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Research Article

BIOETHANOL PRODUCTION FROM HYDROLYSATE DERIVED BY ULTRASONIC PRETREATED DEFATTED BIOMASS OF MUNICIPAL WASTEWATER GROWN MUTANT *TETRADESMUS DIMORPHUS* EMS2

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ABSTRACT. Microalgae biomass is considered an emerging source for future generation feedstock for both biodiesel and bioethanol production due to the accumulation of high amounts of lipids and carbohydrates respectively. In this present investigation, 70% ultrasonic pre-treated municipal wastewater (MWW) grown defatted mutant green microalga, *Tetradesmus dimorphus* EMS2 biomass was ultrasonic pretreated for hydrolysate preparation and its essential process parameters were statistically optimized using CCD-RSM. The prepared hydrolysate used as a cheap culture medium for bioethanol production by fermentation using *Saccharomyces cerevisiae* NITTS1. The maximum bioethanol yield of 51.45±0.12 gL^{-1} was obtained from the hydrolysate prepared from 55 g L^{-1} defatted biomass pretreated at 0.35 WL^{-1} ultrasonic density for 20 min than un-pretreated defatted biomass. The hydrolysate prepared from 55 gL^{-1} defatted biomass primarily contained simple sugars such as glucose (78.17±0.13 % w/w) and xylose (16.02±0.21 % w/w). Further, in this study, the essential physical parameters were optimized by the classical method and found that the maximum bioethanol of 54.36±0.11 g L^{-1} was produced at optimum fermentation conditions of 30 ℃, pH 4 and 150 rpm. This finding suggests that ultrasonic pretreated MWW grown defatted mutant *T. dimorphus* EMS2 biomass could be used as an ecofriendly-sustainable feedstock for bioethanol production after ultrasonic pretreatment.

Keywords: *Bioethanol, defatted biomass, fermentation, Tetradesmus dimorphus, municipal wastewater, ultrasonic pretreatment.*

INTRODUCTION

In recent years, industrialization and urbanization have skyrocketed the world's energy demand. India is the world's fourth prime country in oil consumption mainly for transportation purposes. Therefore, the imports of crude oil increased from 171.73 MTs to 226.95 MTs from 2011-12 to 2019-20 [1]. According to the International Energy Agency (IEA), India's oil requirement expected to increase to 7.1 and 8.7 mb/d by 2030

and 2040, respectively, from 5.0 mb/d in 2019 [2]. Hence, there is an urgent need to explore novel energy sources that are cost-effective and environmentally friendly. At present situation, bio-based energy is one of the renewable energy sources that are vital and imperative to facilitate present society's sustainability as well as deal with the harmful properties and disadvantages of petroleum-based fuels [3]. Therefore, the present researchers are mainly focusing on biofuels production [4] including bioethanol [5], biodiesel, biogas [6], etc., using various renewable sources. Ethanol (CH_3CH_2OH) is considered a promising alternative to gasoline and common alcohol fuel existing in the World market for biofuels. Ethanol is comparatively not as harmful as petroleum-based fuels as by-products of partial oxidation of ethanol are also harmless than the derivatives of petroleum-based fuels [7].

Generally, bioethanol is produced from various edible plants such as sugarcane, maize, wheat, food grains, sorghum and potato. These feedstocks are considered as fuel crops [8]. However, the availability of fuel crops is insufficient to meet up recent demand for ethanol production since the same materials are used as main food sources for humans. Hence, this is a major disadvantage of today's bioethanol production and is not balanced between the proportion of feedstocks (fuel crops) and the quantity of ethanol production. The uses of food crops as feedstock for bioethanol production through microbial fermentation have affected the cost of food prices. Nevertheless, by utilizing the cheapest feedstock, bioethanol commercialization can be economically viable [9, 10].

Nowadays, microalgae biomass is used as an alternative feedstock for both biodiesel and bioethanol production since they have high amounts of lipids and carbohydrates [4]. Microalgae can grow without soil and they need not require freshwater as well as any other substances for their biomass production [11, 12]. Besides, they also are able to grow in various wastewaters with a high rate of growth [13], which is about 10-fold as rapid as sugarcane [14]. The efficient photosynthetic process of microalgae can confiscate carbon dioxide from the atmosphere more than other higher plants [15]. The rapid development of microalgae can be cultivated in various wastewaters as well as in wasteland with yield rate 15-300 folds higher than land-based fuel crop production [16].

