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Research article

Biological nutrient recovery from human urine by enriching mixed microalgal consortium for biodiesel production

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ABSTRACT

Utilization of waste resources is necessary to harness the long-term sustainability of algal technology. The study focused on the use of human urine as the basic nutrient source for culturing native microalgal consortium and further optimized the process parameters using response surface methodology. A full factorial, central composite rotatable design (CCRD) with three variables: urine concentration (1–10% vol of urine/vol of distil water [%v/v]), pH (6.5–9) and light intensity (50–350 $\mu mol\ photons\ m^{-2}sec^{-1}$) was used to evaluate the microalgal biomass and lipid content. Results indicated that at 95% confidence limits, the selected factors influence the biomass and lipid productivity. The maximum biomass productivity of 211.63 ± 1.40 mg l^{-1} d^{-1} was obtained under optimized conditions with 6.50% v/v of urine, pH of 7.69 and at light intensity of 205.40 $\mu mol\ photons\ m^{-2}sec^{-1}$. The lipid content was found to increase from 18.96 ± 1.30% in control media to 26.27 ± 1.94% under optimal conditions. The interactive effect of variables over the microalgal biomass and lipid content has also been elucidated. The data obtained were comparable to the BG11 media (control). Optimized diluted urine media in the presence of ammonium ions and under limited nitrate showed better lipid yields. Significant lipid biomolecules were detected in the algal oil extracts obtained from the diluted urine media characterized by Fourier transform infrared spectroscopy (FTIR) and Nuclear magnetic resonance (NMR). Gas chromatography-mass spectrometry (GCMS) revealed the presence of several monounsaturated and polyunsaturated fatty acids in the transesterified algal oil. Such studies would aid in technically realizing the field scale cultivation of microalgae for biofuels.

1. Introduction

Recently, there is an increased attention towards the novel ways of producing and employing alternative biofuels, owing to the rapid decline in fossil fuels concomitant with issues related to price rise and environmental pollution (Maity, 2015). Microalgae (microphytes) are regarded as one of the promising and sustainable source of biodiesel due to their attractive biochemical composition, with significantly high quantities of lipids (Chisti, 2007; Rangabhashiyam et al., 2017). These photosynthetic organisms are best known for their ease of cultivation, being capable of growing in wastewater sources utilising the atmospheric carbon dioxide (CO_2), thus providing a platform for waste bioremediation with simultaneous biological CO_2 sequestration (Milano et al., 2016; Behera et al., 2018, 2019). Cuellar-Bermudez et al. (2017) described the strategies for remediation of nutrients from wastewater resources and the limitations associated with their scale-up. Most of the conventional large-scale microalgae cultivation involve the use of chemical fertilisers, which are often hindered by the high cost and associated intense energy consumption (Gendy and El-Temtamy, 2013). The use of domestic/municipal waste streams for culturing microalgae via recycling the nutrients present in them can make the integrated process of wastewater treatment and biofuel production more sustainable in terms of costs and energy (Behera et al., 2019). Urinary/sanitary effluents which constitute 1% of the domestic wastewater streams contribute to about 80% nitrogen (N) and 50% phosphorous (P) load (Chang et al., 2013). This situation warrants the idea of utilising the nutrients present in source separated human urine for growing microalgae.

Source separated urine produced at the rate of 0.6–2.5 l per person is a rich source of nitrogen (25–35 g d^{-1}) and phosphorous (2–2.5 g d^{-1}) along with several other microelements like Fe, Mo etc. (Jaatinen et al., 2016). Various innovative techniques employing urine diversion and treatment systems have portrayed the potential of recycling and saving water with the use of underlying nutrients as the source of energy

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(Tuantet et al., 2014a,2014b). Struvite crystallization and ammonia stripping are the common methods currently in use to recover and utilize the recuperated nutrients (Tuantet et al., 2014a,2014b). However, these methods are limited by the higher operational energy and cost requirements (Morales et al., 2013). Microalgae cultivation is regarded as one of the most economical and efficient options for using the recovered nutrients from urine for generation of biomass that can be processed into biofuels (Chang et al., 2013; Jaatinen et al., 2016). Microalgae grown in urine provides added benefits of waste bioremediation clubbed with the production of biofuels and several value-added products like fertilizers in a bio-refinery approach. Use of urine reduces the cultivation costs involved with the usage of chemical fertilisers and dependency over arable lands or freshwater sources (Zhang et al., 2014). Microalgae has also been reported to remove unwanted coliform bacteria and remediate the trace elements present in urine (Tuantet et al., 2014a,2014b). To date, only a few studies have been done utilising urine as the growth media for microalgae. Adamsson (2000) cultivated *Scenedesmus acuminatus* in a 120 L photobioreactor (PBR) using 1:50 diluted urine, supplemented with Mg and Fe, resulting in a biomass concentration of 0.16 g l^{-1}. Chang et al. (2013) predicted a biomass concentration of 0.8 g l^{-1} for *Spirulina platensis* with 1:120 times diluted human urine in a 1.2 L PBR at a temperature of 30 $^{\circ}C$ with CO_2 supply resulting in 96% and 98% elimination of phosphorous and urea respectively. The maximum growth rate of 0.158 h^{-1} was achieved in non-diluted, hydrolysed human urine supplemented with Mg, for *Chlorella sorokiniana* under light-limited conditions of 105 $\mu mol\ photons\ m^{-2} sec^{-1}$ (Tuantet et al., 2014a,2014b). Similar to the above study, Tuantet et al. (2014a,2014b) also reported the microalgal biomass productivity of 14.8 g l^{-1} d^{-1} with significant removal of nitrogen and phosphorous using 2–3 times diluted urine supplemented with Mg, in a short light path (10 mm) PBR. Zhang et al. (2014) proposed that the concentration of *Chlorella sorokiniana* increased from 0.44 g l^{-1} to 0.96 g l^{-1} resulting in 84% nitrogen and 100% phosphorous recovery. Jaatinen et al. (2016) in batch cultivation studies for a period of 21 days with *Chlorella vulgaris* reported a biomass concentration of 0.73 g l^{-1} with 1:100 times diluted urine. *Arthrospira platensis* cultured in 20% nitrified urine, in membrane PBR, eliminated about 10% nitrogen, resulting in accumulation of 62.4% protein (Coppens et al., 2016). Piltz and Melkonian (2017) used immobilized microalgae with 1:1 diluted human urine in porous substrate PBR and reported the productivity of 7.2 g (dry wt.) m^{-2} d^{-1}. Fernandes et al. (2017) reported that *C. sorokiniana* could grow in toilet water with 15–26 N: P ratio. A very recent study by Chatterjee et al. (2019) showed that *Scenedesmus acuminatus* could grow well in 1:15 and 1:20 times diluted source separated urine removing 52% nitrogen and 38% phosphorous. Tuantet et al. (2019) have recently studied the effects of photobioreactor dilution rate, biomass density and photosynthetic efficiency on the growth of *C. sorokiniana* with synthetic urine in short optical length photobioreactor.

In spite of the above studies, the minimal dilution of human urine and other operating conditions required to achieve the maximal microalgal biomass and lipid productivity is still unclear. Most of the studies reported in the literature have usually focussed on culturing microalgae in urine mainly for nutrient recovery to be used as feed/fertilizers, rather than as a source of biofuel except a few studies by Jaatinen et al. (2016); Tuantet et al. (2014a,2014b). Increase in biomass and lipid productivity with *C. vulgaris*, *C. humicola* and *Scenedesmus* sp. in human urine supplemented with metal ions was recently reported by Torres et al. (2018). However, none of the authors have till now utilized multivariable optimization to study the influencing parameters on growing algae in urine. Optimization of process variables are essential to predict the algal productivity under different scenarios. The authors have earlier theoretically evaluated the effect of geographical conditions on microalgal lipid productivity using wastewater in open ponds and tubular photobioreactors based on the site-specific meteorological variables (Aly et al., 2017; Aly and Balasubramanian, 2016, 2017). To reduce the overall production cost of microalgal cultivation it is essential to use cheaper substrates under optimal conditions (Behera et al., 2019).

The current study focused on the potential use of diluted human urine as the growth media of a native microalgal consortium, further optimized the required urine concentration, and the operating variables like pH and light intensity influencing the microalgal growth, thereby affecting biomass and lipid productivity. As one variable at a time (OVAT) approach seems to be time-consuming, costly and often results in misinterpretation of obtained data, a multivariable experimental input based optimization strategy called response surface methodology (RSM) was used to seek the required output. Using RSM, a five-level full factorial central composite rotatable design (CCRD) was formulated, to optimise the operating variables like the concentration of urine (%v/v) [%vol. of urine/vol. of distil water], pH (unitless) and light intensity ($\mu mol\ photons\ m^{-2}\ sec^{-1}$) to enhance the biomass and lipid productivity. The nitrogen and phosphorous content in the optimized media were also analysed to evaluate the possible component responsible for increased biomass and lipid accumulation. The quality of lipids obtained from the algal biomass in the optimized urine media and that of the BG11 were also analysed. To the best of author's knowledge, no optimization study has been carried out so far utilising human urine as the growth media of algae for enhancing the biomass and lipid productivity. Such optimization studies could act as landmark in making the large-scale cultivation and commercialization of microalgal biofuels sustainable and economically feasible in future.

