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1 **Statistical analysis of sustainable production of algal biomass from wastewater**
2 **treatment process**

3

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10

11 **Abstract**

12 Algal biodiesel is one of the most promising renewable and eco-friendly source of energy for
13 transportation, when algae is produced from wastewater. During the process, both goals of
14 biodiesel production and wastewater treatment could be achieved simultaneously. However, the
15 optimal condition for algae production remained unanswered. Algal biodiesel could be produced
16 from various wastewater treatments. In this study the relationship between biomass production
17 versus lipid productivity in various wastewater sources is statistically analyzed. Chemical
18 oxidation demand, total nitrogen, total phosphorus, and CO₂ sequestration could be achieved
19 during the production of different algal biomass in numerous type of wastewater effluent. The
20 regression of different system models and interpretation of linear coefficients were represented in
21 this statistically approached studies. Apart from that the paper also discuss the uncertainty of linear
22 regressions using Monte Carlo method, influence of physical parameters on biomass production,
23 energy potential and efficiency of nutrient removal using different phototrophic systems.

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36 **Key words:** Biomass, Chemical oxidation demand, Total nitrogen, Total phosphorus, Wastewater

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39 **1. Introduction**

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41 The main source of energy for the world is fossil fuels such as petroleum products, methane, and
42 coal. The non-renewable nature of fossil fuels leads to scarcity of energy which have aroused great
43 interest in search for alternative fuels [1]. Rapid increase in population results in expeditious
44 utilization of fossil fuels which lead to two major issues, direct environmental pollution and global
45 warming. To meet the challenges, alternative fuels with renewable, biodegradable and
46 environmental friendly nature are under intensive investigation [2-4]. Biodiesel is one of these
47 renewable fuels because it possess all the features needed as a fossil fuel substitute and it could be
48 produced from numerous feedstocks such as vegetable oil, algal oil and animal fat/oil [5] .

49 The increase of global CO₂ emission demands effective and efficient techniques for the
50 sequestration of CO₂ [6]. In 1997, the Kyoto protocol suggested that, for the reduction of oil and
51 to meet the GHG reduction targets, affordable supplies of clean, secure transportation fuels using
52 low-carbon technologies have to be found [7]. Ever since, significant attention has been devoted
53 to develop biofuels, for example from microalgal sources. Due to growing demand of petroleum
54 and significantly larger issues regarding global warming and greenhouse effect as a part of ignition
55 of fossil fuels, a substantial importance has been given to the concept of using microalgae as a fuel
56 source. Benefits of microalgae-based biofuels are greater production yields and the ability to
57 capture CO₂. Therefore, algal fuel has great importance due to its environmental friendly nature to
58 decrease global warming [8-10]. Biodiesel can be the best renewable energy option because it is
59 free from sulphur and aromatics along with that it also reduced emission carbondioxide,

60 hydrocarbon and particulate matter. Algal biofuels can lower greenhouse gas emission from
61 101,000 grams of CO₂ equivalent per million British thermal units to 55, 440 grams [6].

62 Harder and Witch were the first to propose to use algae for energy and production in 1942. In the
63 1950s, carbohydrate fraction of algal cells was used for the production of methane gas under
64 anaerobic digestion by Meier (1955) and Oswald and Golueke (1960) [11]. In the early 1970s, the
65 sharp raising of energy price led to a push for energy production from aquatic species mainly algae
66 gained major attention [11]. In recent years, the algal cultivation gained greater attention due to its
67 various applications such as an alternative feedstock for biodiesel production, nutrient control in
68 wastewater remediation process as well as low cost method for biomass harvesting[12].

69 Microalgae has great potential to assimilate nutrients efficiently and effectively when algal species
70 were grown in wastewater. Rich nutrients in wastewater provide better growth rate of algal species
71 depending upon the algal strains or species [13]. Recently, there is plenty of work related to
72 treatment of various kinds of wastewater such as dairy wastewater, piggery wastewater, olive oil
73 mill wastewater, brewerywastewater, municipal sewage sludge, molasses wastewaters, soybeans
74 processingwastewater, and petrochemical wastewater using algal culture systems [14-20]. Shoener
75 et al., reported that wastewater treatment can be energy positive with transformation of organic
76 matter by anaerobic digestion and removal of nutrients by phototrophic technologies especially
77 using algae [21]. Combination of wastewater treatment with algae cultivation for biodiesel
78 production could lead to a sustainable, cost effective and eco-friendly algal based energy
79 production process. Algae uses the nutrients present in the wastewater for its growth, which offer
80 an effective nutrient treatment technology along with algal biomass for biodiesel production
81 without fresh water [12, 13, 22].