The economic viability and sustainability of microalgae-based bioenergy development can be enhanced considerably through a biorefinery approach anywhere for a variety of applications. According to the bio-refinery idea, each constituent of microalgae biomass substance could be utilized to manufacture economically important products. In common, the production of microalgal biomass to produce oil towards biodiesel production is significantly raising gradually [10]. Lipid extracted microalgal biomass such as defatted residues could be used for production of bioethanol through fermentation. This defatted waste material is used as an alternative protein source in aquaculture and poultry [17, 18, 19, 20]. Many researchers have reported that from defatted microalgal biomass, bioethanol could be produced after appropriate pretreatment (mechanically, chemically, or enzymatically) to release fermentable sugars [4, 10, 18, 21, 22]. After extracting the fermentable sugar from defatted microalgal biomass using suitable pre-treatment, it can be utilized as a substrate for bioethanol production by yeast. Among the various type of yeast, the strain *Saccharomyces cerevisiae* was the best yeast for maximum bioethanol production through anaerobic fermentation of hydrolysate obtained from defatted biomass of green microalga *Chlorella sorokiniana* NITTS3 by ultrasonic pre-treated [10]. So far, no attempt has been finished for the production of bioethanol from mutant defatted biomass hydrolysate obtained by ultrasonic pre-treatment. Therefore, this study was aimed at bioethanol production from hydrolysate obtained by ultrasonic pre-treated

defatted microalgal biomass of municipal wastewater grown mutant green microalga *Tetradesmus dimorphus* EMS2 using a *Saccharomyces cerevisiae* NITTS1.

MATERIALS AND METHODS

Microalga

The green microalga *Tetradesmus dimorphus* NSA7 (GenBank Accession Number: OP412378.1) was isolated from freshwater Lake in Kancheepuram (Latitude 12.81˚N and Longitude 79.69˚E), Tamil Nadu, India. This wild strain was treated with 2.0 M ethyl methane sulfonate (EMS) for random mutagenesis and obtained *T. dimorphus* EMS2 as a mutant strain. Then this mutant strain was maintained and revived every month in the modified Chu 13 medium.

Ultrasonic pre-treatment of municipal wastewater

Municipal wastewater (MWW) was collected from sewage treatment plant (8.70) MLD, Tiruvannamalai municipality, Tiruvannamalai (Latitude 12.22˚N and Longitude 79.07˚E), Tamil Nadu, India. The collected MWW was transferred to the laboratory and stored in the refrigerator until used. Ultrasonic pre-treatment of MWW was carried out by modified methods of Dhandayuthapani et al., [5]. The probe type sonicator with a 1.2 cm in diameter metal probe was used (Lark Innovative Fine Teknowledge, Chennai, India). The pre-treatment was carried out in a 250 mL stainless steel beaker containing 100 mL of 70% MWW for 20 min at 0.35W mL^{-1} (25kHz). During the sonication, the sample was lightly shacked under a temperature of 30±2 ^{o}C. Finally, ultrasonic pre-treated MWW (UPMWW) was used as a sole culture medium for the cultivation of mutant *T. dimorphus* EMS2.

Cultivation of T. dimorphus EMS2

The cultivation was carried out in 250 mL eerlenmeyer flasks containing 100 mL of UPMWW. About 10% (v/v) of fresh culture was inoculated into UPMWW to initiate the cultivation under incubated at 120 rpm, 25±1 ^{o}C under the light intensity of 33 µE $m^{-2}s^{-1}$ for 12:12 h day and night cycle for 20 days. Every five days once, 5 mL of sample was collected from a culture broth for biomass estimation. Each experiment was carried out in triplicate and the values are represented as mean ± standard deviation of three replications.

