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# Photohydrogen production from dark-fermented palm oil mill effluent (DPOME) and statistical optimization: Renewable substrate for hydrogen

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ABSTRACT

Biological hydrogen production through photo-fermentative process using dark fermented palm oil effluent (DPOME) is a cost effective and environmentally benign process. In this study, effect of various factors like light intensity, agitation rate and dilution of DPOME on the hydrogen productivity of *Rhodopseudomanas palustris* were investigated using batch system. Investigation methods like response surface methodology (RSM) and Box-Behnken design were employed to investigate the optimum conditions for enhanced photo-fermentative hydrogen production. The regression analysis suggested that hydrogen yield was well fitted by a quadratic polynomial equation ($R^2 = 0.92$). The hydrogen production was investigated by varying the intensity levels of these three independent variables, in which all have significant influences on hydrogen yield. The set of 19 experimental runs were conducted to optimize these variables. The highest hydrogen yield of $3.07 \pm 0.66$ $H_2$ yield mol-$H_2$/mol-acetate was obtained under the optimum condition of light intensity 250 W/m$^2$, agitation rate 200 rpm, and 30% dilution of DPOME. The experimentally obtained hydrogen yield found out to be in a good agreement with predicted yield which was about 2.80 mol-$H_2$/mol-acetate. In short, results suggest that experimental strategy using RSM approach along with Box-Behnken design can be a promising approach to achieve enhanced biological hydrogen production.



## 1. Introduction

Hydrogen is considered as an environmentally benign fuel that yields water along with dissipation of fair amount of energy after combustion. Hydrogen is considered as an attractive bioenergy alternative due to its ability to produce fair amount of energy i.e. approx. 122 kJ/g, which is three times higher than the other chemical fuels. Hydrogen production can be achieved using various methods but biological hydrogen production is less energy intensive as compared to chemical and electrochemical routes (Balat and Kırtay, 2010; Mishra et al., 2018). Biological hydrogen production includes fermentative hydrogen production and photo-synthetic hydrogen production (Urbaniec et al., 2010). Photosynthetic bacteria utilizes light as an energy source and use short chain carboxylic acids as their electron donor, which are present in fermentative substrates in plenty amount. The boosted level of electron flow from light enables the plant with the energy required for biohydrogen production. Photosynthetic purples bacteria (e.g. *Rhodopseudomonas, Rhodobacter and Rhodospirillum*) produces biohydrogen using nitrogenases enzyme. The nitrogenases enzyme catalyses the nitrogen-fixation process which is a highly electron/energy intensive process, which yields hydrogen as by-products (Hallenbeck, 2009). Photo-chemotrophic bacteria are promising microbial system armed with the ability to produce hydrogen. This is mainly due to: (a) their ability to yield hydrogen comparable to the theoretical conversion yields (b) the absence of oxygen evolving pathways, which eliminates the problems associated with oxygen sensitive biological pathways (c) ability to use large spectrum of

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**Table 1**
Characteristics of dark fermented palm oil mill effluents.

| Parameters | Characteristics of DPOME before photo-fermentation | Characteristics of DPOME after photo-fermentation |
|---|---|---|
| COD | 17510 | 1676 |
| BOD | 31070 | 4966 |
| Oil | 11308 | 8750 |
| Acetic acid | 6300 | 1110 |
| Butyric acid | 8700 | 3670 |
| pH* | 4.3 | 5.7 |

Values are in g/L except* pH; COD: Chemical Oxygen Demand; BOD: Biological Oxygen Demand.