82 The main objectives of this paper are to: 1) statistically analyze optimal conditions for algae
83 production using wastewater from variety sources such residential or industry; 2) develop
84 predictive equations of algae biomass production using chemical oxygen demand (COD), total
85 nitrogen and total phosphorus, as well as CO₂ fixation rate by different kinds of algal species using
86 regression analysis; and 3) validate the correlation equations using other independently reported
87 research results.

88 **2. Database and statistical methods**

89

90 The data are obtained from published peer reviewed papers. The collected data were organized to
91 present details of biomass production in various kinds of wastewater. In spite of that, there are
92 other various databases, which are shown in this study, such as the influence of nutrient
93 concentration on algal biomass production, relationship between biomass production and lipid
94 productivity in different kinds of waste water and CO₂ sequestration capabilities of several algal
95 species. Therefore, the database helps to perform regression analysis of biomass produced with
96 respect to lipid productivity, COD, total nitrogen and total phosphorus content in wastewater.
97 SPSS was used to obtain linear regression analysis and MatLab was used to determine uncertainty
98 of the linear regression.

99

100 **3. Results and discussion**

101

102

103 *3.1. Biomass production vs. lipid productivity in different types of wastewater resources*

104

105 The algal biomass production and lipid productivity data of various algal species in different
106 wastewater effluents are shown in table 1. Table 1 also explains the ability of several microalgal
107 species to grow in wastewater resources with high lipid content. The key factor in biodiesel
108 production and considerable cost reduction and commercialization of algal biofuel production
109 could be achieved with high lipid productivity. The *Chlamydomonas reinhardtii* (biocoil-grown)
110 grown in municipal centrate effluent showed higher biomass production as well as lipid
111 productivity.

112

113 Table 1. The biomass and lipid productivities of some of the microalgal species grown in different
114 wastewater resources.

Table 1. The biomass and lipid productivity of some of the microalgal species grown in different wastewater resources.

Type of waste water	Type of algal species	Biomass production (mg L ⁻¹ d ⁻¹)	Lipid productivity (mg L ⁻¹ d ⁻¹)	References
Artificial Wastewater	<i>Scenedesmus sp.</i>	126.54	16.2	[1][2][3]
Carpet mill	<i>Scenedesmus sp.</i>	126.54	16.2	[2][3]
Centrate Municipal wastewater	<i>Chlorella sp.</i>	231,4	77,5	[3]
Centrate Municipal wastewater	<i>Hindakia sp.</i>	275	77,8	[3]
Centrate Municipal wastewater	<i>Chlorella sp.</i>	241,7	74,7	[3]

Centrate wastewater	Municipal	<i>Scenedesmus sp.</i>	247,5	75,5	[3]
Concentrated Municipal wastewater		<i>Auxenochlorella protothecoides</i>	268,8	77,7	[3]
Municipal (centrate)		<i>Chlamydomonas reinhardtii</i> (biocoil-grown)	2000	505	[1][4]
Municipal (secondary treated)		<i>Scenedesmus obliquus</i>	26	8	[1][4]
Municipal (secondary treated)		<i>Botryococcus braunii</i>	345,6	62	[1][4]
Municipal treated + CO2)	(primary	Mix of <i>Chlorella sp.</i> , <i>Micractinium sp.</i> , <i>Actinastrum sp.</i>	270,7	24,4	[1][4]
Agricultural manure with high NO3-N)	(piggery with high	<i>B. braunii</i>	34	4,5	[1][4]
Industrial (carpet mill, untreated)		<i>Dunaliella tertiolecta</i>	28	4,3	[1][4]
Industrial (carpet mill, untreated)		<i>Pleurochrysis carterae</i>	33	4	[1][4]