2. Materials and methods

2.1. Strain collection and inoculation in diluted human urine samples

Native microalgal consortium collected from the NIT Rourkela wastewater ponds (enriched with domestic wastewater from the hostels and residential areas) was inoculated and cultured in 250 ml Erlenmeyer flasks with BG11 media at ambient temperature of (30 ± 5 $^{\circ}C$), with artificial white fluorescent lights (36 Watt, 0.44 A) with 16:8 h light-dark cycles. Microalgal inoculum (with concentration of [0.4 ± 0.02] g l^{-1}) was inoculated into 400 ml diluted human urine media contained in 1000 ml Erlenmeyer flasks. Different dilutions ranging from 1 to 10% (v/v) was used for the study (the selection was based on the preliminary study that gave the maximum growth rate at the urine concentration of 10%). The freshly collected human urine was light yellow in colour, having pH of 6.5.

2.2. Design of experiments to study the statistical influence of operating conditions

Apart from the nutrient concentration, environmental parameters like light intensity, temperature and pH might also influences the amount of algal biomass generated, thereby, the quantity of lipid (Behera et al., 2019, Behera et al., 2018; Munir et al., 2015). Since, the main objective is to extrapolate the laboratory scale data to field level, the temperature factor was not considered in this study, and the cultures were kept at ambient temperature (30 ± 5 $^{\circ}C$). The effect of key influencing parameters like light intensity ($\mu mol\ photons\ m^{-2}\ sec^{-1}$), pH (dimensionless), and concentration of urine (%v/v) on biomass (R1/*Bp*) and lipid productivity (R2/*Lp*) were analysed using RSM. The range of selected variables affecting the response has been enlisted in Table 1. The five level full factorial CCRD with $\alpha = 1.682$ was formulated using the Design Expert Software (Version 11, USA Stat Ease). The change in response (biomass and lipid productivity) was studied with that of the influencing variables and the significant process conditions were identified through regression analysis. A total of 20 experiments with 3 variables [A: light intensity ($\mu mol\ photons\ m^{-2}\ sec^{-1}$; B: pH (unitless); C: urine concentration (in %v/v)] generated earlier was experimentally performed in the laboratory. All the experiments were conducted in

Table 1
Range of selected variables for the experimental design using RSM.

Factor	Name	Units	-α	+α	Coded Low (-1)	Coded High (+1)	Mean (0)
A	Light intensity	$\mu mol\ photons\ m^{-2} sec^{-1}$	52.27	452.27	50.00	350.00	200.00
B	pH	Dimensionless	5.65	9.85	6.50	9.00	7.75
C	Urine conc.	% v/v	2.07	13.07	1.00	10.00	5.50

triplicates and then the outputs were analysed and fed into the software. The set of experiments generated along with the response (biomass and lipid productivity in mg l^{-1} d^{-1}) has been given in Table 2.

A generalised quadratic model was used to study the significant parameters affecting the response. The generalised second order polynomial equation for analysing the model is given by Eq. (1).

$$y = \beta + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_i x_i^2 \quad \text{Eq. (1)}$$

where y is the predicted response and x_i represents the level of each of the factors taken into consideration.

The analysis of variance (ANOVA) was used to obtain the regression coefficient (R^2) in each case to study the accuracy of the second order polynomial equation and the quadratic model. The graphical analysis was done with the help of contour and three-dimensional (3D) plots to study the effect of the interaction of variables over the response. The desirability function (D) was used to perform the numerical optimization for obtaining the global maxima (predicted solution). Confirmation and validation of the selected solution was carried out to cross verify the optimal combination of parameters on the biomass and lipid productivity. Parameters like microalgal growth rate, biomass and lipid productivity were analysed in the optimized operating conditions and compared with that of the BG11 media (control).

2.3. Analytical methods

2.3.1. Estimation of biomass content and biomass productivity

After 10 days of incubation [end of stationary phase], the Erlenmeyer flasks containing media were shaken thoroughly and 100 ml of media was filtered through the pre-weighed sterile membrane filter paper (0.11 μm pore size) through vacuum filtration. The filter paper was dried in an oven for 8 h and the final weight was obtained to estimate the final biomass content and productivity as in Eqs. (2) and (3) respectively.

$$Final\ Biomass\ Content\ (B_2),\ \left(mg\ l^{-1}\right) = (W_2 - W_1) \quad \text{Eq. (2)}$$

$$Biomass\ Productivity\ \left(B_p\right),\ \left(mg\ l^{-1} d^{-1}\right) = (B_2 - B_1) / 10 \quad \text{Eq. (3)}$$

where,

W_1 = *Initial weight of filter paper* (mg)

W_2 = *Final weight of the filter paper with dried microalgae* (mg)

B_1 = *Initial biomass content* $\left(mg\ l^{-1}\right)$

2.3.2. Estimation of lipid content, lipid productivity and fatty acid methyl esters (FAMEs)

The lipid present in microalgae was calculated using the Bligh and Dyer method (1959). 15 ml microalgal suspension was filled in a pre-weighed 50 ml falcon tube (W_1), and was centrifuged at 6000 rpm for 10 min. The weight of dried algal biomass was calculated after oven drying at 60 °C, for 24 h by subtracting the final weight (W_2) from initial weight (W_1). To the dried sample 2 ml of methanol, 4 ml of chloroform was added and the mixture was vigorously shaked in a vortex mixer, for 2 min. 2.5 ml of distil water was added, followed by mixing for 2 min using a vortex mixer. The solution was centrifuged at 10,000 rpm, for 15 min and the lower layer was pipetted out through a sterile filter into a pre-weighed vial (W_3) and the chloroform phase was evaporated and the final weight of the vial with lipids (W_4) was measured. The lipid content was obtained by subtracting W_4 from W_3, divided by the weight of biomass (dried) and is finally expressed as %dry cell weight (%dcw). The lipid productivity was estimated using Eq. (4) given as follows.

$$Lipid\ productivity\ \left(L_p\right)\left(mg\ l^{-1} d^{-1}\right) = \left(B_p * lipid\ content\ [\%]\right) / 100 \quad \text{Eq. (4)}$$

Table 2
Experimental design matrix with different combination of variables along with the response.

Std. order	Run Order	Light Intensity (A) $\left(\frac{\mu mol\ photons}{m^{-2} s^{-1}}\right)$	pH (B) (Unitless)	Urine Conc. (C) (%v/v)	B_p : Biomass Prod. (R1) (mg l^{-1} d^{-1}) [Experimental]	B_p : Biomass Prod. (R1) (mg l^{-1} d^{-1} [Predicted]	L_p: Lipid Prod. (R2) (mg l^{-1} d^{-1}) [Experimental]	L_p: Lipid Prod. (R2) (mg l^{-1} d^{-1}) [Predicted]
19	1	200 (0)	7.75 (0)	5.5 (0)	200.97 ± 1.15	196.86	52.47 ± 2.24	51.28
9	2	52.27 (-α)	7.75 (0)	5.5 (0)	29.59 ± 3.93	40.37	6.22 ± 0.50	7.96
18	3	200 (0)	7.75 (0)	5.5 (0)	199.98 ± 4.16	196.86	52.21 ± 3.15	51.28
16	4	200 (0)	7.75 (0)	5.5 (0)	198.71 ± 3.73	196.86	51.88 ± 2.14	51.28
3	5	50 (−1)	9 (+1)	1 (−1)	15.40 ± 3.85	13.47	2.34 ± 0.69	1.09
8	6	350 (+1)	9 (+1)	10 (+1)	74.28 ± 4.66	80.10	12.05 ± 2.47	12.32
12	7	200 (0)	9.85 (+α)	5.5 (0)	38.50 ± 6.49	33.28	4.96 ± 2.24	5.18
1	8	50 (0)	6.5 (−1)	1(-1)	24.75 ± 2.96	20.41	4.74 ± 1.21	2.10
2	9	350 (+1)	6.5 (−1)	1(-1)	61.4 ± 4.24	56.65	13.17 ± 2.18	10.75
14	10	200 (0)	7.75 (0)	13.07 (+α)	170.92 ± 3.82	161.50	28.55 ± 5.51	28.48
5	11	50 (0)	6.5 (−1)	10(+1)	83.40 ± 1.07	85.01	18.17 ± 0.90	17.17
20	12	200 (0)	7.75 (0)	5.5 (0)	200.90 ± 3.71	196.86	52.45 ± 4.93	51.28
4	13	350 (+1)	9 (+1)	1 (−1)	42.80 ± 2.06	42.67	8.67 ± 2.51	7.31
13	14	200 (0)	7.75 (0)	2.06 (-α)	68.36 ± 3.63	75.70	8.19 ± 1.35	11.60
11	15	200 (0)	5.64(-α)	5.5 (0)	30.70 ± 4.18	33.84	6.75 ± 2.52	9.88
17	16	200 (0)	7.75 (0)	5.5 (0)	190.83 ± 2.76	196.86	49.82 ± 6.57	51.28
15	17	200 (0)	7.75 (0)	5.5 (0)	189.40 ± 4.36	196.86	49.45 ± 2.26	51.28
10	18	452.26 (+α)	7.75 (0)	5.5 (0)	57.40 ± 4.05	55.53	11.34 ± 2.01	12.95
6	19	350 (+1)	6.5 (−1)	10 (1)	70.44 ± 3.55	73.84	18.01 ± 2.04	16.89
7	20	50 (−1)	9 (+1)	10 (1)	92.10 ± 4.30	90.32	14.98 ± 3.53	15.03