light intensity (d) ability to use wide range of organic wastes associated with waste waters (Chen et al., 2011). Biomasses or wastewater originated from the various industries can be utilized for dark fermentative hydrogen production. The incomplete degradation of saccharides into hydrogen and $CO_2$ is one of the major bottleneck that need to overcome to improve hydrogen production, which often lowers the hydrogen yield per mole of hexoses. These dark fermented effluents are rich sources of acetic acids, butyric acid and other organic matter, which serves as a suitable carbon sources for the growth of the bacterium responsible for photo fermentative hydrogen production. Hydrogen production using dark fermented effluents are well documented by various researchers (Uyar et al., 2009a, b; Panagiotopoulos et al., 2010; Tawfik et al., 2014). Furthermore, the palm oil industry produces a large amount of wastewater which contains complex carbohydrates and organic acids, which has been used as a feedstock for hydrogen productions by Lam and Lee (2011). The various investigations have been reported for dark -fermentative hydrogen production along with $CO_2$ and volatile fatty acids formation using POME (Mohammadi et al., 2011; Krishnan et al., 2016; Mishra et al., 2017). The organic acids produced by dark fermentative bacteria can further be fermented by using photo-synthetic bacterium *Rhodopseudomanas palustris* (Pott et al., 2014; Mishra et al., 2016). So far, very few researches has been carried out targeting the biohydrogen production using POME and the wide hidden possibilities of biohydrogen production using POME are yet to be explored. For cheaper and enhance hydrogen production requires the optimization of process conditions. The optimization of photo-fermentative hydrogen productions conditions such as light intensity, agitation rate and dilution of dark fermented effluents are very important to get enhanced biohydrogen production from DPOME (Azbar et al., 2010; Liao et al., 2015). The statistical approaches are also considered to be another effective method to optimize these multivariable. Recently, many researchers has extensively used the response surface methodology to optimize various factors responsible to affect the biohydrogen production process (Mangayil et al., 2015). The present study deals with the hydrogen production analysis using DPOME as substrate via photo-fermentation. The effect of process parameters such as light intensity, agitation rate and dilution percentage of DPOME on hydrogen production were investigated using 3 K Box-Behnken factorial design. Finally, the interactive effect of these factors on hydrogen production was analysed by perturbation plots and response surface plots, respectively.

## 2. Materials and methods

### 2.1. Inoculum preparation and fermentative substrate

The strain of photo-fermentative bacterium Rhodopseudomanas palustris was obtained from the China general Microbial Culture Collection, China. Inoculum was activated anaerobically in modified basal medium (Asada et al., 2006) at 30 °C for 72 h under light intensity of 150 W/m$^2$ (continuous illuminations). The medium contains acetate 35 mM (carbon source) and glutamate 20 mM (nitrogen source) with the initial pH of 7.0. Dark fermented palm oil mill effluent was collected from our lab which was fermented using *Bacillus anthracis* isolated from palm sludge (Mishra et al., 2017). The characteristics dark fermented POME used in this study are illustrated in Table 1, before and after treatment. DPOME were centrifuged and sterilized by autoclaving before fermentation to remove the microbial contamination and colloidal materials that can interfere with light penetration. The dilution of DPOME has done using demineralized water.

### 2.2. Experimental set-up for hydrogen production

Photo-fermentation was carried out in 2 L continuous stirrer tank reactor batch mode using 2 L continuous stirrer tank reactor made of PVC with thickness of 0.3 mm (Polyvinyl chloride) having working volume of 1 L. The bioreactor was equipped with a stirrer to provide better mass and heat transfer in the medium, along with a water bath to maintain the temperature of the reactor. Dilution of dark fermented palm oil mill effluents has been done using demineralized water. The reactor was also supplemented with 500 mg of acetate and 150 mg of glutamate for initial acclimatization of inoculum. After inoculation of 10% inoculum ($OD_{660}$~1), the bioreactor was flushed with pure argon gas to create an anaerobic condition. The operations were done under illumination conditions at 30 °C, with initial pH 7.0. Initially, medium pH was adjusted by using 1 M NaOH to DPOME. The reactor was illuminated using external light source (150 W Tungsten lamps) and the intensity of light was control by keeping a distance from the bioreactor. All the experiments were conducted in triplets using the same source of DPOME.

### 2.3. Experimental designing of optimization process using RSM

The Design expert® 7.1.6 was applied to designing the experiment, drawing the RSM plots and analysis of data statistically. The $3^K$ Box- Behnken model applied to investigate the factors for enhanced biohydrogen production and examine the interactive effect of factors (Box and Behnken, 1960). Hydrogen yield was selected as the response variable, while light intensity (X1), agitation rate (X2), and dilution of DPOME (X3) as three independent variables as shown in Table 2. Following to the experimental design Table 3, a total of 19 experiments were conducted. The quadratic model was applied to fit the experimental values produced by RSM

**Table 2**
Levels of independents variables for RSM.

| Independent variables | Parameters | Low levels | High levels |
|---|---|---|---|
| $X_1$ | Light intensity (W/m$^2$) | 100 | 300 |
| $X_2$ | Agitation rate (rpm) | 100 | 300 |
| $X_3$ | Dilution of DPOME (%) | 10 | 50 |