Defatted biomass preparation

For defatted biomass preparation, the biomass was harvested by collecting the sample from the culture broth and centrifuged at 14,000 rpm for 15 min by a centrifuge (Remi Model R-8C BL, Mumbai, Maharashtra, India). Then harvested biomass was cleaned by being treated with deionized water twice and centrifuged. Subsequently, the cleaned biomass was completely dried at 60 ^{o}C by using a hot air oven. Bligh and Dyer's [23] method was adapted for lipid extraction from dried biomass. Finally, defatted biomass (lipid removed) was dried at 28±2 ^{o}C (room temperature) and then it was used for further investigation.

Optimizing ultrasound assisted hydrolysate preparation using defatted biomass for ethanol production

The hydrolysate preparation using defatted biomass was performed by taken slurry in a stainless steel beaker (250 mL) and dipped a 1.2 cm diameter metallic probe up to 1.5 cm depth. This pre-treatment was conducted with a probe type sonicator with a 1.2 cm in diameter metal probe was used (Lark Innovative Fine Teknowledge, Chennai, India). During the sonication process, the sample was lightly shacked under the temperature at 30±2 ^{o}C [24]. Untreated defatted biomass was used as a control. The ultrasound assisted hydrolysate preparation parameters namely defatted biomass concentration (DBC), ultrasound power density (UPD), and exposure time were optimized using 2^3 Central Composite Design (CCD) in Response Surface Methodology (RSM). These three factors were formulated using the MINITAB 12 design expert software. As shown in Table 1, each factor was tested at three different coded levels. The prepared ultrasound assisted hydrolysate was utilized as a sole fermentation medium for ethanol production by batch fermentation. The batch fermentation was performed in duplicates for every run and the ethanol yield was considered as the response. The regression analysis, statistical significance, and analysis of variance (ANOVA) were performed and fitted into a second order polynomial model. The 3D response surface curves also were plotted to study the interaction among the three factors.

Table 1. *Three independent variables levels of CCD for ultrasound assisted hydrolysate preparation from defatted biomass for ethanol production.*

Independent Variables	**Code**	**Levels**		
		Low (-1)	**Medium (0)**	**High (+1)**
Defatted biomass concentration (g L^{-1})	X1	10	55	100
Ultrasound power density (W mL^{-1})	X2	0.2	0.35	0.5
Exposure time (min)	X3	10	20	30

Ethanol production by batch fermentation.

About 100 mL of sterilized hydrolysate was taken in 250 mL Erlenmeyer flasks and inoculated with 10% (v/v) (10^7 cells mL^{-1}) of freshly prepared *S. cerevisiae* NITTS1 (Genbank A/C Number - MG255132.1), obtained from National Institute of Technology Tiruchirappalli, Tamil Nadu, India. Then the cultures were incubated in an orbital shaker at room temperature (28±2 ^{o}C) for 48 h at 120 rpm. End of the experiment, the sample was centrifuged at 14,000 rpm for 20 min using a centrifuge (Remi Model R-8C BL, Mumbai, Maharashtra, India). The cell free sample was used for the estimation of ethanol by the method of Seo et al., [25] and untreated defatted biomass was maintained as control.

Optimizing physical parameters for ethanol production

In order to find the optimum temperature, pH, and agitation speed for enhancing the bioethanol production from hydrolysate obtained from defatted biomass of mutant *T. dimorphus* EMS2, the above physical parameters of batch fermentation were optimized by one parameter at a time approach. The batch fermentation was carried out in 250 mL air-tight Erlenmeyer flasks with 100 mL of ultrasound assisted hydrolysate prepared from defatted biomass and with 10 %(v/v) of freshly prepared *S. cerevisiae* NITTS1 at different temperature ranges 25 to 45 ^{o}C with an increment of 5 ^{o}C. The optimum temperature of this experiment was used for further study. Similarly, the fermentation was performed with the parameters pH (2 to 6 with an increment of pH 1) and agitation speed (100 to 300 rpm with an increment of 50 rpm). Every 12 h once the sample was collected and removed the yeast cells by centrifuged at 14,000 rpm for 15 min. The cell free sample was used for ethanol estimation. Each experiment was performed in triplicate and the values were presented as the mean ± SD.

