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Research paper

The distillers grains with solubles as a perspective substrate for obtaining biomass and producing bio-hydrogen by *Rhodobacter sphaeroides*

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ABSTRACT

The photosynthetic purple non-sulfur bacterium *Rhodobacter sphaeroides* MDC6521, isolated from Armenian mineral spring, and distillers grains with solubles (DGS) (by-product of bio-ethanol fermentation) were applied for obtaining biomass and producing bio-hydrogen (H_2) upon illumination. During growth on diluted DGS media H_2 production was started at 24 h growth, whereas H_2 photoproduction by *R. sphaeroides* cells, grown on Ormerod medium, was detected after 48 h. Moreover, the *R. sphaeroides* produced considerably more H_2 during DGS photo-fermentation: the H_2 yields from 2- fold and 5-fold diluted media were ~4.6–5.5-folds higher in comparison with control. H_2 yield has decreased at higher dilution. The growth yields of *R. sphaeroides* cells, grown in the 2–5-folds diluted DGS media, were considerably higher than those of control cells, grown in Ormerod medium. The results can provide with new cheaper and more effective source of biomass and bio-hydrogen and as well as solve the problem of ethanol by-product utilization.

1. Introduction

Molecular hydrogen (H_2) is an energy carrier with high energy content (−122 kJ g^{-1}). Among the developing alternative energy resources, H_2 is recognized as the most promising alternative to fossil fuels and is expected to play a major role in future energy supply as it is clean, renewable, and efficient [1,2]. Biological H_2 production with microorganisms is considered as one of the perspective field of biotechnology, which suggests the generation of renewable and ecologically clean energy from a variety of substrates and organic wastes [3–7].

Photosynthetic purple non-sulfur bacteria such as *Rhodobacter* species are perspective candidates for the H_2 production due to their high substrates conversion rate [8–10]. Under anaerobic conditions *Rhodobacter sphaeroides*, isolated from Armenian mineral springs, has been shown to perform a photo-fermentation of various carbon- and nitrogen-containing organic substrates with H_2 production [11–14].

The selection of the source for H_2 becomes a serious problem, because it strongly affects the H_2 yield by photosynthetic bacteria. It is important to choose such sources, which can be effectively utilized by bacteria and would be less expensive providing the enhanced H_2 yield. Various organic substrates, generally used in laboratory for research on H_2 production, permit a fast and large amount of H_2 production [8–10]. But one of the key problems in H_2 production technology is the high cost of various organic carbon and nitrogen sources. The use of different organic wastes and new substrates, which are cheaper and more effective, for H_2 production can provide inexpensive energy generation and simultaneous waste utilization.

Practically unlimited source of natural nutrients are the food industry wastes [4–7,15–19]. These sources can include cereal distillers grains (DG) as a by-product of ethanol industry. This source is widely available and very cheap. During ethanol fermentation the conversion yield of one tone wheat or corn to wet DG is ~460–480 kg.

The detailed principles of DG generation are reported in the literature [20–22]. The starch in cereals is transformed into ethanol. The rest of the cereals components such as proteins, lipids,

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minerals, and other remain unchanged chemically. These residual stocks are known as distillers dried grains with solubles (DDGS). The process includes various steps such as fermentation, distillation, by-product recovery and other [21,22]. During by-product recovery the nonvolatile components (obtained during distillation process) are centrifuged to produce a liquid fraction (thin stillage) and a solid fraction (distillers wet grains, DWG). The thin stillage is concentrated into condensed distiller solubles (CDS) via evaporation, and then CDS are mixed with DWG to become distillers wet grains with solubles (DWGS) and then dried into DDGS [21].

Distillers grains with solubles (DGS) contain carbohydrates and essential fatty acids as well as trace elements such as iron, magnesium, manganese, and others, which are necessary for growth of purple bacteria [18–22]. In DGS were also present various amino acids with a predominance of glutamate, including 8 essential amino acids: arginine, lysine, valine, histidine, threonine, phenylalanine, leucine, isoleucine as shown [20–22].

Thus, DGS are a valuable source of natural biological compounds and can be used for H_2 production. However, this aspect of DGS has not been considered yet. Some authors were shown that DGS proved to be a suitable feedstock for bioenergy production [16,17]. Given the possibility of change in the ethanol fermentation, it must be assumed that as a result of deep chemical research can justify the use of DGS as a new source of bio-hydrogen. The solution of this problem will provide a new cheap source of H_2. Increasing energy requirement needs a new substrates and methods of energy generation. Disposal of various by-products makes the H_2 production a new perspective approach towards satisfaction of energy demand.

DGS are a very cheap substrate: their cost in Armenia is 1.5 cent per liter. The use of DGS for H_2 production can be better than the sole carbon and nitrogen sources. Application of DGS can alter the modes of bacterial metabolic pathways and improve the H_2 production.