**Table 3**
Box-Behnken experimental design with three independent variables and one response (Coded).

| Run | Factor $X_1$: Light intensity (W/m$^2$) | Factor $X_2$: Agitation rate (rpm) | Factor $X_3$: Dilution (%) | Response $H_2$ yield (mol $H_2$/mol-Acetate) |
|---|---|---|---|---|
| 1 | −1.000 | −1.000 | −1.000 | 0.37 |
| 2 | 1.000 | −1.000 | −1.000 | 0.32 |
| 3 | −1.000 | 1.000 | −1.000 | 0.67 |
| 4 | 1.000 | 1.000 | −1.000 | 0.364 |
| 5 | −1.000 | −1.000 | 1.000 | 1.77 |
| 6 | 1.000 | −1.000 | 1.000 | 2.89 |
| 7 | −1.000 | 1.000 | 1.000 | 1.46 |
| 8 | 1.000 | 1.000 | 1.000 | 0.96 |
| 9 | −2.000 | 0.000 | 0.000 | 0.27 |
| 10 | 0.667 | 0.000 | 0.000 | 1.89 |
| 11 | 0.000 | −2.000 | 0.000 | – |
| 12 | 0.000 | 2.000 | 0.000 | 0.33 |
| 13a | 0.000 | 0.000 | 0.500 | 3.49 |
| 14 | 0.000 | 0.000 | 1.000 | 3.44 |
| 15 | 0.000 | 0.000 | 0.000 | 2.54 |
| 16 | 0.000 | 0.000 | 0.000 | 2.11 |
| 17 | 0.000 | 0.000 | 0.000 | 3.11 |
| 18 | 0.000 | 0.000 | 0.000 | 3.23 |
| 19 | 0.000 | 0.000 | 0.000 | 2.54 |
| 20 | 0.000 | 0.000 | 0.000 | 3.18 |

(Table 4). The analysis of variance (ANOVA) was used to investigate the significance of fitting model for experimental values, interactive terms, and quadric terms. Effect of factors with central points and corresponded interactions was investigated by perturbation plots and response surface plots respectively.

### 2.4. Analytical methods

The Gas Chromatograph (GC 8500, Perkin) was used to measure hydrogen content, which equipped with 1.5 m stainless steel column (SS350A) packed with the molecular sieve of 80/100 mesh along with thermal conductivity detector and nitrogen as carrier gas. Operational parameters of GC were set according to the previous literature (Mishra et al., 2016). The VFA concentrations were observed using HPLC (Agilent 1200) attached the C-18 column. 0.05 M of $KH_2PO_4$ was applied as mobile phase with a flow rate of 0.4 ml/min 10 ml sample was injected into the column at a controlled temperature of 55 °C. Luxmeter (Tondaj LX1010B) was used to measure the intensity of light. The different parameter of DPOME such as COD, BOD, VSS, and oil contents was measured using APHA standards methods (Eaton et al., 2005). Apart from this, hydrogen production performance was observed using hydrogen yield in corresponds to substrate utilization particularly acetate. The value of hydrogen yield was obtained by dividing amount hydrogen produced in mol over the amount of substrate (acetate) consumed (mol/mol-acetate).

## 3. Results and discussions

### 3.1. Optimization of hydrogen using response surface methodology

RSM has been explored as an effective technique for the optimization of bioprocess technology (Liu et al., 2015). In this study, $3^K$ Box-Behnken model was used to optimized biohydrogen production by applying three independent variables, including light intensity, agitation rate and dilution percentage of DPOME. Light plays an important role during hydrogen evolution process catalyzed by nitrogenase enzyme (Meyer et al., 1978). Agitation rate is another factor which influences hydrogen production as the biogas production may be insufficient in the absence of suitable mixing of substrate and inoculum (Hamdi et al., 1991). Dilution of dark fermented effluents is another factor which affects photo-hydrogen production, as the optimal level of dilution of DPOME is required to get initial desired acetic acid concentration (Özkan et al., 2012).