RESULTS AND DISCUSSION

Optimizing ultrasound assisted hydrolysate preparation from defatted biomass for bioethanol production

To enhance the bioethanol production from hydrolysate, the hydrolysate preparation essential process parameters such as DBC (10-100 gL^{-1}), UPD (0.2-0.5 WmL^{-1}), and exposure time (10-30 min) were optimized using 2^3 CCD in RSM. These three factors were formulated using the MINITAB 12 design expert software. A set of 20 experiments were performed in replicate. Table 2, shows the design matrix incorporated three independent variables of DBC (X_1), UPD (X_2), and exposure time (X_3) with the experimental as well as the predicted values of bioethanol production from the hydrolysate. The Results of CCD were analyzed by coded units (Table 3). This model was expressed with the following regression (1) represents ethanol yield (Y) as a function of DBC (X_1), UPD (X_2), and exposure time (X_3).

$$Y_{CODED} = 51.46 + 1.44X_1 + 2.902X_2 + 3.61X_3 - 6.31X_1^2 - 4.85X_2^2 - 3.74X_3^2 + 1.31X_1X_2 + 0.54X_1X_3 + 0.52X_2X_3 \quad \text{----------(1)}$$

Where Y is the ethanol production (gL^{-1}), X_1, X_2 and X_3 are the coded values of DBC, UPD and exposure time respectively.

The fitness of the model designed for the production of bioethanol was specified by the coefficient of determination (R^2). The estimated value of R^2 was found to be 0.98, which represents the 98% of confidence level in the selected response as explained by the model [5]. The obtained experimental values are more matches with predicted values that state the exactitude of values given. Based on the ANOVA analysis (Table 4) the selected model of this study was more fitting and highly significant for bioethanol production using hydrolysate derived from defatted biomass of mutant *T. dimorphus* EMS2. Also in this study, the individual effect X_1, X_2, and X_3, quadratic effects X_1^2, X_2^2 and X_2^3 and interactive effects X_1X_2, X_1X_3 and X_2X_3,were noteworthy at $p<0.05$.

Table 2. *CCD matrix of three independent variables with experimental and predicted values of ethanol produced from ultrasound assisted hydrolysate of defatted biomass of mutant T. dimorphus EMS2.*

Run order	Defatted biomass conc. (g L^{-1}) (X_1)	Ultrasound power density (WmL^{-1}) (X_2)	Exposure time (min) (X_3)	Ethanol yield (g L^{-1})	
				Experimental value	Predicted value
1	10	0.20	10	31.25±0.12	30.46
2	100	0.20	10	30.12±0.14	30.18
3	10	0.50	10	33.11±0.13	33.16
4	100	0.50	10	37.18±0.21	37.61
5	10	0.20	30	36.45±0.15	36.11
6	100	0.20	30	37.45±0.12	37.44
7	10	0.50	30	40.35±0.13	40.33
8	100	0.50	30	46.59±0.05	46.88
9	10	0.35	20	43.15±0.14	43.73
10	100	0.35	20	47.32±0.11	46.61
11	55	0.20	20	43.15±0.13	43.71
12	55	0.50	20	50.21±0.16	49.52
13	55	0.35	10	44.35±0.11	44.12
14	55	0.35	30	51.23±0.05	51.33
15	55	0.35	20	51.42±0.06	51.46
16	55	0.35	20	51.36±0.11	51.46
17	55	0.35	20	51.45±0.12	51.46
18	55	0.35	20	51.44±0.15	51.46
19	55	0.35	20	51.43±0.14	51.46
20	55	0.35	20	51.32±0.12	51.46

Table 3. *Estimated coefficients and probability values for optimizing ethanol production from ultrasound assisted hydrolysate of defatted biomass of mutant T. dimorphus EMS2.*

Variables	Estimated Coefficients	t-value	p-value
Model	51.4569	321.092	<0.001[a]
X_1	1.4350	9.734	<0.001[a]
X_2	2.9020	19.686	<0.001[a]
X_3	3.6060	24.462	<0.001[a]
X_1^2	-6.2923	-22.384	<0.001[a]
X_2^2	-4.8473	-17.244	<0.001[a]
X_3^2	-3.7373	-13.295	<0.001[a]
X_1X_2	1.3050	7.918	<0.001[a]
X_1X_3	0.5375	3.261	<0.009[a]
X_2X_3	0.5150	3.125	$<0.011_a$