The lipid accumulation in the cells was studied using Nile Red dye (Storms et al., 2014), with fluorescence microscope using cellSens software. The quality of algal lipids obtained were analysed via fourier transform infrared spectroscopy [FTIR] via spectral scan from 400 to 4000 cm^{-1} and via nuclear magnetic resonance study (^1H NMR) using Bruker NMR spectrophotometer using the method as described by Sarpal et al. (2016). The fatty acid methyl esters (FAMEs) obtained via trans-esterification of the algal lipids in the urine based media were analysed using gas chromatography and mass spectrometry (GC-MS) system incorporated with an Agilent 5977 A mass-selective detector (MSD) with the programme as detailed by Sarpal et al. (2016) and compared with that of the BG11 media.

2.3.3. Estimation of nutrients

Nutrients were measured based on spectrophotometric analysis using double beam UV visible spectrophotometer with the use of suitable standard calibration curve. 2 ml of culture media were centrifuged and the supernatant was analysed for the presence of ammonium, nitrate and phosphate ions. The nitrate was estimated based on Cataldo et al. (1975) using salicylic acid at 410 nm. Phosphate ions were measured using ammonium molybdate and stannous chloride method at 650 nm (Murphy and Riley, 1962). Iodophenol blue method (Horn and Squire, 1966) was used to calculate ammonium ions in the supernatant of culture media with absorbance at 630 nm. The nutrient uptake rate was estimated using Eq. (5).

$$Nutrient\ Uptake\ Rate\ (NUR) = [C_0 - C_t] / t \quad \text{Eq. (5)}$$

where,

$C_o = Initial\ concentration\ of\ nutrient\ \left(mg\ l^{-1}\ or\ mg\ ml^{-1}\right)$

$C_t = Concentration\ of\ nutrient\ after\ time\ interval\ (t)\left(mg\ l^{-1}\ or\ mg\ ml^{-1}\right)$

$t = Time\ interval\ (days)$

As the concentration of ammonium and nitrate is higher in diluted urine compared to that of the concentration of phosphate, the Nutrient uptake rate (NUR) for ammonium and nitrate has been presented in terms of mg $(NH_4)^+$ ml^{-1} and mg $(NO_3)^{-1}$ ml but in case of phosphate it has been given in terms of mg (PO_4) l^{-1}.

3. Results and discussion

3.1. Statistical interpretation of parameters influencing microalgal biomass and lipid productivity

Preliminary experiments with source separated human urine as nutrient media for microalgal cultivation were carried out at 10–100% dilution. The highest microalgal growth was observed with 10% diluted urine. With increase in human urine concentration, the microalgal growth rate was found to decline. Hence, the range of urine concentration has been chosen as 1–10% in the present study for further optimization. The pH has been kept in the range of 6.5–9 as microalgae has been postulated to grow best within this range (Munir et al., 2015). The light intensity for algal growth has been selected in the range of 50–350 $\mu mol\ photons\ m^{-2}\ sec^{-1}$ based on the literature (Behera et al., 2018, 2019; He et al., 2015b).

The CCRD was used to identify the essential factors at their best level of combination affecting the output (biomass and lipid productivity) with the experimental design as represented in Table 2. The experimental runs were randomized in order to negate the effects of the uncontrolled parameters and reduce the level of noise and unwanted errors. Multiple regression analysis was done by the Design Expert software. The regression analysis was used to correlate the relative effect of each of the independent variables along with their quadratic and interactive effects on B_p and L_p via the coded equation as shown in Eq. (6) and Eq. (7) respectively.

$$B_p = +196.80 + 4.51A - 0.17B + 25.51C - 1.76AB - 11.85AC + 5.06BC - 52.65A^2 - 57.74B^2 - 27.67C^2 \quad \text{Eq. (6)}$$

$$L_p = +51.28 + 1.49A - 1.40B + 5.02C - 0.61AB - 2.23AC - 0.28BC - 14.44A^2 - 15.47B^2 - 11.05C^2 \quad \text{Eq. (7)}$$

From Eq. (6), it could be projected that the urine concentration (C), light intensity (A) are the primary influencing factors for microalgal biomass productivity with relatively lesser influence by pH (B). The positive coefficients indicate synergistic effect of the influencing factors while the negative coefficients indicate antagonistic effect. Interaction effects of light intensity (A) and urine concentration (C) was more prominent (antagonistic) followed by the interactive effects of pH (B) and urine concentration (C) which was found to be synergistic. The interactive effects of light intensity (A) and pH (B) was antagonistic. The quadratic effects of each of the factors were also found to be significant indicating that the optimal regions for the selected factors lies inside the experimental space and not in the extremes, and the relation between the response and the variable could be represented by a complex surface.

In case of lipid productivity as indicated by Eq. (7), all the selected factors are significantly influential, with maximum effects exhibited by urine concentration (C), followed by light intensity (A) and pH (B). The interactive effects of the selected factors were found to be mostly antagonistic with maximum influence by light intensity (A) and urine concentration (C), followed by the interactive effects of light intensity (A) and pH (B), and that of pH (B) and urine concentration (C). The quadratic effects of the selected linear factors were also found to be significant as shown by their extremely lower P values (P value $<$ 0.0001).

Tables 3 and 4 shows the ANOVA for the formulated second order response model. The statistical evaluation of the model was done in terms of the predicted F values (Fisher Test Values) and P values (Probabilistic values). The F and the P values represent the goodness of fit, showing the accuracy of how the experimental data fits the model. At 95% confidence interval, the F value for biomass and lipid productivity was found to be 233.19 and 147.31 respectively, with P value $<$ 0.0001 in both the cases. Higher the F values, lower will be the P values and the quadratic regression model would be significant. F values greater than 3.94 usually shows adequacy of the model.

In case of biomass productivity, coefficients for urine concentration (C) and light intensity (A), were found to be significant with P value $<$ 0.05. Higher F value of 195.92 for urine concentration (C) showed that it was the most significant factor influencing the model, followed by light intensity (A) with F value of 6.11. Significant interactive effects were also observed between the light intensity and urine concentration (AC) with F value of 24.79 and P value $<$ 0.0001. Quadratic effects of all the factors were also found to be significant. The non-significant lack of fit with P value of 0.1936 (P value $>$ 0.05), implied that the quadratic model was adequate and the response surface satisfactorily fitted the model using the selected process variables. The actual (experimental results fed into the design matrix) and predicted values (model) of biomass productivity plotted in Fig. 1a showed that the experimental values were closely related to the predicted values of response. The experimental regression coefficient (R^2) of 0.9953 invocated adequacy of the model. The difference of less than 0.2 between the adjusted R^2 (0.9910) and predicted R^2 (0.9728) showed excellent correlation between the output and selected model parameters. The relatively lower percentage of covariance (% CV) of 6.56, representing the ratio of standard error of the estimate to the mean value of the observed response, justified the accuracy and reproducibility of the experimentally collected data. The value of adequate precision (signal: noise ratio) of 38.51 (>4) indicated that the unwanted errors were relatively less

Table 3
ANOVA table of microalgal biomass productivity (R1) for the experimental design adopted in the study.

Source	Sum of Squares	Df	Mean Square	F value	p Value	
Model	95184.60	9	10576.06	233.19	2.02E-10	significant
A - Light intensity	277.31	1	277.31	6.11	0.032948	
B – pH	0.38	1	0.38	0.01	0.928441	
C - urine conc.	8885.71	1	8885.71	195.92	6.79E-08	
AB	24.88	1	24.88	0.54	0.475874	
AC	1124.09	1	1124.09	24.78	0.000555	
BC	204.93	1	204.93	4.52	0.059453	
A^2	39942.02	1	39942.02	880.68	4.41E-11	
B^2	48037.89	1	48037.89	1059.11	1.77E-11	
C^2	11032.90	1	11032.90	243.26	2.4E-08	
Residual	453.53	10	45.35			
Lack of Fit	315.17	5	63.03	2.27	0.193642	not significant
Pure Error	138.36	5	27.67			
Corrective Total	95638.13	19				

R2 = 0.9953.
Predicted R2 = 0.9728.
Adjusted R2 = 0.9910.
% CV = 6.56.
Adequate Precision = 38.51.
p value < 0.05 indicates significant.