In this study the photosynthetic purple bacterium *R. sphaeroides*, isolated from Armenian mineral spring, was employed to produce H_2 during the photo-fermentation of new substrates such as DGS. It is very important to choose such source, which can be effectively utilized by *R. sphaeroides* for obtaining biomass and provide the enhanced H_2 yield technology.

2. Materials and methods

2.1. Bacterial strains, cultivation conditions and determination of growth

Phototrophic bacterium *R. sphaeroides* strain MDC6521 (Microbial Depository Center, National Academy of Sciences of Armenia, Yerevan, Armenia, WDCM803), isolated from Arzni mineral spring in Armenian mountains, was cultivated in glass vessels of 150 ml capacities with plastic press caps in anaerobic conditions on Ormerod medium with succinate as carbon source upon illumination (~36 W m^{-2}) as described previously [12–14]. The growth of batch culture was monitored by changes in optical density (OD) by Spectro UV–Vis Auto spectrophotometer (Labomed, USA), and by determining bacterial biomass dry weight (DW), which was correlated with OD at 660 nm: DW (g L^{-1}) = $OD_{660} \times 0.50$. Specific growth rate was calculated as ln2/doubling time of OD within an interval, where the logarithm of culture OD increased with time in a linear manner (logarithmic growth phase), and it was expressed as h^{-1} [12,13].

DGS from common (bread) wheat *Triticum aestivum* L. were obtained from "Alex Grig" Alcohol Plant Co. LTD (Yerevan, Armenia). DGS are a yellow-brown color polydisperse system with yeast scent, in which compounds are dissolved and suspended (with visible milled caryopsis). DGS were obtained during ethanol fermentation, which was performed by yeast *Saccharomyces cerevisiae*. The untreated DGS were filtered through cotton wool, and then paper filter, next filtrate was sterilized at 120 °C by autoclaving for 20 min, and then used during experiments. As pH of DGS was ~3.5, before autoclaving the pH of the DGS was adjusted to 7.0 ± 0.1 by means of 10^2 mol m^{-3} NaOH. DGS were diluted at 2-fold, 5-fold, 10-fold, and 25-fold using distilled water. Diluted DGS were used without any medium supplements, because DGS contained various carbon-containing compounds. DW of undiluted DGS was 20 g L^{-1}.

2.2. Determination of medium pH, redox potential (E_h) and H_2 yield

The initial pH of the culture growth medium was measured at time intervals 0 h–96 h by a pH-potentiometer (HI 122-02, HANNA Instruments, Portugal) with pH electrode (HJ1131B) as described [13,14].

The medium E_h was determined during *R. sphaeroides* anaerobic growth by potentiometric method using a pair of redox electrodes: platinum (Pt) and titanium-silicate (Ti–Si) electrodes as described [11–14]. The kinetics of E_h measured simultaneously by both electrodes during bacterial growth provides information about general redox processes and also H_2 production [14,23–25]. The electrochemical approach, although indirect, is a useful means of determining H_2 production and gives accurate and reproducible data [24,26].

The H_2 yield was calculated by the decrease of E_h to low negative values during bacterial growth and expressed in mmol g^{-1} DW as described [12–14]. This determination of H_2 is close to the method with Clark-type electrode used by the other authors [27]. The correlation between E_h and H_2 production was shown; the supplementation of H_2 didn't affect medium pH [14,27]. H_2 production in gas phase was also confirmed by the chemical method based on the bleaching of $KMnO_4$ solution in H_2SO_4 with H_2 as described [11,12,28]: $2KMnO_4 + 3H_2SO_4 + H_2 \rightarrow K_2SO_4 + 2MnSO_4 + 4H_2O$.

The pH and redox electrodes were immersed into the glass vessels, and the electrodes readings were registered each 24 h as shown in Figures.

2.3. Reagents, data processing and others

Various reagents of analytical grade were used in this study. Each experiment was repeated three times to determine deviation, which is presented as error bars on the figures. Standard errors were calculated using Microsoft Excel 2013. The standard errors and Student criteria (p) were employed to validate the difference in average data between various series of experiments as described previously [13,14].

3. Results and discussion

3.1. H_2 yield in R. sphaeroides during DGS photo-fermentation

The bio-hydrogen production ability of *R. sphaeroides* during DGS photo-fermentation was tested. H_2 production was measured till 96 h growth (Table 1). The results showed that H_2 production by *R. sphaeroides* control cells, grown on Ormerod medium, was detected at 48 h growth.

No H_2 production was observed, when undiluted DGS were used (not shown). This is possible due to high organic compounds content in DGS [20–22]. It is known, that high content of sugars has inhibitory effect on bacteria growth and generation of H_2 [29]. Thus, dilution of DGS is necessary to optimize the organic compounds concentration for the growth and H_2 production by purple bacteria.

The results obtained have shown that in diluted 2-fold, 5-fold, and 10-fold DGS media H_2 production was started at 24 h and