



# Influence of laser irradiation on rumen fluid for biogas production from dairy manure

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## ABSTRACT

The irradiation of rumen fluid (RF) with laser source was hypothesized to enhance the anaerobic process and accelerate the manure digestion, which increases the biogas and methane production. The photobiotostimulating effects of laser irradiation on biogas and methane production were investigated by irradiating the RF for 0.5, 1 and 2 h with 532 nm laser source compared with 1 h incandescent light, non-irradiated RF and the control. The highest significant values of the biogas and methane production were found to be 583 ml Biogas g<sup>-1</sup> VS and 367.9 ml CH<sub>4</sub> g<sup>-1</sup> VS when RF was irradiated for 0.5 h with 532 nm laser source ( $p < 0.05$ ) compared with the other irradiation times with laser, incandescent light source, non-irradiated RF, and the control which yielded only 357 ml Biogas g<sup>-1</sup> VS and 196 ml CH<sub>4</sub> g<sup>-1</sup> VS, respectively. Moreover, the biogas and methane production rates were found to be inversely proportional with the irradiation time using laser source. The results showed that the lag phase was reduced from 4 days to 1 day. Additionally, the time to achieve the highest biogas production (peak) was reduced from day 28 to day 16 of the Hydraulic Retention Time (HRT) compared with the control.

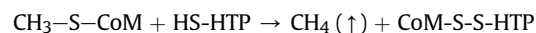
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## 1. Introduction

Anaerobic digestion (AD) is one of the most important techniques that convert biomass to renewable bioenergy in the form of methane which is a biofuel with reduced treatment costs [1,2]. Anaerobic digestion has numerous advantages such as low energy and nutrients requirements as well as low sludge production [3]. Furthermore, AD enables higher loading rates than aerobic treatment, and more destruction of pathogens [4]. AD can be divided into 4 major bioprocesses which are: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [5,6]. The temperature of the digester is a main factor which affects the biogas production [7]. AD consists of a series of microbial processes that convert organic matters into CH<sub>4</sub> and CO<sub>2</sub>; under psychrophilic (<20 °C), mesophilic (25–40 °C) or thermophilic (50–65 °C) conditions.

Most conventional systems of anaerobic digestion are operated under dark conditions. However, photoenhancement by

incandescent lighting found to significantly increase methane production [8]. Argun and Kargi [9] stated that few studies have investigated the effects of sunlight as an external artificial factor on fermentation. It was suggested that sequential dark and light conditions could enhance anaerobic fermentation. There have been few studies regarding optimum light conditions of methane production from AD under illumination. The methane production was investigated under different illumination times via acetate as a carbon substrate. The lighted upflow anaerobic sludge blanket (LUASB) system was investigated by Sawayama et al. [10] under mesophilic conditions. The photoenhancement by incandescent lighting of the methane production process from thermophilic anaerobic digestion was reported by Tada and Sawayama [8]. Inactive 2-(methylthio)ethanesulfonic acid reductase from *Methanothermobacter* sp. was partially activated by exposure to light in vitro [11]. CH<sub>3</sub>-S-CoM reductase catalyzes the last step of methanogenesis, and this includes the reductive demethylation of CH<sub>3</sub>-S-CoM with reducing equivalents from *N*-(7-mercaptoheptanoyl)-L-threonine O<sup>3</sup>-phosphate (HS-HTP) as follows [12]:



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This enzyme catalyzes  $\text{CH}_3\text{-S-CoM}$  to form  $\text{CH}_4$  at the terminal step of methanogenesis only in the presence of light.

The photoactivation of a reaction mixture containing the isolated prosthetic group, native  $\text{F}_{430}$ , or its analogues could not be accomplished [11]. The growth of methanogens was inhibited by the blue end of the visible spectrum from 370 to 430 nm [13]. Sawayama et al. [10] reported the use of the lighted upflow anaerobic sludge blanket method under mesophilic conditions. The results obtained by Tada and Sawayama [8] indicated that light at a wavelength between 390 and 540 nm enhanced the methane production from thermophilic anaerobic digestion. The irradiation with low-intensity blue [14] and red [15] light accelerated the growth of cultures of *E. coli* WP2. Visible light, especially in the blue region, found to be inducing an enormous variety of physiological responses. Light growth response of *E. coli* is a rather well documented phenomenon [16,17].

Karu et al. [18] mentioned that mitochondria are sensitive to irradiation with monochromatic visible light. Irradiation with light at wavelengths of 415 nm [19] 602 nm, 633 nm, 650 nm and 725 nm enhances ATP synthesis [20,21]. The exposure to light at 633 nm augments the mitochondrial membrane potential and proton gradient [21], which leads to modifications in the optical properties of mitochondria, changes a number of NADH linked (NADH, reduced form of nicotinamide adenine dinucleotide) dehydrogenase biochemical reactions [22] and maximizes the ADP/ATP exchange rate (ADP: adenosine diphosphate; ATP: adenosine triphosphate) [23]. It has been proved that red monochromatic light can accelerate the proliferation of yeast [24] and mammalian cells [25], and increase the activity of mitochondrial respiratory chain enzymes [26] and ATP synthesis was revealed in remote progeny cells [18]. Karu [27] confirmed that cytochrome *c* oxidase; the terminal enzyme of the mitochondrial respiratory chain, is a photoacceptor of red–near-IR light. The absorption of light photon by this enzyme molecule was proposed to accelerate electron transport in the respiratory chain, which increases the transmembrane electric potential of mitochondria and activates ATP synthesis. This exposure modified the reactions of dehydrogenases associated with NADH and increased the rate of ATP synthesis [22,23,28].

