were observed when 9 and 13 klx were applied.

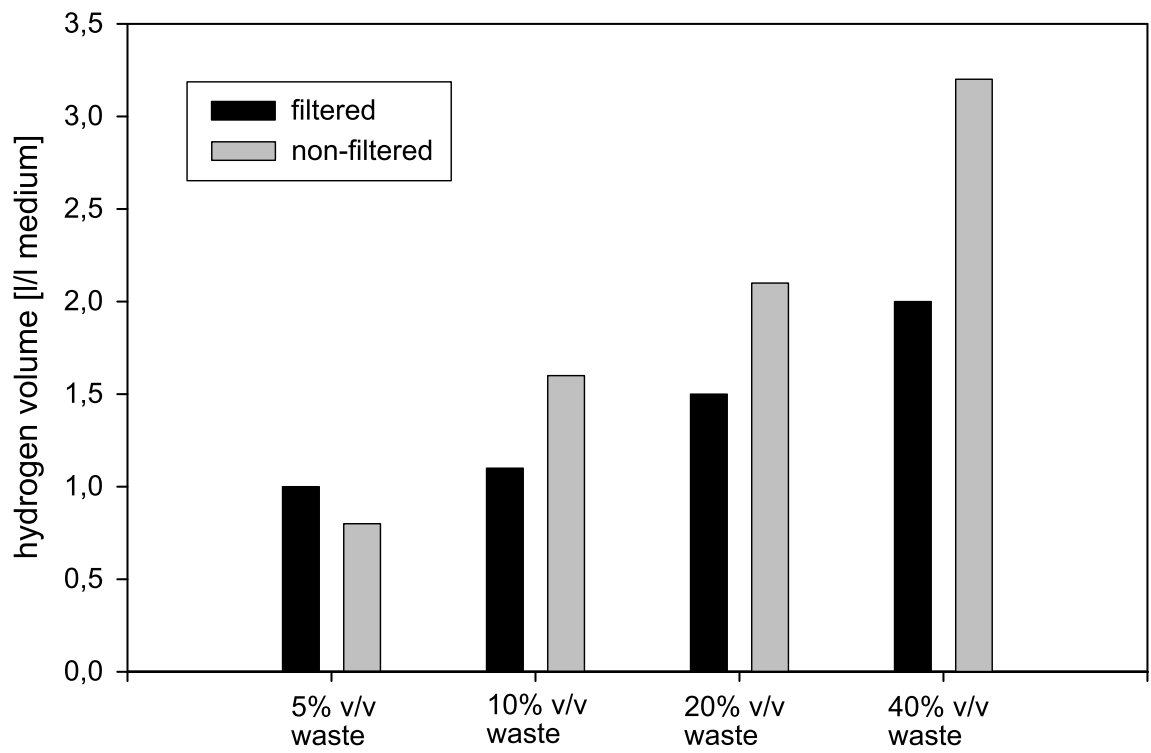


Fig. 3. Influence of filtration of dairy wastewaters on hydrogen generation.

Similar light intensity (8klx) was use by Zhu et al. for tofu wastewaters treated with *Rhodobacter sphaeroides*. Volume of hydrogen obtained for these conditions was 1.5 l H₂/l medium (2.8 l H₂/l medium when glucose was used) (Zhu, 1999). Nath et al. showed the best results of hydrogen generation when 10 klx was applied (Nath, 2009). Li et al., however, studying the photofermantation of glycerol with *Rhodobacter sphaeroides* ZX-5 proved the highest hydrogen production with light intensity not exceeding 5 klx (Li, 2009). Surprisingly, high light intensity was tested by Obeid et al. for photofermentation of lactate medium and *Rhodobacter capsulatus* IR3 (up to 50 klx). Highest effectiveness and rate of hydrogen production were obtained when 30-50 klx were used. These tests are essential taking into account that light intensity on sunny day can be higher than 100 klx.