Table 4
ANOVA table for microalgal lipid productivity (R2) for the experimental design adopted in the study.

Source	Sum of Squares	Df	Mean Square	F value	p Value	
Model	7336.67	9	815.18	147.94	1.92E-09	significant
A - Light intensity	30.11	1	30.11	5.46	0.041487	
B – pH	26.60	1	26.60	4.82	0.052687	
C - urine conc.	343.89	1	343.89	62.41	1.31E-05	
AB	2.96	1	2.96	0.53	0.480093	
AC	39.82	1	39.82	7.22	0.022758	
BC	0.63	1	0.63	0.11	0.741702	
A^2	3003.24	1	3003.24	545.04	4.71E-10	
B^2	3448.94	1	3448.94	625.93	2.38E-10	
C^2	1758.16	1	1758.16	319.08	6.46E-09	
Residual	55.10	10	5.510			
Lack of Fit	45.67077	5	9.13	4.84	0.05417	not significant
Pure Error	9.4304	5	1.88			
Corrective Total	7391.776	19				

R^2 = 0.9925.
Predicted R^2 = 0.9514.
Adjusted R^2 = 0.9858.
% CV = 10.57.
Adequate Precision = 30.2387.
#p value < 0.05 indicates significant.

and the predicted model could be used to navigate the design space. The normal probability plot of residual as illustrated in Fig. 1c followed a straight-line trend showing that the errors have been normally distributed. Fig. 1e shows the plot of predicted and standardized residual values, and the absence of any specific pattern revealed the adequacy of the model with no significant violation of constant variance assumption. Residual analysis of the model has been detailed in the supplementary section.

Lipid productivity of microalgae was found to be influenced by all the selected parameters, more prominently affected by urine concentration (C) having *F* values of 62.41 and with *P* value < 0.0001. The light intensity (A) and pH (B) also have considerable effects as evident from the *F* values of 5.47 and 4.83 respectively. The quadratic effects of the selected factors were also found to be significant with *P* values < 0.0001. The interactive effects of light intensity (A) and urine concentration (C) also influenced the output as indicated by the *F* value of 7.23 and *P* value of 0.0228. The interaction between light intensity (A) and pH (B) [*P* value = 0.4801] was also found to be essential in influencing the lipid productivity. Relatively lower *F* values for interaction effects compared to the quadratic effects indicated that the later has a more prominent influence than the former.

Since lipid productivity is a function of biomass productivity, almost similar conclusion was obtained for the statistical evaluation of the effect of parameters. The *P* value of 0.05417 for the lack of fit indicated that it is non-significant and the experimental data appropriately described the model. The experimental R^2 value of 0.9925 as represented by the predicted versus actual plot (Fig. 1b), showed accurate correlation between the parameters. The reasonable agreement between the adjusted and predicted R^2 established that the model could be used to propagate the design space. The % CV of 10.67 and acceptable precision value of 30.24 indicated adequate signal and relatively lower standard error. The normal plot of residuals and the plot of predicted versus standardized residuals (as in Fig. 1d and f) showed that the errors are distributed normally and there was no suspect of violation of independent constant variance. Residual analysis as detailed in the supplementary section also showed the second order regression model to be accurate with no heteroscedasticity.

3.2. Effect of interaction of variables on biomass and lipid productivity

The operating culture conditions as light intensity, pH and urine concentration influences the biomass productivity and lipid content, thereby the lipid productivity. The one-factor plot obtained from the design expert clearly indicated that the selected factors are prominently involved in mutual interaction. The following sections explain the mutual interaction of the factors and their effect on the biomass and lipid productivity.

3.2.1. Mutual interaction between urine concentration and light intensity

The contour and 3D plots in Fig. (2a and 2b) and Fig. (2c and 2d) depicts the combined effects of light intensity (A) and urine concentration (C) on biomass and lipid productivity respectively. At low light intensity (50 $\mu mol\, photons\, m^{-2}\, sec^{-1}$) and low urine concentration (1% v/v), the biomass productivity was found to be 29.59 ± 3.50 mg l^{-1} d^{-1}, with lipid productivity of 6.22 ± 0.50 mg l^{-1} d^{-1} (Run 2). On increasing the light intensity till 200 $\mu mol\, photons\, m^{-2}\, sec^{-1}$ and the urine concentration to 5.5% v/v, the maximum biomass productivity of 200.97 ± 1.14 mg l^{-1} d^{-1} was obtained with lipid productivity of 52.47 ± 2.24 mg l^{-1} d^{-1} (Run 1) [which was also more or less same in the replicate

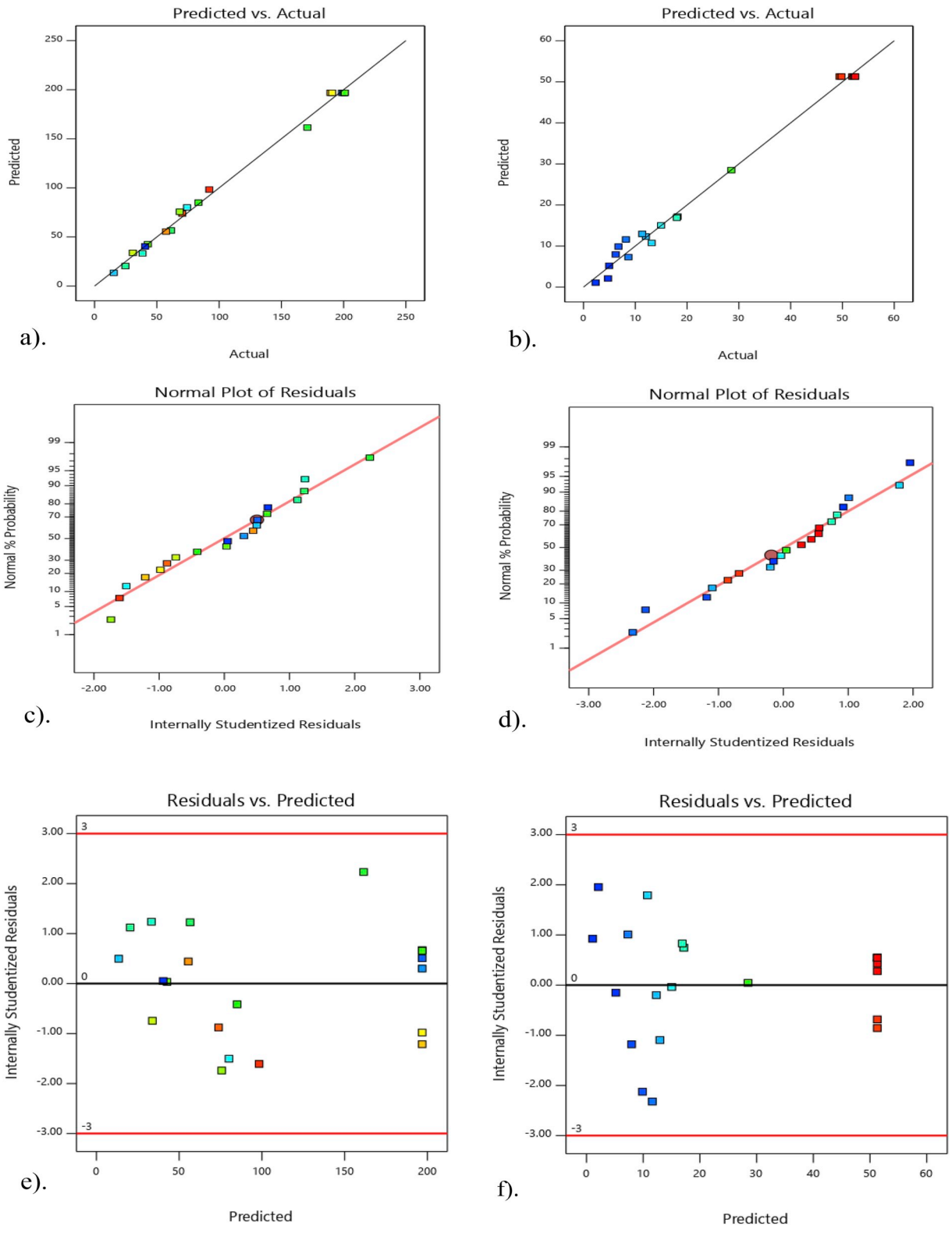

Fig. 1. a). Predicted versus actual plot for biomass productivity b). Predicted versus actual plot for lipid productivity c). Normal plot of residuals for biomass productivity d). Normal plot of residuals for lipid productivity e). Residuals versus predicted plot for biomass productivity f). Residuals versus predicted plot for lipid productivity [Scale: R1/*Bp* 15.4 200.97; R2/*Lp*. 2.34 52.47.

runs of 3, 4, 12, 16, 17]. Beyond 200 $\mu mol\ photons\ m^{-2}\ sec^{-1}$, and urine concentration of 5.5% v/v, the biomass and lipid productivity was found to decline as evident from the Run 18 and 10 respectively. It might be due to the irrevocable damage of PS II caused by increasing light intensity beyond the threshold/saturation level, which thereby decreases the growth rate (Tan and Lee, 2016). The increase in urine concentration also decreases the growth rate due to increase in ammonium toxicity (Jaatinen et al., 2016). It is expected that the ammonium toxicity can cause significant decline in biomass productivity, but the range of difference is less due to control of the media pH in the present study.