[a] *Significant at $p<0.05$ level*

Table 4. *ANOVA for ethanol production from ultrasound assisted hydrolysate of defatted biomass of mutant T. dimorphus EMS2.*

Source	**Degree of freedom**	**Sum of Squares**	**Mean Square**	**F-value**	***p*-value**
Regression	9	1070.97	119.00	547.60	<0.001*
Linear	3	234.84	78.30	360.23	<0.001*
Square	3	818.07	273.00	1000	<0.001*
Interaction	3	18.06	6.02	27.70	<0.001*
Residual Error	10	2.17	0.22		
Lack of Fit	5	2.16	0.431	0.54	0.645
Pure Error	5	0.02	0.003		
Total	19	1073.14			

[a] Significant at p<0.05 level
R-Sq = 98.5% R-Sq(adj) = 98.3%

The CCD in RSM results was used to plot the three-dimensional (3D) response surface curve to find out the interaction effect of DBC, UPD and exposure time on bioethanol production is given in Fig. 1, 2, 3. On a surface plot, the responses were studied, taking two factors at a time while keeping the other one at a fixed level. The surface plots (Fig. 1, 2, 3) shows the combined effect of two factors on the bioethanol yield, while another one is a constant factor. It is observed that the activity value increased on increasing the DBC, UPD, and exposure time from the low to middle points such as 55 g L^{-1}, 0.35 W mL^{-1}, and 20 min respectively, after which further increase caused a decrease in the bioethanol yield. From the surface plots (Fig. 1, 2, 3) the 55 g L^{-1} defatted biomass, 0.35 W mL^{-1} UPD, and 20 min exposure time were found as the most favourable conditions for the production of maximum bioethanol of 51.45±0.12 g L^{-1} (run no. 17), while untreated defatted biomass yielded 22.15±0.21 g L^{-1} bioethanol. Because the monosaccharide glucose 78.17±0.13% was obtained from 55 g L^{-1} defatted biomass by ultrasonic pre-treatment at 0.35 W^{-1} mL for 20 min.

Analysis of carbohydrate content in hydrolysate of ultrasonic pre-treated defatted biomass

The composition of sugar molecules of ultrasound-assisted hydrolysate prepared using 55 g L^{-1} defatted biomass of mutant *T. dimorphus* EMS2 by ultrasonic pre-treatment at 0.35 W mL^{-1} for 20 min is given in Table 5. The carbohydrate concentration of carbohydrate in the ultrasound assisted hydrolysate obtained using defatted biomass of mutant *T. dimorphus* EMS2 is highly similar to the results reported by [10]. The carbohydrate obtained from the defatted biomass mainly contained simple sugar glucose (78.17±0.13 %) and xylose (16.02±0.21%). Whereas the total carbohydrate of unsonicated defatted biomass of mutant *T. dimorphus* EMS2 was measured as 22.36±0.13%. About 2.3-fold carbohydrate was extracted from 55 g L^{-1} defatted biomass of mutant *T. dimorphus* EMS2 by ultrasonic pre-treatment at 0.35 W mL^{-1} for 20 min. Besides, the degradation products of sugar namely acetic acid, lactic acid, formic acid, and propionic acid were not detected in hydrolysate using HPLC.

In this study, the glucose could be successfully converted into bioethanol by *S. cerevisiae* NITTS1 by fermentation as stated. Microalgae contains a substantial quantity

of carbohydrates in the form of fermentable sugars that are the best feedstock for bioethanol production [26]. Using second-generation feedstock for example food crops for bioethanol production has many challenges due to their direct effect on deforestation and food prices. Microalgal biomass can be used as an alternative feedstock to overcome the aforesaid problem. Because the microalgae produce carbohydrates as a primary metabolite by photosynthesis. In addition, the carbohydrates recovered from microalgae, for bioethanol production, are very simple. Whereas a strong pre-treatment is required for recovery, the carbohydrates from a lignocellulosic feedstock for fermentation. Therefore defatted microalgal biomass and non-defatted microalgal biomass could be used as sustainable feedstock for bioethanol production by fermentation.