Light intensity seems to be an important factor in hydrogen photogenerating process. On one hand increase at light intensity stimulates hydrogen production and biomass growth, on the other hand too high intensity may cause the reduction of nitrogenase activity or even damage of the cells (Asada, 1999, Uvar, 2005). An important parameter which shows the relationship between light intensity, irradiation area, duration of H₂ production and total H₂ amount is the light conversion efficiency (η , equation 1). It is the ratio of the total energy of the obtained hydrogen to the total energy input of the photobioreactor by solar radiation (Eroğlu, 2007). In our tests η reached the highest value when 9 klx was applied (2.4 % for dairy waste, 1.7 for brewery waste, Table 2) Results in Table 2 show that illumination with

5 klx leads also to high light conversion efficiency. However, in this case the duration of the process significantly increases. For this reason 9 klx seemed to be optimal and has been used for further experiments.

Light intensity [klx]	standard			40% dairy waste			10% brewery waste		
	final time [h]	hydrogen [l/l medium]	η [%]	final time [h]	hydrogen [l/l medium]	η [%]	final time [h]	hydrogen [l/l medium]	η [%]
5	106	2.26	1.76	72	1.7	1.9	96	2.0	1.7
9	76	2.3	1.73	60	3.2	2.4	60	2.26	1.7
13	48	2.0	1.33	60	3.15	1.7	60	2.3	1.2

Table 2. The effect of light intensity on duration of the process, hydrogen production and light conversion efficiency (η) (30% v/v inoculum).

3.3 The effect of inoculum concentration

In these series of experiments we tested several concentrations of inoculum introduced to the medium: 5-40 % v/v (0.086 g dry wt/l – 0.48 g dry wt/l) for standard medium and 10% and 30% (0.086 g dry wt/l and 0.36 dry wt/l) in case of medium containing wastes (Fig. 5). The optimum inoculum concentration in all cases turned out to be 30% v/v. Data in Table 3 indicate that the second higher concentration produces more hydrogen, shorter lag phase