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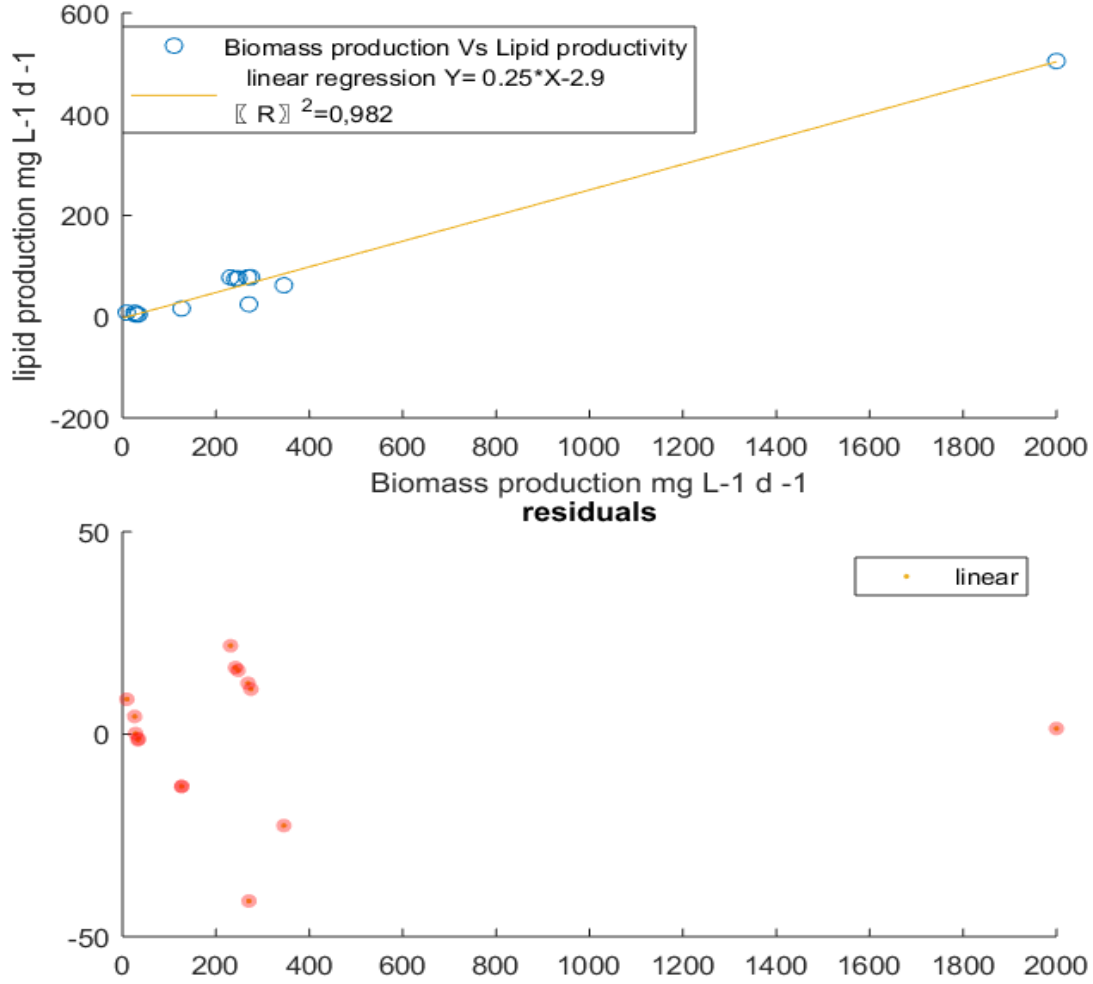
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119 The Fig1, shows the linear regression between biomass and lipid productivity, which can be
120 expressed as follows:

$$121 \quad Y = 0.25 x - 2.9 \quad R^2 = 0.982 \quad (1)$$

122 Where: y is biomass production ($\text{mg L}^{-1} \text{d}^{-1}$) and x is lipid productivity rate ($\text{mg L}^{-1} \text{d}^{-1}$)

123 The regression equation shows linear correlation between lipid productivity in various waste water
124 resources depends on biomass production. Biomass production can explain 93.7% of the
125 variability of our dependent variable, which is lipid productivity. Residual plots denotes the
126 difference between the observed value of the dependent variable, lipid productivity of algal species
127 in different wastewater resources and the predicted value. The residual plots shows random pattern
128 indicating good fit to the linear model.