Table 5. *Estimation of sugar composition of hydrolysate obtained from defatted biomass of mutant T. dimorphus EMS2.*

Sugar composition	**Concentration (%, w/w)**	
	Before ultrasound treatment	**After ultrasound treatment**
Total carbohydrate content	22.36±0.13	48.56±0.21
Maltose	n.d	n.d
Glucose	37.59±0.12	78.17±0.13
Xylose	5.86±0.11	16.02±0.21
Ramnose	2.22±0.21	3.11±0.22
Fucose	1.89±0.12	2.14±0.11
Other	0.31±0.14	0.56±0.12

n.d- Not detected

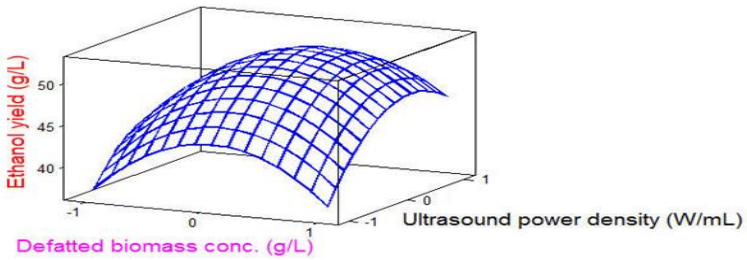

Fig. 1. *Three-dimensional response surface plot showing the interaction effect of DBC and UPD on bioethanol production.*

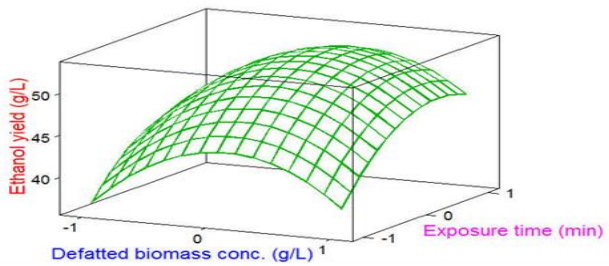

Fig.2. *Three-dimensional response surface plot showing the interaction effect of DBC and exposure time on bioethanol production.*

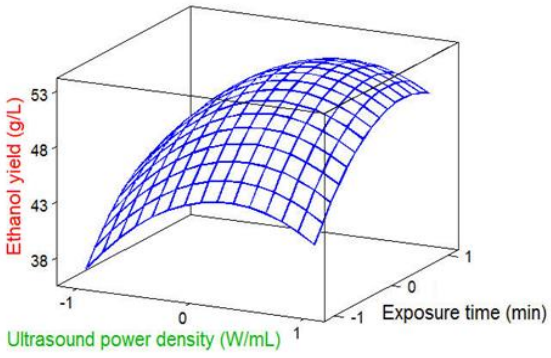

Fig. 3. *Three-dimensional response surface plot showing the interaction effect of UPD and exposure time on bioethanol production***.**

Optimizing the temperature for bioethanol production

This study was conducted with various temperature ranges from 25 ^{o}C to 45 ^{o}C with an increment of 5 ^{o}C. 100% hydrolysate was used as a bioethanol production medium, and it was inoculated with 10% v/v, of fresh yeast strain *S. cervevisiea* NITTS1. As observed in Figure 4, yeast strain *S. cervevisiea* NITTS1 very effectively produced the maximum bioethanol of 51.89±0.14 g L^{-1} at 30 ^{o}C by converting sugars, which are present in the hydrolysate. It is noticed from Fig. 4, that rising the temperature from 25 ^{o}C to 30 ^{o}C has resulted in a clear increase in bioethanol production from 38.12±0.11 to 51.89±0.14 g L^{-1}. When increasing the temperature above 30 ^{o}C, a decrease in bioethanol production is observed. This tendency of changes in bioethanol production with fermentation temperature is in accordance with the results expressed by [10] Dhandayuthapani et al., (2021). Generally, the yeast *Saccharomyces* strains are fermenting well and produce bioethanol at the temperature range of 20 to 35 ^{o}C [27, 28]. The calculated 'r' value of correlation analysis is significant at 0.05 levels at df_5. Therefore, the fermentation temperature and ethanol production are interdependent. In this study, the highest bioethanol production was obtained at 30 ^{o}C. Hence, the temperature of 30^{o}C was used as the optimum temperature for further study.