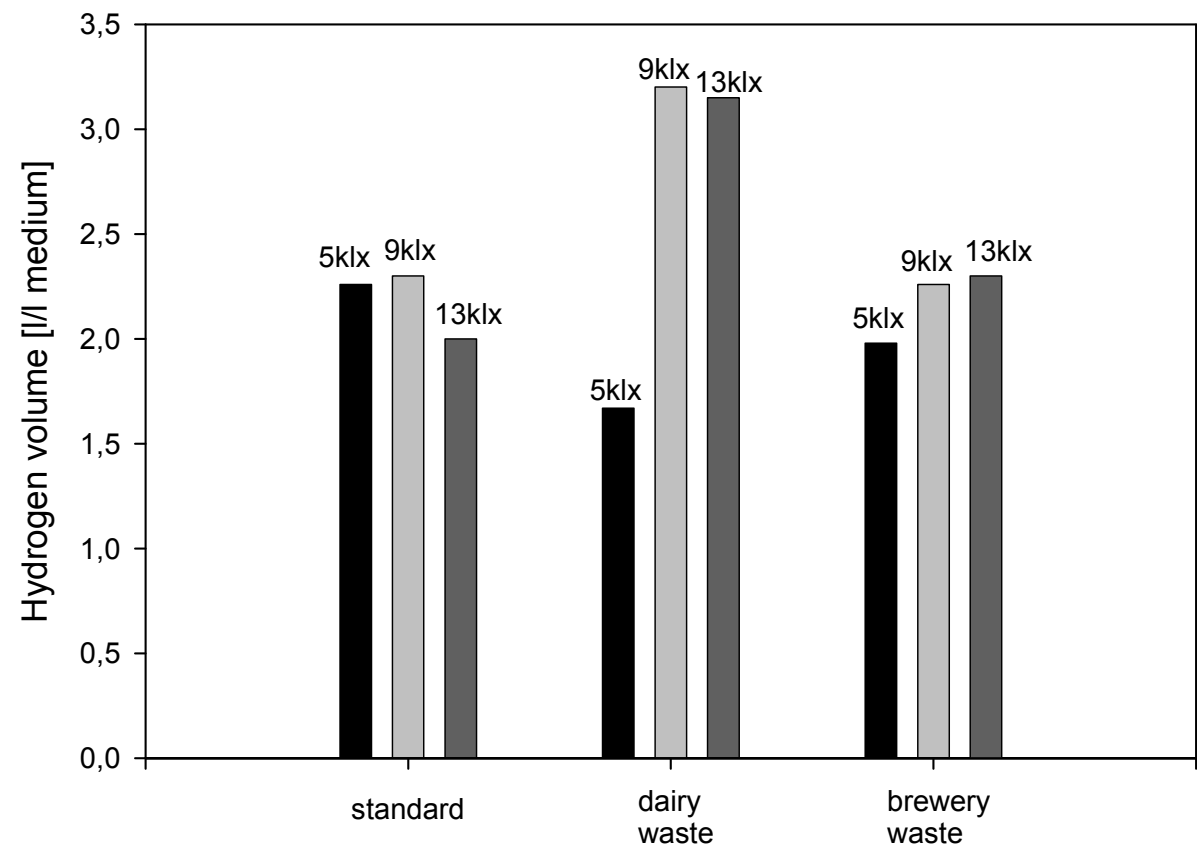


Fig. 4. The effect of light intensity on hydrogen production in photofermentation process (30% v/v inoculum, 40% v/v dairy waste, 10% v/v brewery waste)

and bigger COD loss. Increase to 40% lead to smaller amount of produced H₂. This effect seems to be caused by the fact that with the inoculum, apart from biomass, we also introduced metabolites which in high concentrations negatively influence the efficiency of photofermentation (Waligórska, 2006, Koku, 2002).

Inoculum concentration (g dry wt/l)	H _{max} (l/l)	R _{max,H2} (l/l/h)	λ _{H2} (h)	Y (l H ₂ /l waste)	pH final	COD loss (g O ₂ /l)	Bio-mass (g/l)
Dairy waste							
0.086	2.52±0.17	0.057±0.018	18.0±7.6	5.8	7.3	3.5	2.0
0.36	3.23±0.21	0.049±0.007	14.5±4.3	7.6	6.9	4.2	2.2
Brewery waste I							
0.086	1.41±0.04	0.034±0.004	11.6±2.9	13.6	6.1	3.1	2.7
0.36	2.24±0.09	0.061±0.009	9.4±2.6	19	6.2	3.8	2.6

Table 3. Kinetic parameters of cumulative hydrogen production at different concentration of inoculum

3.4 The effect of waste concentration on hydrogen production

The effect of waste concentration was studied with inoculum concentration of 30% (0.36 dry wt/l) and light intensity of 9 klx. The following waste concentration were used: 5, 10, 20, 40, 60% v/v in case of dairy waste, 1, 3, 5, 10, 20% v/v in case of brewery waste I and 5, 10, 20, 40, 80% v/v in case of brewery waste II. The results in Tabl.4 show the maximum hydrogen production of 3.2 l/l medium occurring when 40% of dairy waste was used. When brewery waste with high