**Table 4**
ANOVA for Response Surface Quadratic model [Analysis of variance table, Partial sum of squares-Type III].

| Source | Sum of Squares | df | Mean square | F Value | p-value Probe F | |
|---|---|---|---|---|---|---|
| Model | 27.36 | 9 | 3.04 | 13.10 | 0.0002 | significant |
| $X_1$-light intensity (W/m2) | 0.021 | 1 | 0.021 | 0.092 | 0.7678 | |
| $X_2$-agitation rate (rpm) | 0.095 | 1 | 0.095 | 0.41 | 0.5357 | |
| $X_3$- DPOME dilution (%) | 4.66 | 1 | 4.66 | 20.09 | 0.0012 | |
| $X_1$ $X_2$ | 0.44 | 1 | 0.44 | 1.90 | 0.1987 | |
| $X_1$ $X_3$ | 0.12 | 1 | 0.12 | 0.51 | 0.4902 | |
| $X_2$ $X_3$ | 0.83 | 1 | 0.83 | 3.60 | 0.0872 | |
| $X_1^2$ | 5.20 | 1 | 5.20 | 22.38 | 0.0008 | |
| $X_2^2$ | 11.74 | 1 | 11.74 | 50.56 | <0.0001 | |
| $X_3^2$ | 0.34 | 1 | 0.34 | 1.48 | 0.2510 | |
| Residual | 2.32 | 10 | 0.23 | | | |
| Lack of Fit | 1.29 | 5 | 0.26 | 1.24 | 0.4089 | not significant |
| Pure Error | 1.04 | 5 | 0.21 | | | |
| Cor Total | 29.68 | 19 | | | | |

Note: The Model F-value of 13.10 implies the model is significant. There is only a 0.021% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 1.00 indicate model terms are significant. In this case C, AB, $A^2$, $B^2$, $C^2$ are significant model terms. The "Lack of Fit F-value" of 1.24 implies the Lack of Fit is not significant relative to the pure error. There is a 40.89% chance that a "Lack of Fit F-value" this large could occur due to noise. Std. Dev.; 0.48, R-Squared; 0.9218, Mean; 1.75, Adj R-Sequared; 0.8514, C.V. %; 27.58, Pred R-Sequared; 0.2146, Press; 23.31, Adeq precision; 9.534.

Software "Design-expert[7.1.6]" was used to set the series experiment of 19 runs, where three variables were varied at different levels and hydrogen yield was investigated as response Table 3. The optimal levels of variables and their interactions with response were further investigated by Box-Behnken design. The multiple regression analysis of experimental values (Table 5), following second order polynomial equation was suggested to explain the hydrogen yield (eq. (1)).

$$Y = 2.78 - 0.049X_1 - 0.077X_2 + 0.72X_3 - 0.023X_1X_2 + 0.12X_1X_3 - 0.32X_2X_3 - 0.69X_1^2 - 0.66X_2^2 - 0.29X_3^2 \quad (1)$$

Where, Y represents the hydrogen yield and X1, X2 and X3 are the value of light intensity, agitation rate and dilution of DPOME respectively. Analysis of variance (ANOVA) suggested that response surface for hydrogen yield are well fit for a quadratic model with an *F-value* of 13.10 (Table 4). The model *F-value* of 13.10 implies the model is significant. There are only 13.40% chances that an *F-value* large because of noise. The software predicted that [dilution%], [light intensity]$^2$, and [agitation rate]$^2$ to be significant model terms. ANOVA suggested that the experimental data is statistically significant.

### 3.2. Perturbation plot analysis

The effect of variables at one point in RSM generated design can be compared using perturbation plot. It traces out the variations in the response generated due to variations in a factor as it is varied away from the reference point meanwhile the ratio of all other factors remains constant. In this prospectus, the possible variables are shown on a plot (single), which allows the visualization of the sensitivity of response to deviation from a central point. Indispensably, it shows the straight visualization of how sensitive system is to deviation from the central point. Therefore, perturbation plot or trace plots in response surface methodology helps in the comparatively effect of all variables in a design space. The sheerness of the curvature of given factor is indicated the sensitiveness of response is to changes in one variable. Thus, relatively flat lines can be interpreted as a general lack of significant effect on the response by variation in the factor under investigation (Bauer et al., 1999). In the present study this perturbation plot was used to investigate the hydrogen yield as three factors are studied as variables; light intensity, agitation rate and dilution of DPOME in design central point. From the perturbation plot obtained in present study shows that dilution (C) of dark fermented palm oil mill effluent had a significant effect on photo fermentative hydrogen yield (Fig. 1). On the other hand factors such as light intensity (A) and agitation rate (B) did not have a significant role in hydrogen yield from DPOME. Component C has a major role in the hydrogen production and B