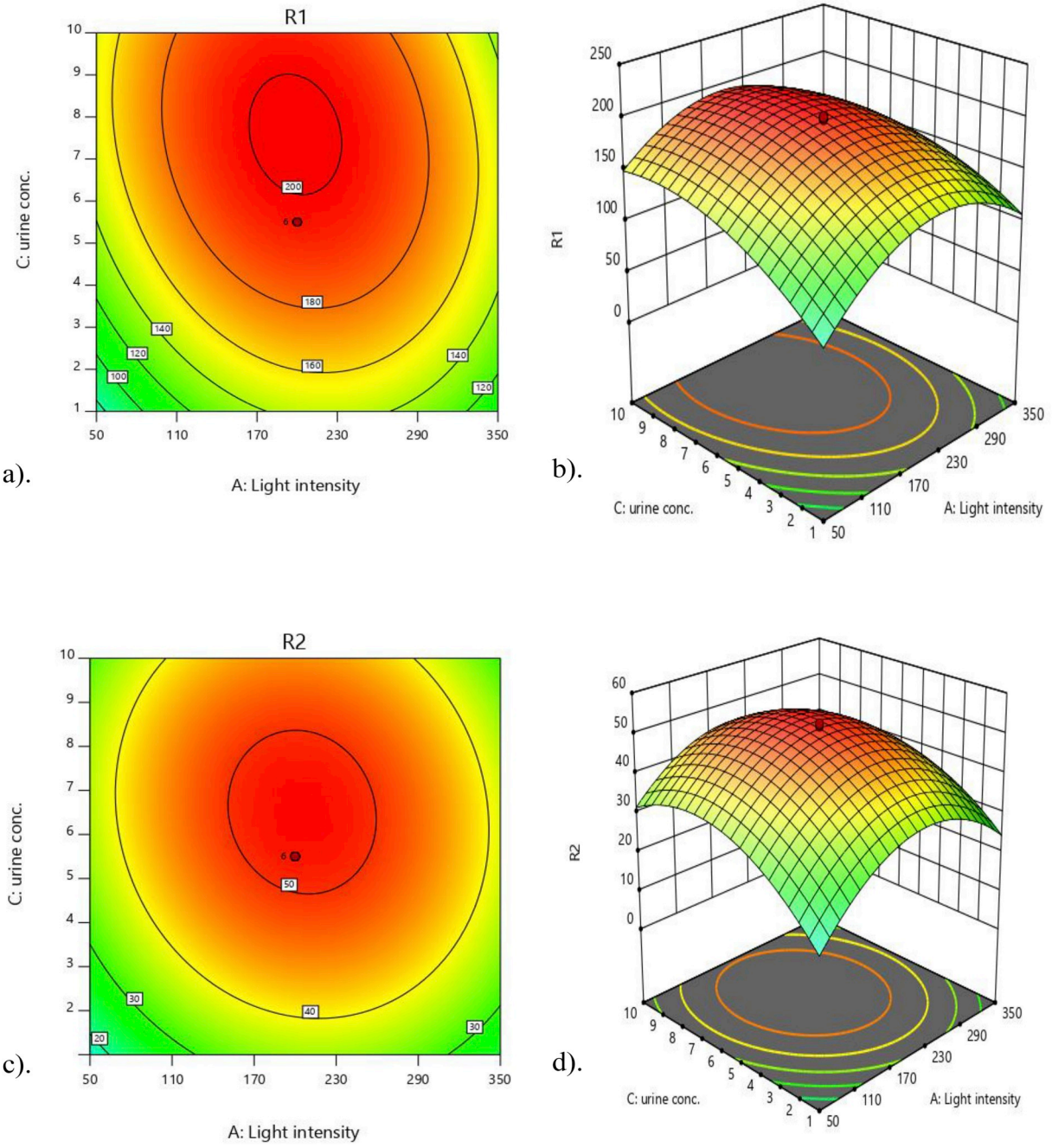

Fig. 2. a). Contour plot for the effects of mutual interaction of light intensity and urine concentration on biomass productivity b). 3D plot for the effects of mutual interaction of light intensity and urine concentration on biomass productivity c). Contour plot for the effects of mutual interaction of light intensity and urine concentration on lipid productivity d). 3D plot for the effects of mutual interaction of light intensity and urine concentration on lipid productivity [Scale: R1/*Bp* 15.4 200.97; R2/*Lp*. 2.34 52.47.

3.2.2. *Mutual interaction between urine concentration and pH*

Studies have reported that the microalgae can grow and metabolise best at an optimum pH and nutrient concentration (Munir et al., 2015; Binnal and Babu, 2017). At low pH range (below 7.75) and with low urine concentration of below 5.5% (v/v) as evident in Run 8, 9, the biomass as well as lipid productivity were found to be less. At the pH level of 7.75, with urine concentration of 5.5% (v/v), the maximum biomass and lipid productivity has been predicted (Run 1, 3, 4, 12, 14, 16). Increasing the pH to 9 or decreasing it beyond 7.75, at the constant urine concentration of 5.5% (v/v) a declining trend in biomass and lipid productivity was obtained (Run 7 and 15). Similarly, as evident from run 10 and 15, keeping the pH constant at 7.75, altering the urine concentration to lower or higher levels often resulted in lower biomass and lipid productivity. Cabello et al. (2015); Binnal and Babu (2017) reported that algae grow best at a pH near to 8, the growth rate and biomass productivity decreases at pH below 6 and at pH 9. This signifies the need of an appropriate concentration of urine and optimum pH for achieving desirable microalgae growth, below which there might be nutrient shortage and above which inhibitory levels of ammonium ions damage the cell causing cell death. It is well evident from the contour and 3D plots shown in (Fig. 3a–d), that both the factors are involved in mutual interaction. Elevating pH along with an increase in urine concentration predominantly inhibits the growth rate due to the excessive release of free ammonia (Tuantet et al., 2014a,2014b). Though it is

expected that the H^+ ions released at high ammonium concentration would be neutralized by the increase in pH, since the effect of increase in urine concentration is more prominent over pH, the neutralization might not be sufficient. This effect resulted in decreased productivity as illustrated in Fig. 3a–d. However, due to the control of media pH, the interaction effect was comparatively less significant compared to the previously explained effect.

3.2.3. Mutual interaction of light intensity and pH

As illustrated by the contour and 3D plots (Fig. 4a–d), keeping the urine concentration constant at 5.5% (v/v), the maximum biomass and lipid productivity was obtained at the light intensity of 200 $\mu mol\ photons\ m^{-2}\ sec^{-1}$ and at pH of 7.75. The biomass and the lipid content was found to decline with further increase in light intensity keeping the pH at 7.75 (Run 18), owing to the saturation of PS II and decline in cell concentration, though the lipid content was found to be 19.75%. As postulated by previous studies at high light intensity and at alkaline pH of around 8, diversion of internal chemical energy and carbon flux occurs generating lipids, but the decrease in cell concentration antagonistically influences the process causing a decline in