COD (220 g O₂/l) was applied, 2.2 l of H₂ per l medium was produced (waste concentration10% v/v). In case of brewery waste with low COD (27 g O₂/l) only 0.67 l of H₂ per l medium was produced (waste concentration 80 % v/v). If higher concentrations of wastes were applied, the efficiency of hydrogen production was lower, which was caused by and inhibiting concentration of N-NH₄⁺ (40 mg/l for dairy waste and 96 mg/l for brewery waste) (Waligórska, 2009, Melis, 2006). Such concentration of ammonium ions can diminish significantly the overall generation of hydrogen . The presence of ammonium ions as well as N₂ causes reduction of nitrogen *via* nitrogenase into gaseous NH₃ instead of required hydrogen. The amount of evolved CO₂ never exceeded 10 vol. %. Additionally, higher concentrations of wastes caused acidification of medium during the process and darkens the medium, which makes the access of the light into the medium more difficult and negatively impact on hydrogen production. The final pH values presented on fig. 6 show the drop from 7.1 to 5.2 in case of brewery waste and 7.5 to 5.7 in case of dairy waste. This effect is caused mainly by formation of organic acids (lactic and acetic) (Koku, 2002). The higher was the concentration of the waste the higher was the amount of detected acids and lower value of pH. This can be explained by higher ability of transfer of undissociated form of acids towards the cell, followed by dissociation inside the cell, proton release and final inhibition of the process (Van Ginkel, 2005).