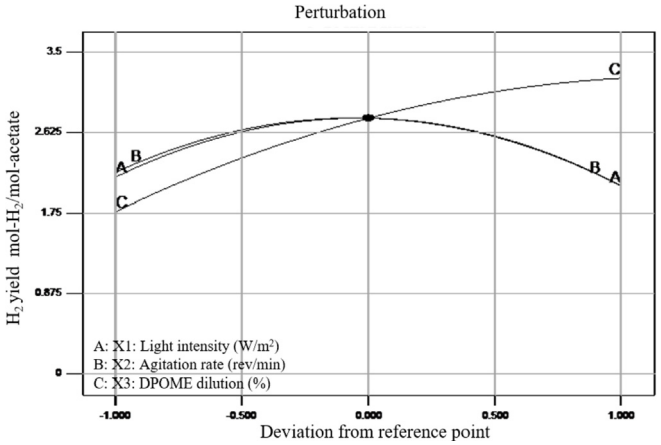


**Fig. 1.** Pertubation plot showing the effect of process parameters on hydrogen yield; Interaction plot.

and C had less drastic effect during operation. The optimal maximum hydrogen yield of 3.11 was observed at 30% dilution of DPOME when light intensity and agitation rate was 250 W/m$^2$ and 200 rpm respectively. The least hydrogen yield of 0.32 mol-$H_2$/mol-acetate was observed at 10% dilution of DPOME when light intensity and agitation rate was 300 W/m$^2$ and 100 rpm respectively. The analysis suggests that actual hydrogen yield of 3.1 mol-$H_2$/mol-acetate lies slightly higher with the predicted one of 2.80 mol-$H_2$/mol-acetate at 250 W/m$^2$, 200 rpm agitation rate, and 30% dilution of DPOME. The impact of dilution on photo-hydrogen production using dark fermented effluent has been previously investigated (Uyar et al., 2009a, b). The main concept behind this is to maintain an optimal acetic concentration for bacterial growth, which influences hydrogen production. Another study reported that dark fermented effluent of sugar beet thick juice fermented by *C. saccharolyticus* had dilated 3 times to enhance the photobiological hydrogen production by *R. capsulatus* (Özkan et al., 2012). Finally, the perturbation plots demonstrate that dilution of DPOME played an important role in photo fermentative hydrogen production. This can be due to concentrate substrate which includes high organic matter which inhibits inoculum growth as well as hydrogen production.

### 3.3. RSM plots for hydrogen yield and variables interactions

The produced three-dimensional response surface plots allow a pairwise comparison of variables effects on hydrogen yield. In every plot, the variable which is not represented by the two horizontal axes remains fixed at their central actual levels (Guo et al., 2009). Liu et al. (2015) showed that the highest predicted values are

**Table 5**
Regression analysis of the central composite rotary design.

| Factor | Coefficient Estimate | df | Standard Error | 95% CI Low | 95% CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 2.78 | 1 | 0.17 | 2.39 | 3.17 | |
| A-$X_1$ | −0.049 | 1 | 0.16 | −0.41 | 0.31 | 1.39 |
| B-$X_2$ | −0.077 | 1 | 0.12 | −0.35 | 0.19 | 1.00 |
| C-$X_3$ | 0.72 | 1 | 0.16 | 0.36 | 1.08 | 1.03 |
| $X_1$ $X_2$ | −0.23 | 1 | 0.17 | −0.61 | 0.15 | 1.00 |
| $X_1$ $X_3$ | 0.12 | 1 | 0.17 | −0.26 | 0.50 | 1.00 |
| $X_2$ $X_3$ | −0.32 | 1 | 0.17 | −0.70 | 0.057 | 1.00 |
| $A^2$ | −0.69 | 1 | 0.15 | −1.01 | −0.36 | 1.50 |
| $B^2$ | −0.66 | 1 | 0.093 | −0.87 | −0.46 | 1.02 |
| $C^2$ | −0.29 | 1 | 0.24 | −0.81 | 0.24 | 1.15 |