129

130 Fig1. Regression between biomass production mg L⁻¹ d⁻¹ with respect to lipid productivity mg L⁻¹ d⁻¹ of various algal species in different wastewater resources

132

133 *3.2.Relationship between biomass production with COD ,TN and TP concentration in*
 134 *piggery wastewater*

135 Zhu et al., (2013) cultivated the microalgae, *Chlorella zofingiensis*, in piggery wastewater effluent
 136 under different concentration of nutrients such as COD, TN and TP as depicted in Table 2.
 137 Therefore, the effect of nutrients concentration on biomass production could be quantified. The
 138 maximum biomass production of *Chlorella zofingiensis*, 296.16 mgL⁻¹d⁻¹ was observed with
 139 concentrations of COD, TN, TP 1,900mg L⁻¹, 80mg L⁻¹, 85mg L⁻¹ respectively [23].

140
 141 Table 2. Effect of COD, TN, TP concentration in piggery wastewater for biomass production of
 142 *Chlorella zofingiensis* [23].

Table 2. Effect of COD, TN, TP concentration in piggery wastewater for biomass production of *Chlorella zofingiensis* [5].

COD (mg L ⁻¹)	TN (mg L ⁻¹)	TP (mg L ⁻¹)	Biomass(mg L ⁻¹ d ⁻¹)
3500	148	156	267.81
2500	106	111	273.33
1900	80	85	296.16
1300	55	58	216.63
800	34	36	160.34
400	17	18	106.28

143
 144
 145 The Fig 2 represents that the linear regression between biomass production and COD
 146 concentration in piggery wastewater system can be obtained as follows:

147 $Y = 0.0528 x + 128,64$ $R^2 = 0,667$ (2)

148 Where: y is biomass production (mg L⁻¹ d⁻¹) and x is COD concentration (mg L⁻¹)

149 The linear regression between biomass production and TN concentration in piggery wastewater
150 system can be obtained as follows:

$$151 \quad Y = 1.2462 x + 128.71 \quad R^2 = 0.665 \quad (3)$$

152 Where: y is biomass production ($\text{mg L}^{-1} \text{d}^{-1}$) and x is TN concentration (mg L^{-1})

153 The linear regression between biomass production and TP concentration in piggery wastewater
154 system was shown in Figure 5 and can be depicted as follows:

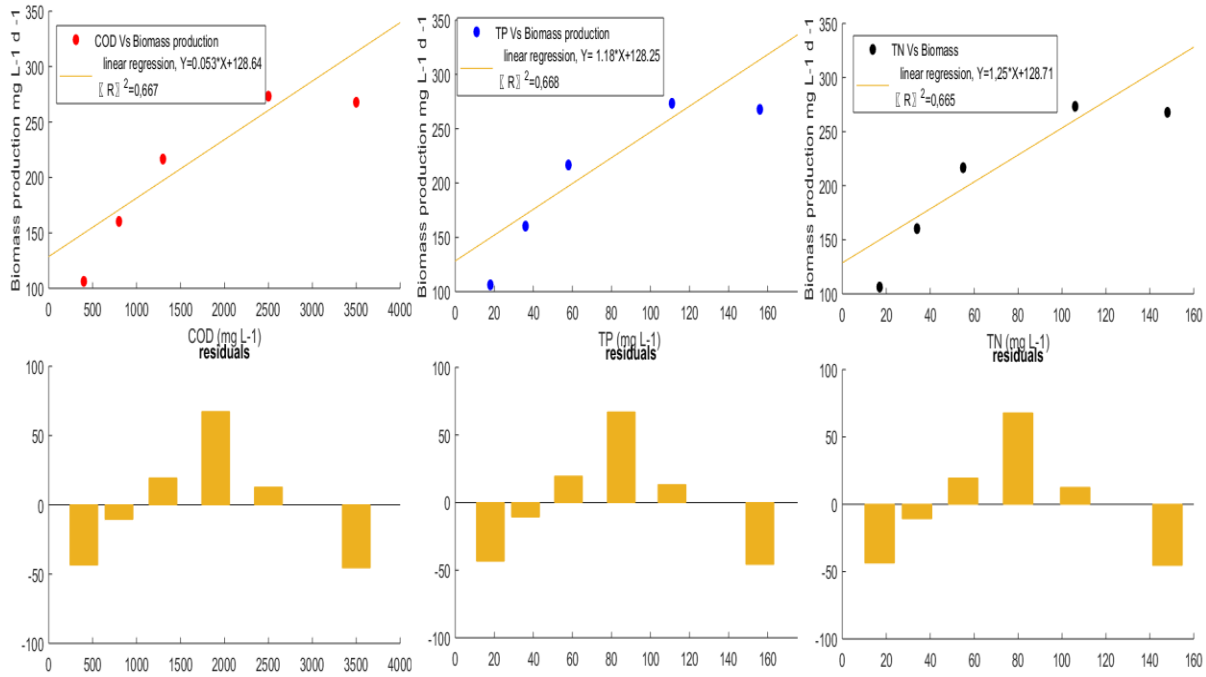
$$155 \quad Y = 1.1876 x + 128.25 \quad R^2 = 0.668 \quad (4)$$