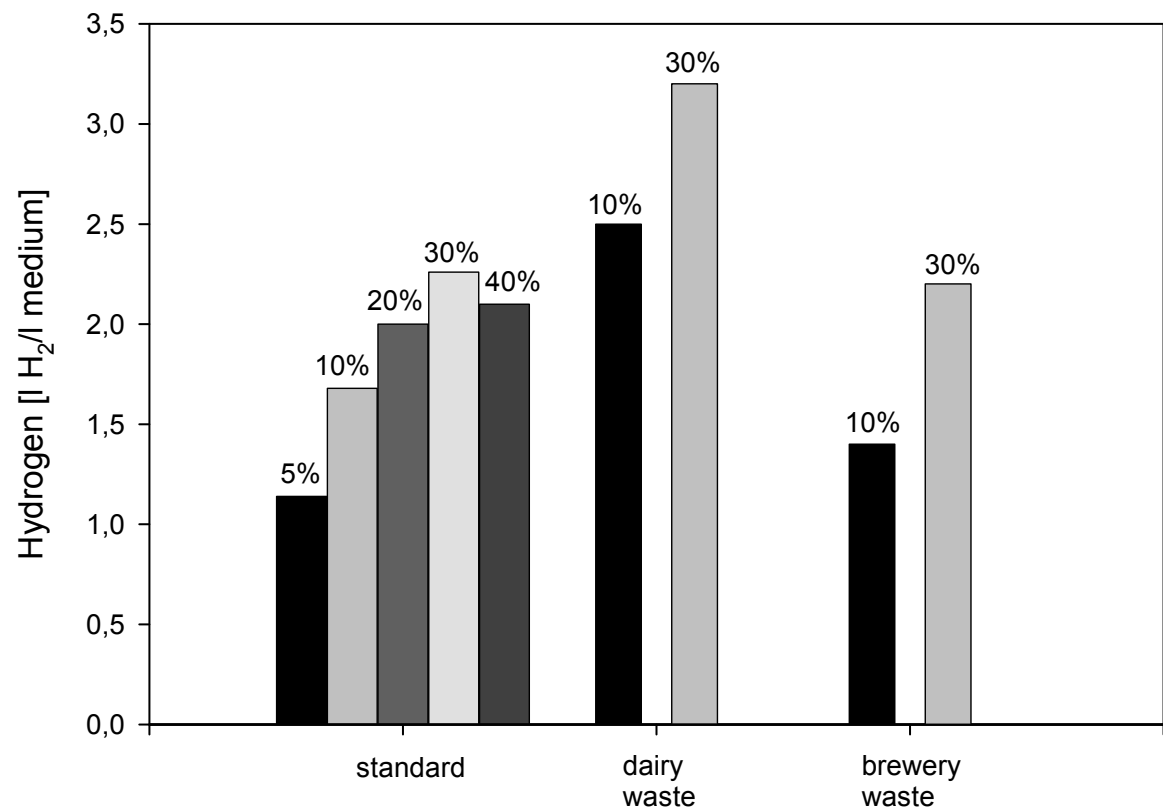


Fig. 5. The effect of inoculum concentration on hydrogen production

Dairy waste (COD = 46 g O ₂ /l)				
Concentration of dairy waste (% v/v)	H _{max} (l/l medium)	COD loss (gO ₂ /l medium)	Y _{sub} (l H ₂ /l waste)	Y _{sp} (l H ₂ / COD _{loss})
5	0.77	1.3	11.3	0.6
10	1.58	1.8	13.7	0.78
20	2.1	2.8	9.4	0.75
40	3.2	4.2	7.6	0.76
60	0	-	-	-
Brewery waste I (COD = 220 g O ₂ /l)				
1	0.86	1.9	56	0.45
3	1.17	2.4	29	0.49
5	1.4	2.8	22	0.51
10	2.24	3.8	19	0.59
20	0.52	2.3	1.1	0.23
Brewery waste II (COD = 27 g O ₂ /l)				
5	0.38	1.3	3.6	0.29
10	0.4	1.5	2.0	0.27
20	0.4	1.6	1.0	0.25
40	0.56	2.4	0.9	0.23
80 (concentrated)	0.67	2.8	0.59	0.24
Standard (L-malic acid)				
0.2	2.3	1.9	-	1.2

Table 4. The correlation between waste concentration, amount of hydrogen produced, COD loss and efficiencies.