indicated by the response surface plots. They reported that the elliptical projection reflects the interactive interaction between two variables (Liu et al., 2015). The response plot using was CCD has been successfully applied for optimization of fermentative hydrogen production using palm oil mill effluent as substrate. Mohammadi et al., 2017, investigated the effect of up-flow velocity ($V_{up}$) and feed flow rate ($Q_F$) as independent variables on bio-hydrogen production (response) from POME and suggested the "well model prediction" (RSM) for parameters optimizations (Mohammadi et al., 2017). The response plots of the present investigation are illustrated in Figs. 2–4. In this study, when light intensity and agitation rates were varied at constant dilution of DPOME (30%), the hydrogen yield observed low at low light intensity and high agitation rate (Fig. 2 a&b). The maximum hydrogen yield of 3.0 mol-$H_2$/mol-acetate was observed at intermediate levels of these two variables. The appearance of the concentric ellipse of two-dimensional projections shows the interactive effects between variables (Fig. 2 a&b). With the variations in light intensities and dilution of DPOME at an intermediate agitation, 200 rpm showed an adequate effect on hydrogen yields (Fig. 3 a&b). The hydrogen yields decreased at high levels of light intensity and dilution of DPOME. The variation of light intensities is relatively more important than that of agitation rates on hydrogen (Fig. 3 a&b). Furthermore, as shown in response plot in Fig. 4 a&b, hydrogen yield increased with increasing the dilution of DPOME and influenced by agitation rate. This appears as interactive effects between dilutions and agitation rate. The highest optimal hydrogen yield of 3.11 mol-$H_2$/mol-acetate was achieved when dilation of DPOME increased to 30% and agitation rate increased to 220 rpm. Further increase in both the factors had shown the negative impact on hydrogen yield.

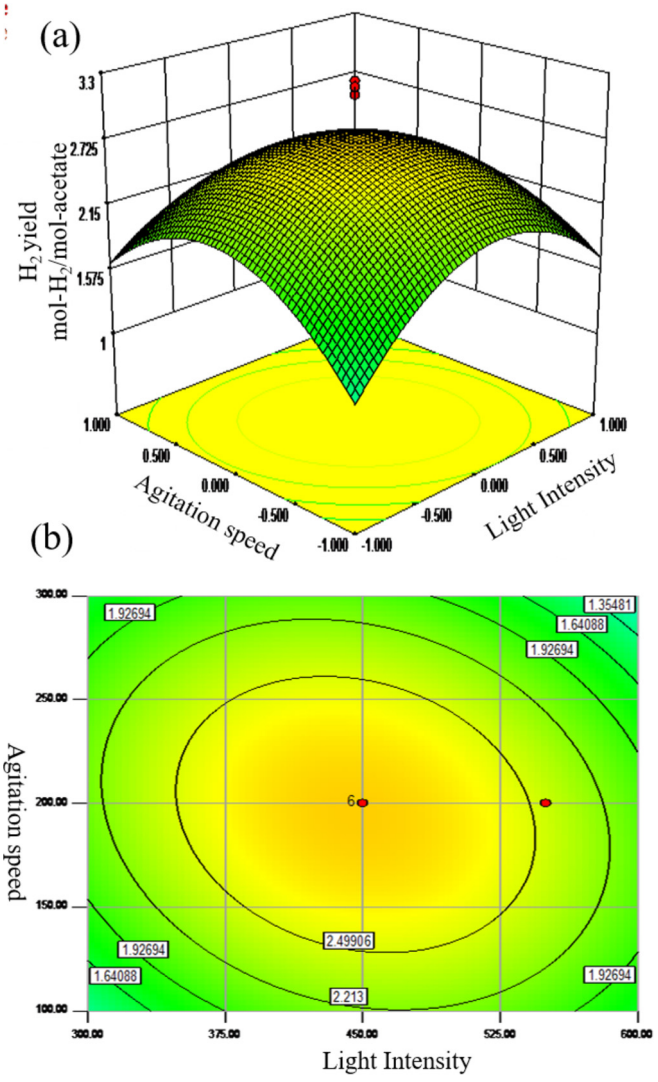


**Fig. 2.** (a) Response surface plot and (b) Its contour plot for $H_2$ yield: effect of agitation speed and light intensity.