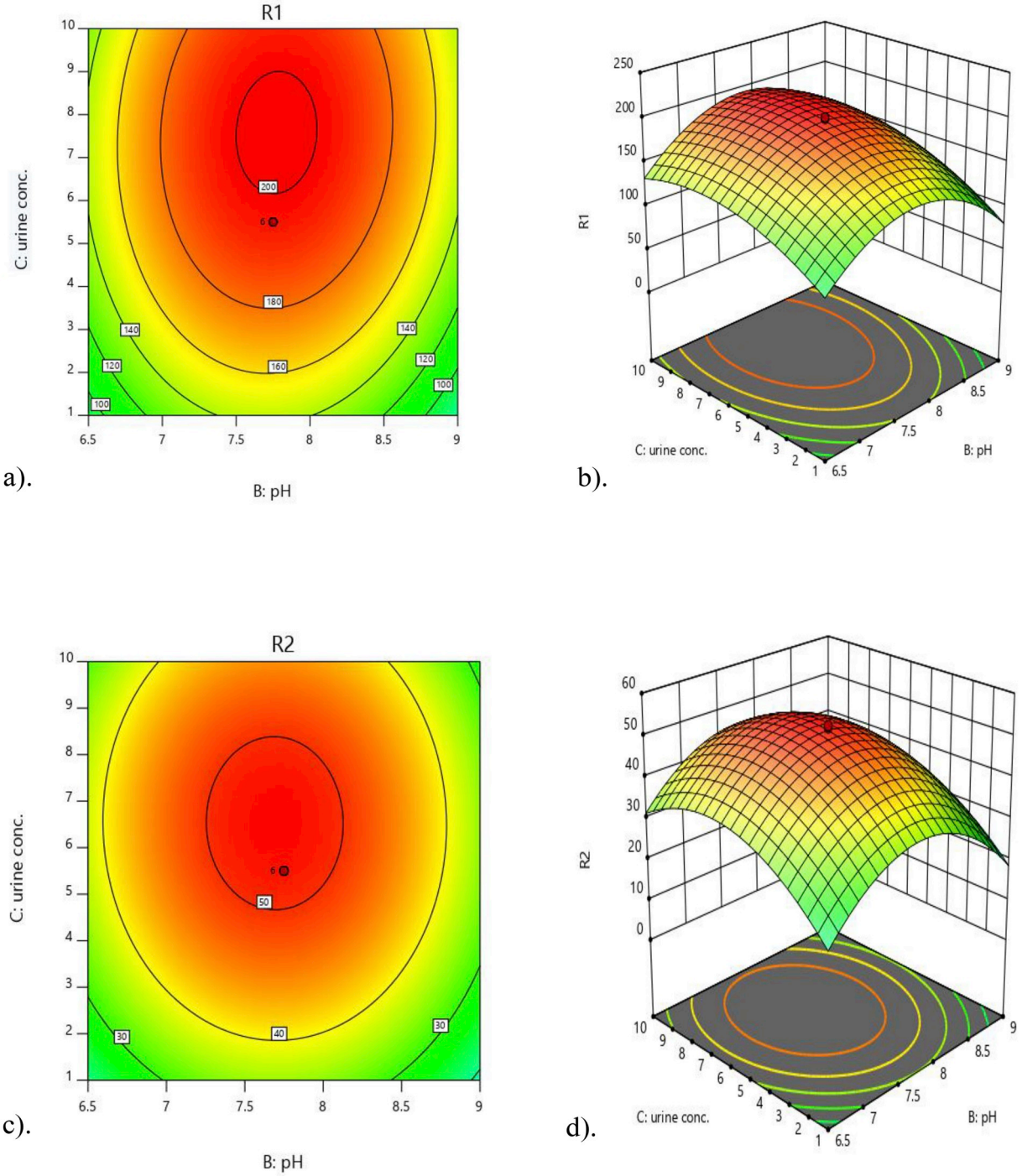

Fig. 3. a). Contour plot for the effects of mutual interaction of urine concentration and pH on biomass productivity b). 3D plot for the effects of mutual interaction of urine concentration and pH on biomass productivity c). Contour plot for the effects of mutual interaction of urine concentration and pH on lipid productivity d). 3D plot for the effects of mutual interaction of urine concentration and pH on lipid productivity [Scale: R1/*Bp* 15.4 200.97; R2/*Lp*. 2.34 52.47.

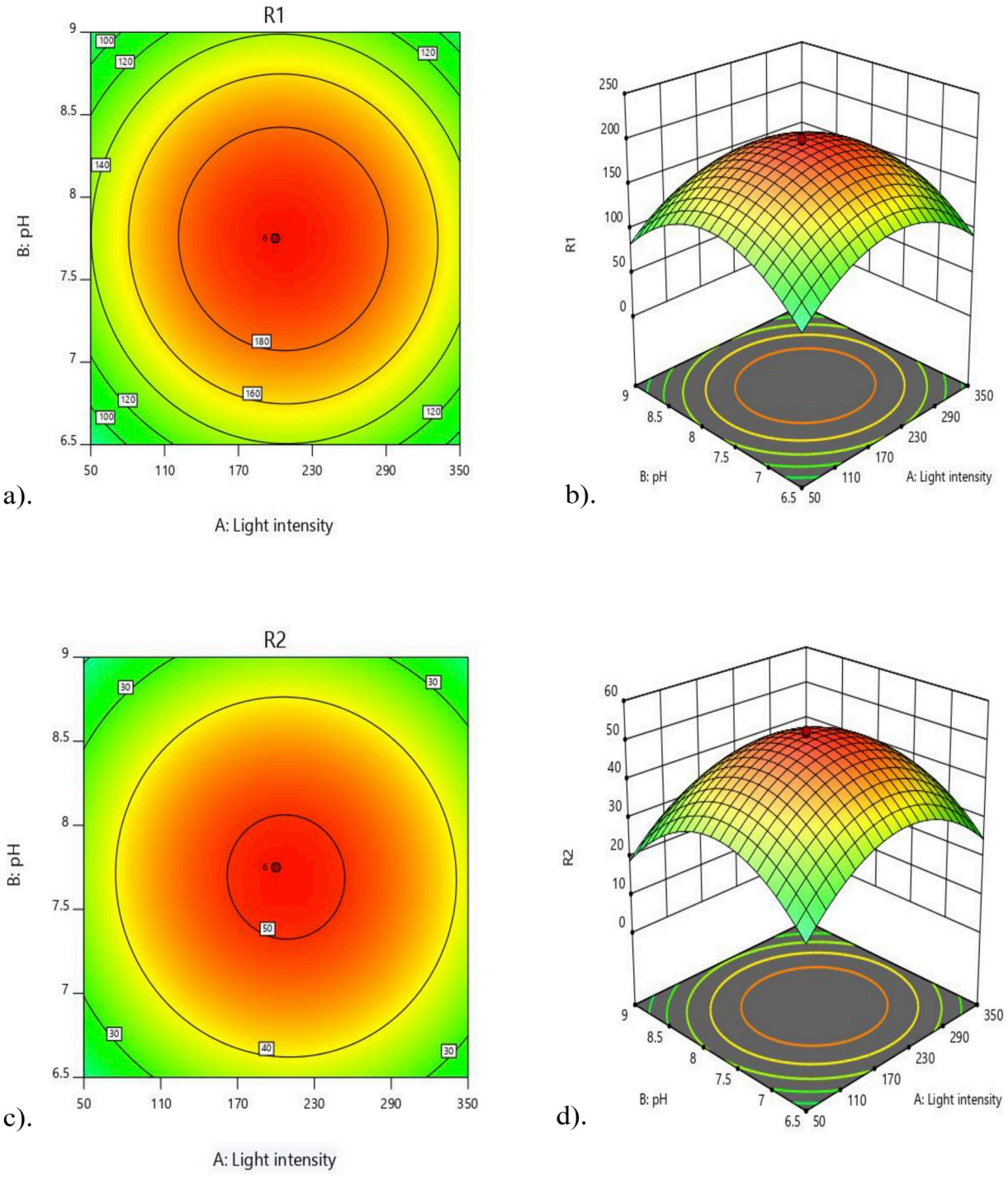

Fig. 4. a). Contour plot for the effects of mutual interaction of pH and light intensity on biomass productivity b). 3D plot for the effects of mutual interaction of pH and light intensity on biomass productivity c). Contour plot for the effects of mutual interaction of pH and light intensity on lipid productivity d). 3D plot for the effects of mutual interaction of pH and light intensity on lipid productivity [Scale: R1/*Bp* 15.4 200.97; R2/*Lp*. 2.34 52.47.

productivity (He et al., 2015a; 2015b; Zhu et al., 2016). Reports depicted that at slightly alkaline pH near 8, there is an increase in the release of lipids from autospore (Munir et al., 2015). The decrease in intensity of light, with pH of 7.75 and 5.5% (v/v) (Run 2) primarily lowers the amount of chemical energy available for photosynthesis, reducing biomass and lipid content (He et al., 2015a,2015b). Altering the pH beyond 7.75 keeping other parameters constant also projected a probable decline in productivity (Run 7 and 15). The present study is in accordance with the previous literature where it has been reported that it is essential to maintain a specific level of pH and light intensity to maximise the biomass and lipid productivity (Munir et al., 2015; Binnal and Babu, 2017).

3.3. Numerical optimization, point prediction and confirmation

Numerical optimization involves the use of desirability function (D) to select the appropriate combination of parameters in order to achieve the required responses/outputs. The desirability function ranges from 0 to 1 (ideal conditions) for different goals (Harrington, 1965; Candioti et al., 2014). The individual desirability are often combined in order to

evaluate D for the response. In the present study, numerical optimization has been done by setting each of the parameters at the selected range giving them equal weightage/importance (+++/3) and by fixing the biomass productivity at the target value of 200.97 mg l^{-1} d^{-1}, further seeking to maximise the lipid productivity. Binnal and Babu (2017) and Amini et al. (2008) also followed a similar kind of procedure of numerical optimization. In the present study, the criteria for optimization started with 100 random points, with 50 design points, a simplex fraction of 0.1 and the duplicate solution filter was kept at epsilon, which provided the minimum difference and omitted several identical solutions. 6 solutions were obtained with the different desirability as shown in Table 5. The 1st solution was selected as the global maxima with B_p of 200.97 mg l^{-1} d^{-1} and L_p of 51.91 mg l^{-1} d^{-1}, at reasonable and feasible combination of parameters i.e. light intensity of 205.40 $\mu mol\ photons\ m^{-2}\ sec^{-1}$; pH of 7.69 and urine concentration of 6.5% (v/v). The ramp desirability for solution has been shown in Supplementary Fig. 2a. The individual desirability of the factors and responses were combined to give the overall desirability of 0.994 as shown in Supplementary Fig. 2b. The validation of the solution was thus obtained by running the confirmatory experiment in laboratory, resulting in the B_p of 211.63 ± 1.40 mg l^{-1} d^{-1} and L_p of 55.56 ± 4.74 mg l^{-1} d^{-1} with standard error of 5.33% and 1.85% respectively compared to the predicted value in the model. Since the response predicted by the model is comparable to that of the experimental data, the accuracy of the model can be established.