156 Where: y is biomass production ($\text{mg L}^{-1} \text{d}^{-1}$) and x is TP concentration (mg L^{-1})

157 The above regression analysis clearly shows that biomass production in piggery wastewater system
158 depends on COD, TN and TP concentration correspondingly. COD, TN and TP can explain 66.7%,
159 66.5% and 66.8% of the variability of our dependent variable, biomass production respectively.

160 The residual plots denotes the difference between the observed value of the dependent variable,
161 biomass production of *Chlorella zofingiensis* in piggery wastewater resources and the predicted
162 value. The residual plots shows random pattern's decent fit to the linear model.

163



164

165 Fig2. Regression between biomass productions of *Chlorella zofingiensis* ($\text{mg L}^{-1} \text{d}^{-1}$) with respect
 166 to COD (mg L^{-1}), TN (mg L^{-1}), TP (mg L^{-1}) in piggery wastewater effluent

167

168 *3.3. Relationship between various algal biomass production and CO₂*

169

170 Based on table 3, the majority of algal species preferred lower concentration of CO₂ where as some
 171 algal species especially *Chlorella* species showed capacity to withstand high concentration of CO₂.
 172 Moreover the highest biomass production was observed at lower concentration of CO₂.
 173 Carbondioxide tolerance limits were specific for algal species, so several studies aimed at
 174 determining the optimum CO₂ concentration for each algal species [24]. Table 3 shows the
 175 capabilities of various microalgal species in CO₂ sequestration under various CO₂ (%v/v)
 176 concentration [24].

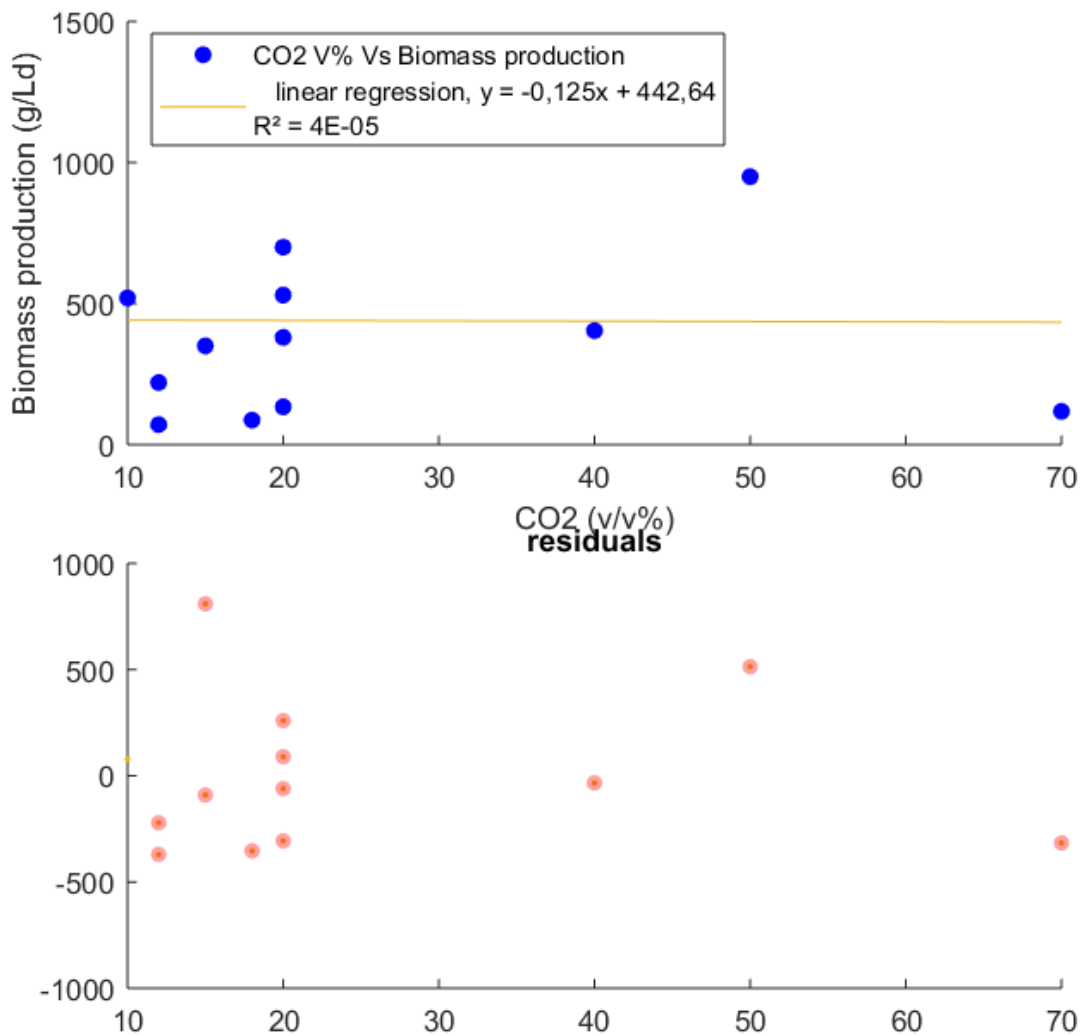
177 Table 3. Biomass production for different microalgal species under various CO₂ (%v/v)
 178 concentration