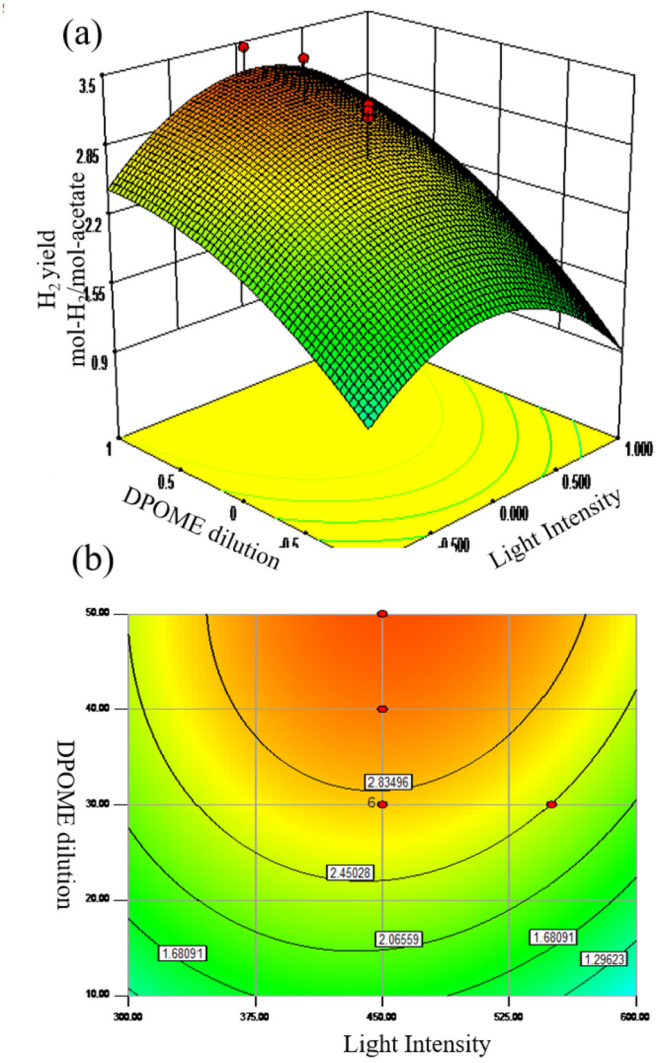


**Fig. 3.** (a) Response surface plot and (b) Its contour plot for $H_2$ yield: effect of DPOME dilution with tap water and light intensity.

### 3.4. Validation of the model equation

The optimal results as suggested by statistical design, furthermore verified by performing photo-fermentation at set parameters with: 30% DPOME, light intensity of 250 W/$m^2$ and agitation rate of 200 rpm. Based on these optimal conditions hydrogen production was conducted in triplicates to get significant hydrogen yield. Under the optimized conditions the hydrogen yield was 3.07 ± 0.66 mol-$H_2$/mol-acetate, suggesting that experimental and predicted yields (2.80 mol-$H_2$/mol-acetate) were in good agreement. The correlation between the actual and predicted conditions verifies the model validation and existence of an optimal point.