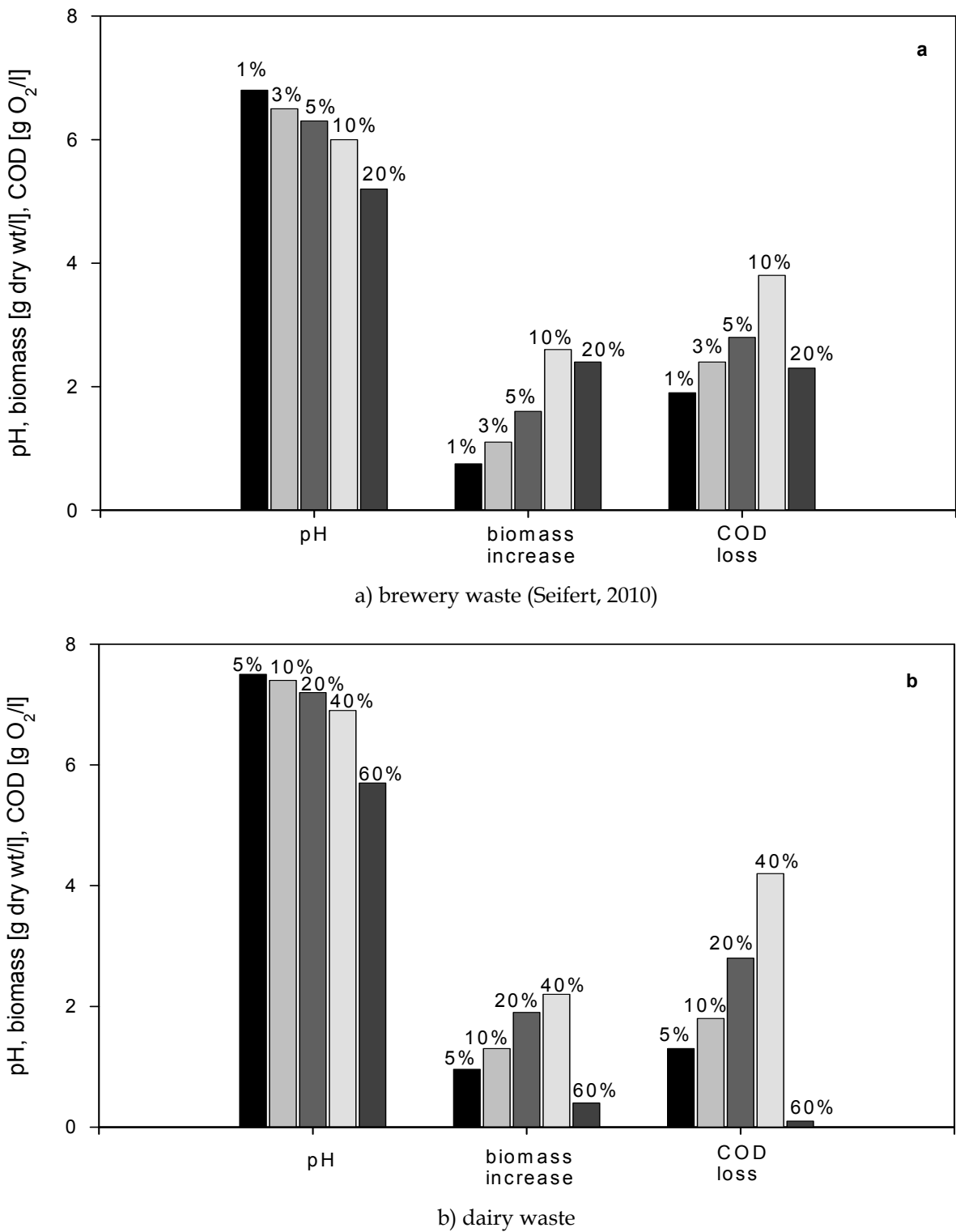


Fig. 6. Influence of food wastewater concentration on pH, biomass increase and COD loss.

With the rising concentration of wastes we observed higher COD loss, biomass increase and increase of specific efficiency (Table 4, fig.6). With further increase of waste concentration COD loss and specific efficiency were lower. However substrate efficiency decreases with higher waste concentration. Similar results showed Eroglu et al. obtaining the best substrate

efficiency of hydrogen generation (0.1g/l waste) for low waste concentration (olive mill wastewater 2%) however maximum volume of hydrogen production (0.45 l/l) and highest COD loss (40%) were observed when higher waste concentrations were used (Eroğlu, 2004). Also Mohan et al. studying hydrogen production from vegetable based market waste, obtained good specific efficiency when low waste concentrations were used, however highest COD loss (almost 60%) occurred when higher waste concentrations were introduced to the media (Mohan , 2009). Comparing the above results with the ones obtained for hydrogen generation on standard medium with L-malic acid, it can be seen that total amount of produced hydrogen is by 30% higher when dairy waste in concentration of 40%v/v was used and comparable when brewery waste with high COD was used (Table 4, Fig 7). Different papers published so far have proved that organic substrates such as glucose, sucrose, malic acid have been more effective than the waste containing media (Yetis2000, Zhu, 1999, Basak, 2009). However based on our results we can state that wastes studied in this paper represent an effective nutrient for photobiological hydrogen production.