Table 3. Biomass production for different microalgal species under various CO₂ (%v/v) concentration

Microalgal species	CO ₂ (% v/v)	Biomass production (mg L ⁻¹ d ⁻¹)
<i>Chlorella sp. KRI</i>	70	118
<i>Dunaliella sp</i>	12	71
<i>Scenedesmus obliquus AS-6-1</i>	20	380
<i>Nannochloris sp.</i>	15	350
<i>Chlorella sp.</i>	50	950
<i>Chlorella sp.</i>	20	700
<i>Chlorococcum littorale</i>	20	530
<i>Aphanothece microscopic Nageli</i>	15	1250
<i>Chlorella kessleri</i>	12	220
<i>Chlorella vulgaris</i>	18	87
<i>Scenedesmus obliquus SJTU</i>	20	134
<i>S. obtusiusculus</i>	10	520
<i>Scenedesmus sp.</i>	40	404

179
 180 The Fig. 3 show that there is no apparent correlation between the unit biomass productions with
 181 the volume concentration of CO₂. The correlation coefficients (R²) for the linear relationships of
 182 the biomass production, and the CO₂ concentration are extremely low of 4×10⁻⁵. The major reason

183 for no correlation could be different experimental reactors and processes. Therefore, different CO₂
184 utilization rate and efficiencies could not be used as a predictor for algal biomass production.



185
186 Fig3. Regression between biomass production (mg L⁻¹ d⁻¹) with respect to CO₂ concentration
187 (%v/v) of various algal species [24].

188

189 The carbon dioxide capturing efficiency of microalgal species based on significant research studies
 190 were represented in Table 4. The maximum CO₂ fixation rate 1.45 gL⁻¹d⁻¹ was shown by *Anabaena*
 191 *sp. ATCC 33047* with a biomass production of 0.31 gL⁻¹d⁻¹. The main advantage of CO₂
 192 sequestration using microalgae is that the trapped carbon dioxide is combined with carbohydrates
 193 and lipids which results in production of value added products such as biomass for biodiesel and
 194 other chemicals [6].

195 Table 4. Unit biomass production of various microalgal species in CO₂ sequestration [6].

Table 4. Unit biomass production of various microalgal species in CO₂ sequestration [6].