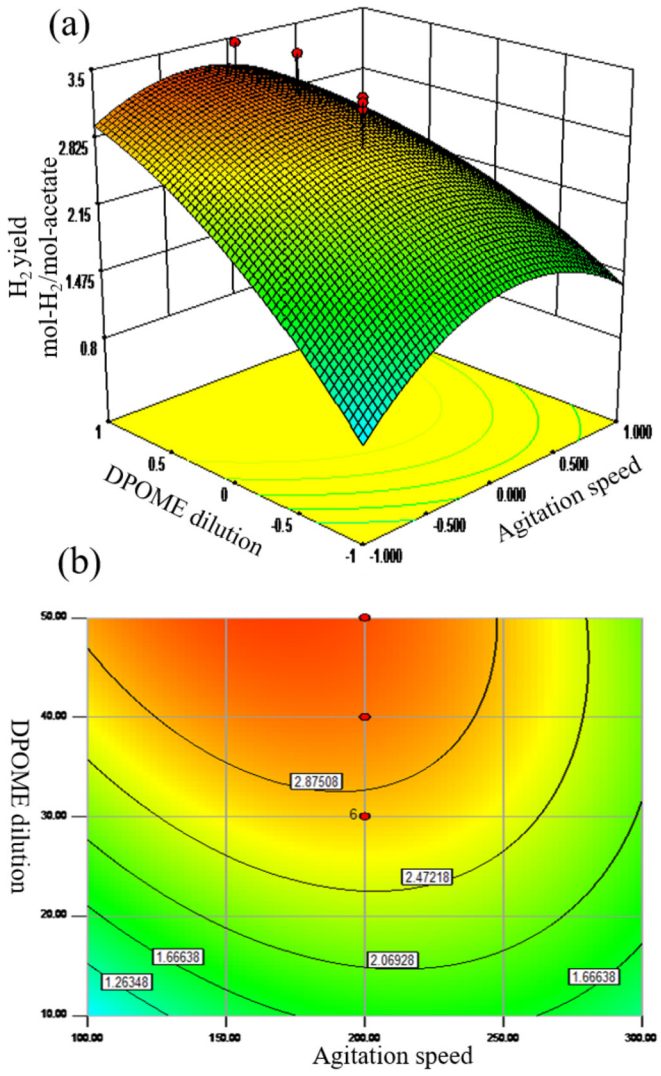


**Fig. 4.** (a) Response surface plot and (b) Its contour plot for $H_2$ yield: effect of DPOME dilution with tap water and agitation speed.

Light energy plays an important role along with organic substrates during photo synthetic hydrogen production. Several researchers been carried out to find the optimal light intensity for hydrogen production using various microorganism (Kim et al., 2006; Basak et al., 2014; Adessi et al., 2014). Photofermentation hydrogen production at optimum light intensity 270 W/m$^2$ by *R. sphaeroides* O.U. 001 have been reported from the prepared medium containing glutamate 2 mM and malate 15 mM (Uyar et al., 2007). In another experiment, the optimum light intensity of 4170 lux was reported for hydrogen from acetate, butyrate and propionate mixtures by *Rhodopseudomonas capsulate* using response surface methodology (Shi et al., 2005). The optimal light intensity of photo-fermentative hydrogen production also has been investigated using industrial wastewaters. For instances, Seifert et al. investigated the effects of light intensity on photo-fermentative hydrogen production from dairy wastewater. They suggested the optimum light intensity of 9000 lux when *R. sphaeroides* O.U.001 was applied as inoculum (Seifert et al., 2010a). Furthermore, Brewery wastewater was subjected as a substrate for hydrogen production by *R. sphaeroides* O.U.001. Their results suggested that at a light intensity of 116 W/m$^2$, inoculum had the optimal hydrogen production efficiency (Seifert et al., 2010b). In this study, shown by RSM results, light intensity impacts the hydrogen production by *Rohopesudomans pultuirs* from DPOME and high intensity of has the negative effects.

Factors such as agitation rates and dilution of dark fermented effluent effectively influence the photo-hydrogen production. Ozager et al. reported that the three-time dilution of dark fermented effluents of sugar beet molasses had the positive impact on enhanced photo hydrogen production (Özgür et al., 2010). While in another study 10% dilution of the dark fermented effluent of brewery waste water had the enhanced hydrogen production effect (Seifert et al., 2010b). In contrast to these results, our results suggested that 40% dilution is optimal for hydrogen from DPOME. The variation in dilution percentages can be attributed to the complexity of waste waters, as each type of waste water is significantly different in their COD, BOD, VSS etc. concentrations. Various investigations have been done to effects of agitation on biogas production (Karim et al., 2005a). Karim et al. successfully investigated enhanced biogas production from the agitated substrate as compared to unmixed one (Karim et al., 2005b). Moreover, Kaparaju et al. investigated the influences of mixing on biogas production cattle slurry from 6.5 to 7.5% TS and reported the improved process performance in CSTR reactor (Kaparaju et al., 2008). By comparing the effect of agitation rate on hydrogen production with these results, our investigation has an agreement that an agitation rate can improve the hydrogen production efficiency as shown in the present study.

## 4. Conclusions

In batch experiments, dark fermented palm oil mill effluents (DPOME) were used as a substrate for photo-fermentative hydrogen using *Rhodopseudomanas palustris*. The hydrogen yield was investigated at various levels of light intensity, agitation rate, and dilution of DPOME by response surface methodology. The maximum hydrogen yield of $3.07 \pm 0.66$ mol-$H_2$/mol-acetate was achieved under the optimized conditions: light intensity 250 W/m$^2$, agitation rate 200 rpm, and 30% dilution of DPOME, respectively. The validation test of obtained optimal conditions of hydrogen yield from DPOME had good agreement with the predicted values (2.80 mol-$H_2$/mol-acetate). The experimental results suggested that statistical design for process optimization by RSM is an efficient and feasible technique to get enhanced hydrogen production through biological processes. The future work on use of DPOME for photohydrogen production at large-scale seems more advantageous application prospects in enhancing hydrogen productivity from photo-fermentation.

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