Optimizing the pH for bioethanol production

The effect of fermentation pH on bioethanol production using 100% hydrolysate of defatted biomass was investigated. The fermentation was carried out at different pH ranges from 2 to 6 with an increment of pH 1. Fig. 5, shows that the *S. cervevisiea* NITTS1 fermented effectively the sugars of hydrolysate obtained from defatted biomass of mutant *T. dimorphus* EMS2 by ultrasonic pre-treatment at 0.35 W mL^{-1} for 20 min and produced the highest bioethanol of 52.45±0.12 g L^{-1} at pH 4.0, which is good agreement with the results of Lin *et al.,* [29] and Dhandayuthapani et al., [10]. The calculated 'r' value of correlation analysis is significant at 0.05 levels at df_5 and it suggests that the fermentation capability of *S. cervevisiea* NITTS1 is pH dependent. In this study, the highest bioethanol production was obtained at pH 4.0. Hence, pH 4.0 was used as the optimum pH for further study.

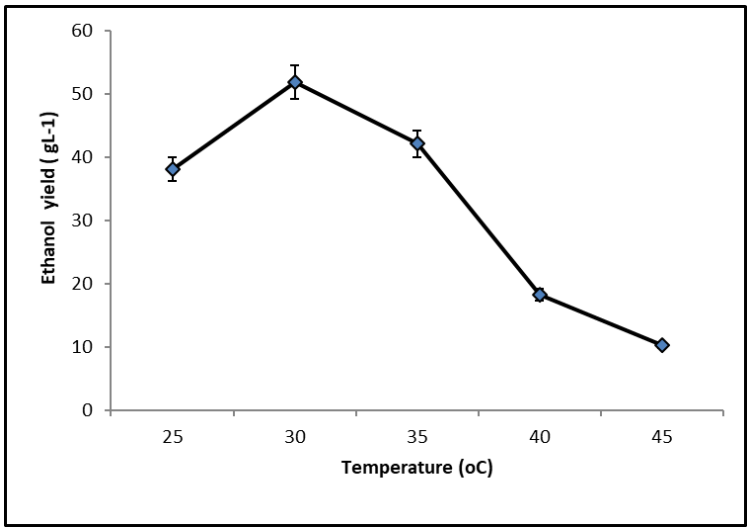

Fig. 4. *Effect of temperature on ethanol production using hydrolysate of ultrasonic pre-treated defatted biomass.*

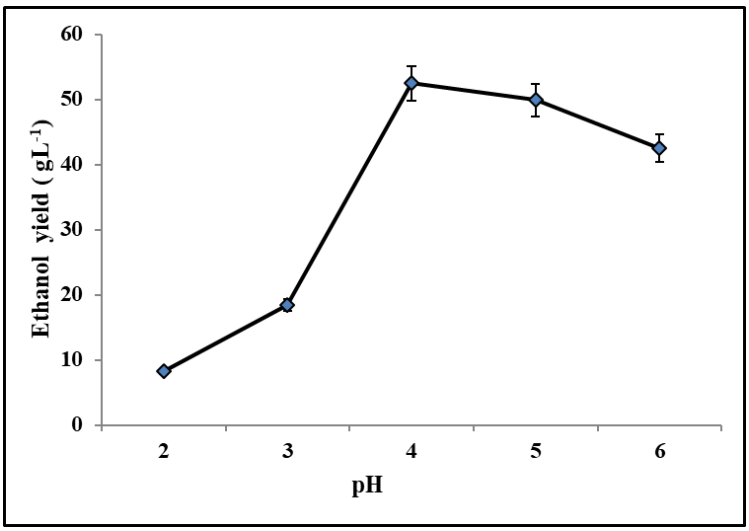

Fig. 5. *Effect of pH on ethanol production using hydrolysate of ultrasonic Pre-treated defatted biomass.*