3.4. Validation of the bioprocess optimization of microalgal lipid productivity

Use of human urine as media for microalgae cultivation is a noble approach that synergistically combines the strategy of waste bioremediation with biofuel production. As far as the author's knowledge goes, there has been no reports on optimization of microalgae cultivation with diluted human urine as the sole nutrient source and the influencing operating parameters over the biomass and lipid productivity.

The growth rate and productivity of the microalgal consortium under the optimized conditions was compared to that of the control media (BG11) under similar conditions as represented in Fig. 5a. The growth rate in the optimized media was comparable to that of the control media. The specific growth rate of 0.26 d^{-1} was obtained compared to the 0.18 d^{-1} obtained with BG11. The reported maximum biomass content was 2.71 ± 0.00452 mg ml^{-1} in optimized media while it was 2.52 ± 0.00421 mg ml^{-1} with BG11. The maximum biomass and lipid productivity obtained under optimized conditions were 211.63 ± 1.4 mg l^{-1} d^{-1} and 55.60 ± 4.47 mg l^{-1} d^{-1} respectively, which was comparable to that of the control media (B_p = 225.90 ± 2.78) mg l^{-1} d^{-1} and (L_p = 42.86 ± 3.46 mg l^{-1} d^{-1}). Lipid content in microalgal cells also improved from (18.96 ± 1.3) % to (26.27 ± 1.94) %. Several studies have been done to cultivate different microalgal strains in urine as outlined in Table 6. The data obtained in the present study was well comparable to most of the previous studies. However, the biomass productivity was found to be lower compared to other studies. This might be attributed to the fact that addition of supplementary trace elements or carbon sources resulting in mixotrophic conditions enhanced the biomass content further. Algal consortia requires mostly macronutrients like nitrogen, phosphorous along with the microelements like iron, magnesium, copper, manganese in trace amount. Availability of non-mineral elements, macro and micro nutrients along with the environmental factors greatly influence the photosynthetic metabolism, thereby the carbon fixation and allocation in different carbohydrate, protein and lipid molecules (Joniyas et al., 2016). Thus, the bio-macromolecular composition of microalgae is influenced by simultaneous activity of these factors.

Table 5
Solutions suggested by numerical optimization function.

S. No.	Light Intensity (μmol m^{-2} sec^{-1})	pH (Unitless)	Urine Conc. (%)	Biomass Prod. (mg l^{-1} d^{-1})	Lipid Prod. (mg l^{-1} d^{-1})	Desirability
1	205.40	7.69	6.50	200.97	51.91	0.994361
2	201.26	7.69	6.49	200.97	51.89	0.994250
3	210.45	7.67	6.62	200.97	51.87	0.994022
4	198.85	7.66	6.57	200.97	51.87	0.994001
5	207.84	7.76	6.45	200.97	51.85	0.993812
6	185.66	7.71	6.68	200.97	51.65	0.991780

Growth rate of microalgae is a function of irradiance and the nutrient uptake rate, the assimilation rates are correlated to the external availability and their demand for growth. Studies have reported an increase in nutrient utilization rate with an increasing light gradient until the threshold limit. Joniyas et al. (2016) reported that light provides energy and power in terms of ATP to initiate intracellular nutrient transport. It also facilitates the reduction of nitrite and nitrates into ammonium. Optimal irradiation also produces carbon skeleton for incorporation of micro and macro elements in the larger macromolecule. The uptake of macronutrients like nitrate and phosphate can be positively correlated with the increase in light intensity, however no direct correlation between ammonium uptake and utilization has been established. Similarly pH affects the nutrient solubility, their ionic form and thereby diffusion and availability. Most nutrients are unavailable at high alkaline pH. da Silva Cerozi and Fitzsimmons, 2016 have described in detail the influence of pH over the concentration of different macro and micro elements in the nutrient solution. At optimum pH, most microelements remain in ionic form that could be easily diffused and assimilated by cells. The present study highlights the impact of the influencing parameters like light intensity and pH over the elemental availability. Nevertheless, the data presented here establishes a cost efficient approach of culturing microalgae, without the use of additional supplements. Further, the synergistic combination of different nutritional sources and addition of trace elements to improvise the microalgal productivity could be carried out in the future.

3.5. Effect of diluted urine on biomass and lipid productivity of microalgae

Operational conditions especially the source and thereby the concentration of nitrogen and phosphate in the nutrient media influences the microalgal growth rate and thus the biomass and lipid productivity (Behera et al., 2018, 2019). The nutrient depletion with respect to the time in both the optimized diluted urine and BG11 as control has been shown in Fig. 5b-d. Conversion of nitrogen has to be optimum for algal uptake and metabolism, thereby enhancing the algal growth rate and lipid content (Zhuang et al., 2017). In case of optimized media with diluted urine, the concentration of nitrate and phosphate was found be much lower compared to BG11. Phosphate uptake rate was 0.16 mg l^{-1} d^{-1} in the diluted urine based media compared to 0.42 mg l^{-1} d^{-1} in BG11. About 80% nitrogen and 40% phosphorous were recovered in the algal biomass. The presence of limited nitrate and phosphate in the optimized diluted urine media, results in enhanced lipid accumulation in algal cells, thus the lipid content increased from 18.96% to 26.27%. Binnal and Babu (2017); Tan and Lee (2016) also reported similar results of enhanced microalgal growth rate under nutrient limited conditions. During the lag phase in case of diluted urine media, the ammonium concentration was found to rise, which gradually declined as microalgal growth progressed through the exponential phase. Urea in the urine hydrolyses into ammonia that combines with H^+ ions providing ammonium ions, that is assimilated by microalgae in the presence of light, before being converted into nitrate (Dhup et al., 2016). During metabolism, the ammonium ions are partly diffused out from the cells and are oxidized into nitrate, which is being further assimilated by the microalgae. The ammonia uptake rate in diluted urine media

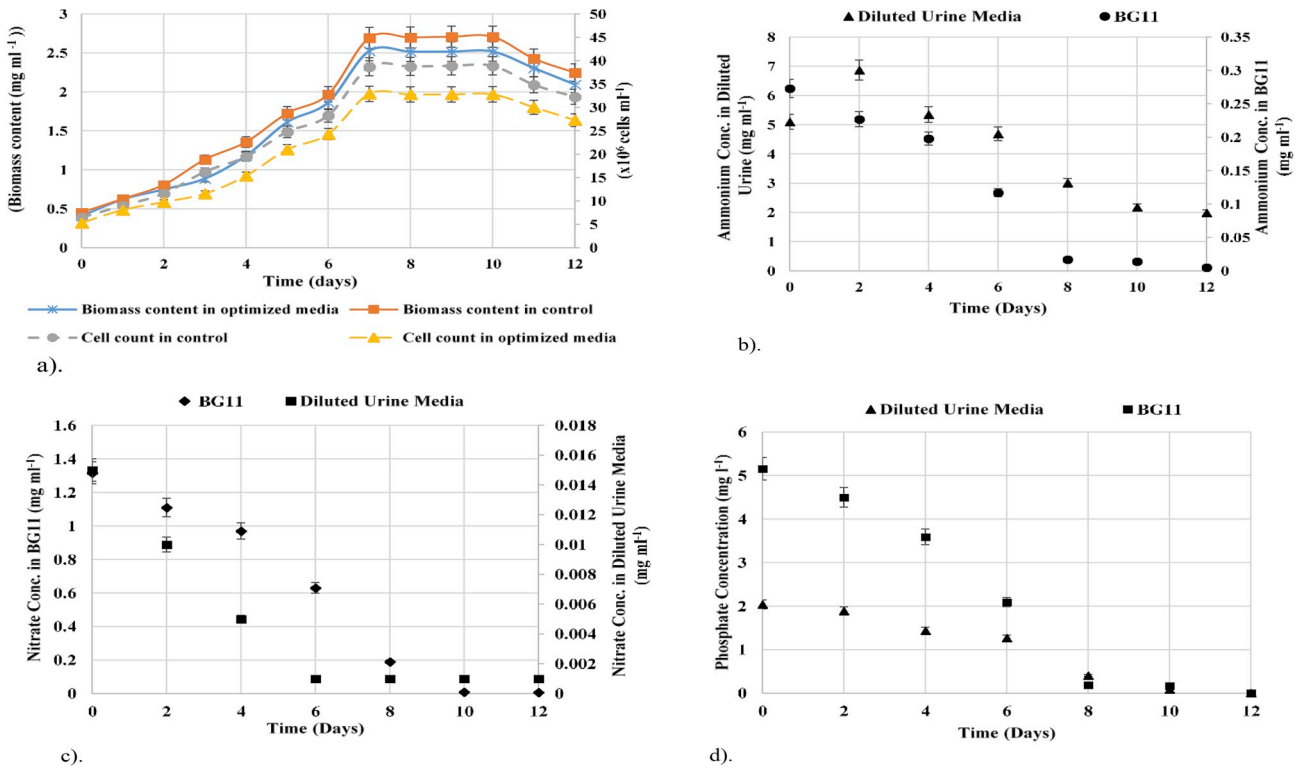

Fig. 5. a). Comparison of growth rate of microalgae in diluted urine media and BG11 b). Ammonium depletion rate in diluted urine media and BG11 c). Nitrate depletion rate in diluted urine media and BG11 d). Phosphate depletion rate in diluted urine media and BG11.