Many studies have been carried out to increase the efficiency of anaerobic process by biological, chemical and thermal pre-treatments of wastes to increase biogas production [29–33]. Ruminant animals produce significant amounts of methane from their normal digestive processes [34,35]. In the rumen (large forestomach), microbial fermentation converts organics into products which can be digested and utilized by the animal [36,37]. Rumen microorganisms showed higher ability to degrade the lignocellulosic biomass, such as organic fraction of municipal waste and grass, compared to other usual anaerobic microorganisms [38,39]. In addition, Budiyo et al. [40] and Sunarso et al. [41] have also reported that 50% rumen fluid (RF) inoculated to biodigester gave significant effect on biogas production. Where, RF inoculums can increase biogas production rate efficiently about 3 times in comparison to manure substrate without rumen fluid.

The photoactivation was found to photobiostimulate and, therefore, improve the microbial activity. In the present study, the rumen fluid exposure to 532 nm laser has been hypothesized to improve the anaerobic bioprocesses and accelerate the digestion of slurry, which maximizes the biogas and methane production. Therefore, the objectives of this study were to investigate the effects of laser irradiation on biogas and methane production in comparison with incandescent light and the control. Therefore, a set of biodigesters and a biogas production system were designed and manufactured for conducting the experiments in the bioenergy laboratory at the National Institute of Laser Enhanced Sciences at

Cairo University. This application has been hypothesized to maximize the biogas yield, methane percentage and shorten the lag phase (startup) of the anaerobic process.

## 2. Materials and methods

### 2.1. Sample preparation and analysis

The raw manure samples were collected manually from West-ern Farm of the faculty of Agriculture, Cairo University, Giza City, Egypt. The samples were mixed for 30 min to obtain one sample. On the other hand, the rumen fluid (RF) was prepared according to the method elucidated by Budiyo et al. [40], where the rumen contents were obtained from the slaughterhouse and mixed with 25 L of distilled water. The obtained slurry was filtered by cloth (textile) filter to separate the solid contents. The rumen fluid (RF) is semi-transparent and has high contents of microorganisms (methanogens) which is suitable for irradiation. All samples were stored at 4 °C for one day prior to analysis and preparation. Total solids (TS), volatile solid (VS) and ash were determined by the standard methods using muffle furnace (Vulcan D-550, Ney Tech, York, USA) as shown in Table 1. The total and volatile solids can be calculated using method 1684 [42].

### 2.2. Experimental set up

A batch anaerobic system was designed and manufactured according to Samer [43], and implemented in this study. The experimental setup of the batch anaerobic system consists of; 1) 2L Biodigester (Pyrex, FV2L, Scilabware, Staffordshire, UK), 2) Thermostatic water bath (HOME MADE, 60 L, 0–100 °C), 3) Biogas holder unit with 1 and 2L graduated cylinders (Azlon, Staffordshire, UK) and 4) Portable gas analyzer (Geotech, GA2000, Warwickshire, UK) as shown in Abdelsalam et al. [44–46]. The experiments were carried out in the Biogas Laboratory at National Institute of Laser Enhanced Sciences (NILES), Cairo University.

### 2.3. Irradiation setup

The effects of light and laser irradiation on biogas production were investigated using batch anaerobic system. For this purpose, RF was irradiated by incandescent light and laser sources. The irradiation setup consists of either laser source (250 mW, Nd:Y second harmonic, 532 nm, China) or incandescent light source (i.e. 200 W incandescent lamp), magnetic hot plate stirrer (SCIOGEX, MS7-H550-S, Connecticut, USA), and beam expander to expand the laser beam diameter to 0.01 m. The light intensity which attained the surface of rumen fluid was 5000 lux equivalent to  $100 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  [47], which was measured using a digital light meter (Lutron, LX-101, Taiwan). The incandescent light source was set apart from the bio-digester to deliver the same light intensity of 5000 lux. During irradiation time, the bio-digester was wrapped with aluminum foil to benefit from the reflected rays as shown in

**Table 1**  
Composition of biomass used in irradiation experiment.

Parameter	Fresh manure	Slurry	Rumen Fluid (RF)
TS (%)	15.19	7.40	1.82
VS (%)	12.57	5.75	1.12
Ash (%)	2.61	2.03	0.41
Organic carbon (% from VS)	48.02	43.2	45.6
Total Nitrogen (%)	1.84	1.76	3.61
C:N ratio	26:1	24: 1	12:1
pH	5.94	6.2	6.1

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