Optimizing the agitation speed for bioethanol production

The effect of various agitation speeds ranging from 100 to 300 rpm on bioethanol production from 100% hydrolysate was studied. When the agitation speed was raised from 100 to 150 rpm the bioethanol yield was increased from 52.34±0.12 to 54.36±0.11 g L^{-1}. Further increase in the agitation speed resulted in decreased bioethanol yield. However, the highest bioethanol production of 54.36±0.11 g L^{-1} was obtained at 150 rpm at 30 ^{o}C and pH 4.0, which is in good agreement with the results of Dhandayuthapani et al., [10]. The results of this study state that the higher agitation speed reduces the bioethanol yield because the over mixing may interrupt the *S. cervevisiea* NITTS1 growth and its fermentation capacity. The calculated 'r' value of correlation analysis is significant at 0.05 levels at df_5 and it suggests that the fermentation capability of *S. cervevisiea*

NITTS1 is medium mixing dependent. In this study, the highest bioethanol production of 54.36±0.11 g L^{-1} was obtained at 150 rpm. Hence, 150 rpm was used as the optimum agitation speed for further study.

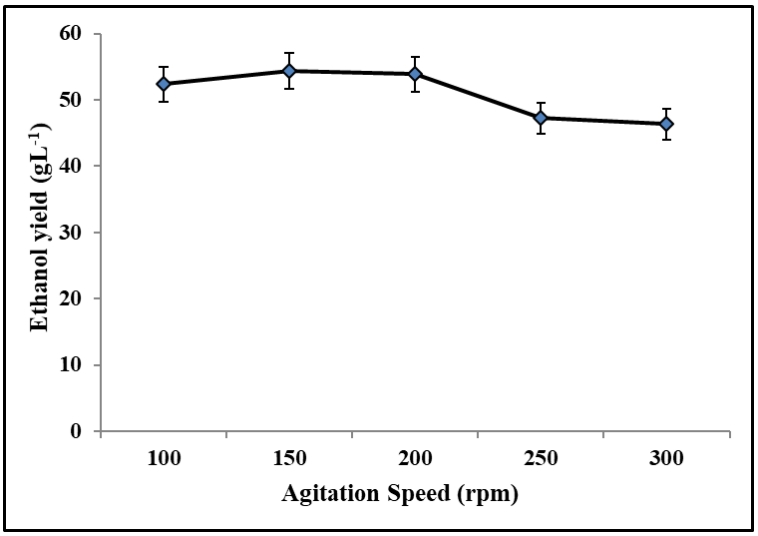

Fig. 6. *Effect of agitation speed on ethanol production using hydrolysate of ultrasonic pre-treated defatted biomass.*

CONCLUSION

The mutant green microalga *T. dimorphus* EMS2 was cultivated in 70% ultrasonic pre-treated MWW and its defatted biomass was used for hydrolysate preparation. The hydrolysate obtained from ultrasonic pre-treated defatted biomass was effectively utilized for bioethanol production by fermentation using yeast *S. cerevisiae* NITTS1. The ultrasonic pre-treatment process parameters were statistically optimized using CCD-RSM and found DBC 55 g L^{-1}, UPD 0.35 W mL^{-1} and the exposure time 20 min as optimum conditions with maximum bioethanol yield of 51.45±0.12 g L^{-1}. After ultrasonic per-treatment the hydrolysate carbohydrate content was analyzed and it was found that glucose and xylose are the major simple sugars. Besides the effect of physical parameters on bioethanol production was optimized and found to be 30 ^{o}C, pH 4 and 150 rpm with the highest bioethanol yield of 54.36±0.11 g L^{-1}. The outcome of this study demonstrates that the ultrasonic pre-treated defatted biomass of mutant *T. dimorphus* EMS2 is a biorefinery approach thus could be a overcome the bottleneck of the sustainable renewable eco-friendly-biofuel production from the wastewater grown indigenous microalgae at large scale. Moreover, the findings show that the treated microalgae defatted biomass can be an ideal feedstock for bioethanol production thus may be used as future fuel for the E-20 bikes where the blended of fuel 20 percentage ethanol and 80 percentage petrol that could be used for a carbon neutral future for mobility. However, more research has to be carried out to identify microalgae defatted biomass which are bring down price of fuel as a go green eco-friendly technology for the future applications.

Conflict of Interest. The authors declared that there is no conflict of interest.

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