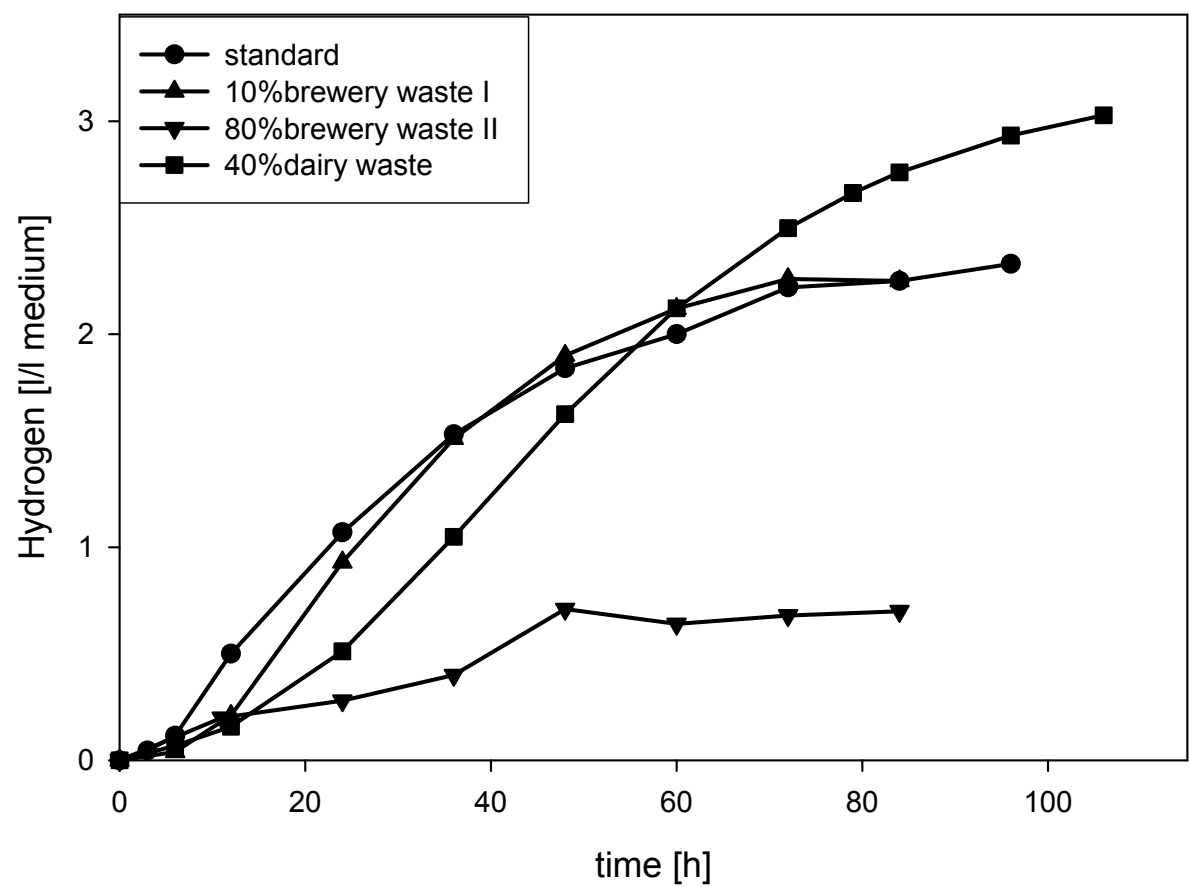


Fig. 7. The effect of optimum waste concentration on hydrogen production (30% v/v inoculum, 9 klx)

3.5 The influence of pH correction on hydrogen production

The untreated “raw” dairy wastewater with low value of pH (4.27) was completely non-active in hydrogen generation by microbiological method. However, we assumed that the same wastewater under controlled pH can generate hydrogen similarly as a sterilized one. Therefore, in order to achieve similar conditions like in bioreactor operating under controlled pH we performed our batch tests in small photoreactors (capacity of 60 ml with working capacity of 30 ml) correcting pH with 0.5M solution of NaOH every 12 h. Medium containing non-sterilized dairy wastewater with concentration of 40 v/v % was inoculated with bacteria at two different concentrations: 0.086g dry wt/l (10 vol.%) or 0.36 g dry wt/l (30 vol.%). Data presented in table 5 indicate that stabilization of the system at pH close to 7 allows for hydrogen generation even from the untreated dairy wastewater. Application of inoculums with concentration at the 0.36 g dry wt/l level generates 3.6 l H₂/l. The four-fold dilution of microorganisms reduces the volume of hydrogen to 2.6 l H₂/l. Although the starting time was relatively long (about 20 h) savings which could arise from the application of untreated waste can be significant. Performing the same experiment with brewery waste II (concentration 40 v/v %) the yield of the generated hydrogen has not been improved. In this case the value of pH rapidly grew to 7.5- 7.9 in the first two days. However , it can not be excluded that in the system with controlled pH this yield could be much higher. Preliminary experiments performed under such conditions confirm this assumption.

Inoculum conc.	H _{max} (l/l)	R _{max,H2} (l/l/h)	λ _{H2} (h)	Y (l H ₂ /l waste)	pH final	COD loss (g O ₂ /l)	COD loss (%)	Biomass (g/l)
0.086	2.58±0.16	0.038±0.005	20.7±3.6	6.0	6.8	3.8	20	2.2
0.36	3.62±0.24	0.056±0.009	17.0±4.5	8.6	6.7	4.6	23	2.8

* expressed in g dry wt/l
** biomass increase

Table 5. Kinetic parameters of cumulative hydrogen production for non-treated 40 % dairy wastewater, with correction of pH for different concentration of inoculums (Seifert, 2010).