Table 6
Comparative analysis of the microalgal productivity data with the existing literature of microalgal growth with urine as nutrient source.

Sl No.	Culture conditions	Biomass content (mg l^{-1})	Lipid content (% dcw)	Prod. (mg l^{-1} d^{-1})	References
1.	*Scenedesmus acuminatus* [120 l (PBR) using 1:50 diluted urine, with addition of Mg and Fe	160	–	–	Adamsson (2000)
2.	*Spirulina platensis* [1:120 times diluted human urine in a 1.2 l PBRs at 30 °*C* with CO_2 supply]	800	–	–	Chang et al. (2013)
3.	*Chlorella sorokiniana* [2–3 times diluted urine supplemented with a particular N: P ratio and Mg, in a PBR with a shorter light path of 10 mm]	9800	–	14,800: B_p	Tuantet et al. (2014a,2014b)
4.	*Chlorella vulgaris*, [Batch culture with 1:100 times diluted urine without trace elements]	730	–	–	Jaatinen et al. (2016)
5.	**Native algal consortium isolated from NIT Rourkela [Light intensity of 205.40 *μmol photons* m^{-2} *sec*$^{-1}$; urine concentration to 6.50% (v/v); pH-7.69 (Experimental)]**	**2520**	**26.27**	**211.63: B_p 55.60: L_p**	**Present study**

(optimized) was 0.26 mg ml^{-1} d^{-1}, while the nitrate uptake rate was 0.001 mg ml^{-1} d^{-1}. Ammonium ions with higher uptake efficiency was assimilated at ease and further metabolized into cellular biomass and lipids. The increased accumulation of lipids with diluted urine under optimal conditions compared to the BG11 media has been confirmed under fluorescence microscopic analysis (Supplementary Fig. 3). Urea in urine also provides an optimal source of organic carbon, which was also evident with the gradual increase in dissolved CO_2 in the media, resulting in increased specific growth rate. Lipid productivity thereby increases under suitable combination of organic nitrogen and carbon. Urea containing 46.7% as nitrogen with the price of 0.14 USD kg^{-1} is much cheaper compared to other nitrate and phosphate sources (Dhup et al., 2016). Further, the use of diluted urine as the source of urea would provide an environmentally benign substrate with zero costs for the culture of algae. The study establishes the fact that the diluted urine under optimal conditions provides ammonium ions as the direct nitrogen source for metabolism, which in combination with limited nitrate and phosphate facilitates the increased lipid content in microalgae. Since cost-economics constitute an essential aspect for successful functioning of biorefinery models. Achieving an increase in lipids with utilization of cheaper nutrients from urine that could be processed into biodiesel is expected to reduce the process costs, thus the overall economics.

3.6. Characterization of the lipids obtained in diluted human urine as nutrient media

The properties of the lipids obtained with the diluted urine media under the optimized conditions were compared to that of the lipids obtained via cultivation in BG11 media using FTIR, ^{1}H NMR and GC-MS analysis.

Characteristics and distinct fingerprints corresponding to the lipids were observed in the optimized media in the FTIR spectrum (Supplementary Fig. 4). Peaks at 2954–2854 cm^{-1} correspond to – C–H stretching in $–CH_3$ and $–CH_2$ groups present in the lipid extracts. Carboxylic acid groups could be detected in the peaks pertaining from 1524

to 1510 cm^{-1}. CH_2 bending in the lipid extract is evident from the peaks at 1459 cm^{-1}. Similar kind of peaks were also found in the lipid extracts obtained by growing microalgae in BG11 media, showing that the diluted urine media could act as a suitable alternative for culturing algae. Similar kind of FTIR spectra for algal lipid extracts were also detected by Laurens and Wolfrum (2011) for *Nannochloropsis* sp., *Chlorococcum* sp., *Spirulina* sp..

The presence of lipids in the algal extracts after Bligh and Dyer extraction was also confirmed via NMR (Supplementary Fig. 5). The presence of unsaturated fatty acid chains could be confirmed by signals obtained between 5.32 and 5.36. Peak overlapping could be seen below 5 ppm corresponding to the regions of olefinic protons. Multiplet signals between 4.02 and 4.90 ppm refers to the anomeric protons of mono, di and trigylcerides. Signals spread in the region from 3.27 to 3.39 ppm were due to the presence of $–N(CH_3)$ group of mono and diglycerides. Signal at 2.19 is because of the allylic groups of fatty acids/glycerides. Prominent peaks obtained at 0.018, 1.27–1.73 ppm may be assigned to the terminal methyl chains corresponding to the components of fatty acids belonging to C14:0-C18:4. The NMR spectra of lipids were comparable to that of the lipids obtained with the BG11 media, showing that the optimized urine media could act as a potential replacement for synthetic media. Similar results and group assignments has also been reported by Nuzzo et al. (2013) in algal oil extracts of *Thalassiosira weissflogii*, *Cyclotella cryptica* and *Nannochloropsis salina*. The study by Sarpal et al. (2016) also reported similar groups of neutral lipids in the algal oils.

The presence of FAMEs in the biodiesel obtained after transesterification of lipid extracts obtained in the optimized urine media and that of the BG11 media were also confirmed by the GCMS analysis. The biodiesel obtained by processing the algal lipids from the urine based media showed the presence of oleic acid (C18:1) [40.57%], linoleic acid (C18:2) [3.59%], stearic acid (C18:0) [7.42%] and also vaccenic (C18:1, cis) [1.45%], docosadienoic (C22: 2) [1.16%] and palmitic (C16:0) [0.89%] acids. Similar kinds of FAMEs were also detected in the biodiesel obtained via the transesterification of the lipid extracts obtained from the culture of algal consortium with BG11 (Supplementary Table 3). Sarpal et al. (2016) also reported the presence of FAMEs ranging from C14–C22 in algal oil obtained from *Chlorella vulgaris*, *Spirulina platensis* and *Tetraselmis affchuii*. Similar to the study of Patil et al. (2011), the presence of olefins, sterols, aldehydes along with poly and mono unstaturated fatty acids were also detected in the transesterified algal oil.

4. Conclusion

Utilization of human urine as nutrient media for microalgal cultivation was carried out and validated after numerical optimization resulting in the B_p of 211.63 ± 1.40 mg l^{-1} d^{-1} and L_p of 55.60 ± 4.47 mg l^{-1} d^{-1} at light intensity of 205.40 $\mu mol\ photons\ m^{-2}\ sec^{-1}$; pH of 7.69 and urine concentration of 6.50% (v/v). High R^2 and F value signifies the adequacy of the model to predict responses. The selected parameters had a significant influence (both linear as well as interactive) over the biomass and lipid content of microalgae. The productivity obtained under optimal operating conditions was well comparable to that of control (BG11 media). The lipid accumulation in the optimized diluted urine media was higher (26.27%) compared to the BG11 due to the presence of ammonium and limited nitrate and phosphate. Spectral analysis via FTIR and NMR showed peaks the presence of significant lipid biomolecules in the algal oil extracts obtained from the microalgae grown with urine. GC-MS analysis also showed the presence of PUFAs, MUFAs and other saturated fatty acids in the transesterified oil obtained from the culture grown with diluted urine well comparable to that grown with BG11 media. The study not only unravels the potential of diluted human urine as the low cost substrate for cultivating microalgae, but also signifies the importance of precise experimental optimization to achieve the desired productivity.

Authors' declaration

PB has initiated the concept of the project. Experiments and acquisition of data was carried out by BB and SP. BB and PB have done the data analysis and interpretation. BB have drafted the manuscript. PB has reviewed and finalized the manuscript. All authors read and approved the final manuscript for peer review and possible publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.110111.

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