The results presented in this section suggest that hydrogen generation can be effectively performed under solar radiation in photobioreactor operating under continuous conditions.

3.6 Kinetic of hydrogen generation

The results of kinetic considerations based on modified Gompertz equation (Eq. 4) are shown in table 6. Independently from the kind of food waste (in the active of concentration) it was observed that the increase of the volume of generated hydrogen, small drops in reaction rate and prolongation of the lag phase.

These results showed that higher substrate yield increases the reaction rate. Moreover, these values are well correlated with the lag phase in systems with higher concentration of wastes are caused probably by longer adaptation of microorganisms to the bed.

Concentration of waste (% v/v)	H _{max} (l/l)	R _{max} (l/l/h)	λ _{H2} (h)
Dairy waste			
5	0.77±0.03	0.08±0.05	6.5±3.1
10	1.58±0.11	0.058±0.019	7.3±6.2
20	2.10±0.06	0.055±0.021	10.0±4.8
40	3.23±0.21	0.049±0.007	14.5±4.3
Brewery waste			
1	0.86±0.02	0.046±0.007	8.0±1.4
3	1.17±0.05	0.045±0.009	6.1±2.7
5	1.40±0.05	0.042±0.008	6.1±2.1
10	2.24±0.09	0.061±0.009	9.4±2.6
20	0.52±0.02	0.040±0.015	18.7±2.2
standard	2.3±0.2	0.047±0.004	2.7±1.8

Table 6. Kinetic parameters of cumulative hydrogen production for different initial concentration of food waste

4. Conclusions

The presented results shows that the waste studied in this paper represent a vary good substrate in photophermentation by *Rhodobacter sphaeroides*. Light intensity of 9 klx and inoculum concentration of 0.36 g dry wt/l (30% v/v) were used as the most effective (high light conversion efficiency and short duration of the process). The studied wastes has to be treated with high temperature (20 min in 120°C). This pretreatment significantly increases H₂ production. The optimum concentrations of wastes were estimated: 40% v/v for dairy waste and 10% v/v for brewery waste with high COD. These wastes represent the effective (comparable with L-malic acid) nutrient for hydrogen production. Higher wastes concentrations inhibit the process as it initiate fermentation which starts to compete with hydrogen production and additionally increases NH₄⁺ concentration, which also negatively affect the process. Brewery waste with low COD shows low efficiencies and needs to be concentrated to supply sufficient concentration of organic compounds. An application of untreated dairy wastewater containing suspensions in efficient hydrogen generation process can be performed only at controlled acidity (pH = 7.0). Kinetic measurements proved that the rate of hydrogen generation drops with concentration of the waste and prolongs the lag phase.

5. Acknowledgements

These studies were supported by Polish Ministry of Science and Higher Education (grant no: N N204 185440).

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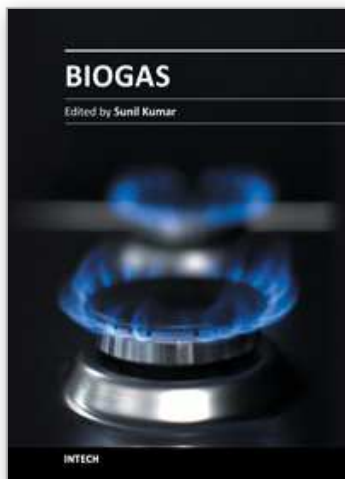
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Biogas

Edited by Dr. Sunil Kumar

ISBN 978-953-51-0204-5

Hard cover, 408 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

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How to reference

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Krystyna Seifert, Roman Zagrodnik, Mikołaj Stodolny and Marek Łaniecki (2012). Photofermentative Hydrogen Generation in Presence of Waste Water from Food Industry, Biogas, Dr. Sunil Kumar (Ed.), ISBN: 978-953-51-0204-5, InTech, Available from: <http://www.intechopen.com/books/biogas/photofermentative-hydrogen-generation-in-presence-of-wastewaters-from-food-industry>

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