Algal species	Biomass production (g L ⁻¹ d ⁻¹)	CO ₂ fixation rate (g L ⁻¹ d ⁻¹)
<i>Chlorella vulgaris</i>	2,03	0,43
<i>Chlorella kessleri</i>	0,87	0,163
<i>Scenedesmus obliquus</i>	0,142	0,253
<i>Chlorococcum littorale</i>	0,12	0,2
<i>Chlorella sorokiniana</i>	0,338	0,619
<i>Anabaena sp. ATCC 33047</i>	0,31	1,45
<i>Spirulina platensis</i>	2,18	0,32
<i>Haematococcus pluvialis</i>	0,076	0,143
<i>Botryococcus braunii SI-30</i>	1,1	1

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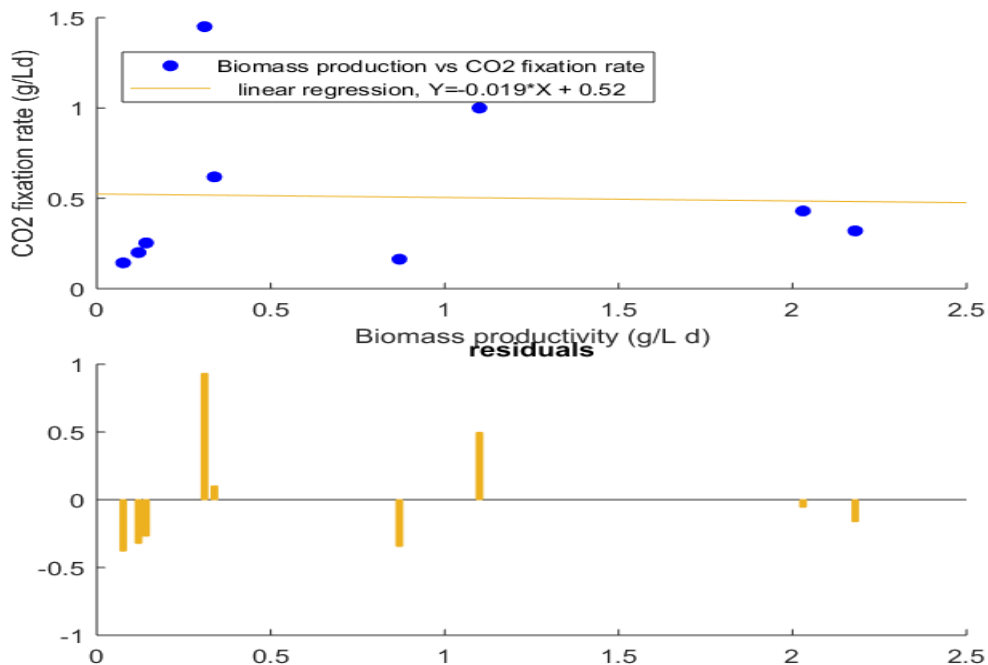
197 The linear regression between biomass production and CO₂ fixation rate by different algal species
 198 was shown in Figure 4 and can be depicted as follows:

199
$$Y = -0.019 x + 0.52 \quad R^2 = 0,0012 \quad (5)$$

200 Where: y is CO₂ fixation rate (g L⁻¹d⁻¹) and x is biomass production (g L⁻¹d⁻¹)

201 In this regression analysis, biomass production and CO₂ fixation rate less depends on each other.
 202 Biomass production of various algal species can only explain 0.12% of the variability of our
 203 dependent variable, CO₂ fixation rate.

204



205

206

207 Fig4. Regression between biomass production $g L^{-1}d^{-1}$ with respect to CO₂ fixation rate $g L^{-1}d^{-1}$
 208 of various algal species in wastewater resources [6].

209

210

211 3.4. Interpretation of regression analysis parameter and linear correlation coefficients

212 Correlation equation 1 in table 5 shows summary and parameter estimates of multiple regression
 213 analysis of biomass production with respect to COD, TN, TP in piggery wastewater and model 2

214 represents the CO₂ fixation rate by different algal species. The correlation coefficient, R²,
 215 measures the quality of the prediction of the biomass production, the dependent variable. When
 216 multivariable regression is used, parameters such as COD, TN, TP, and CO₂ explain 98.5%
 217 biomass production. On the other hand, CO₂ does not show any linear relationship with biomass
 218 production as in model 2.

219 Table 5. Model summary and parameter estimates of regression analysis of biomass production
 220 with respect to various predictors

Table 5. Model summary and parameter estimates of regression analysis of biomass production with respect to various predictors				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0,992 ^a	0,985	0,962	14,352
2	0,035 ^a	0,001	0,141	0,477

a. Predictors: (Constant), TP, TN, COD,CO₂
 b. Dependent variable: Biomass production[23,24]

221
 222
 223 Based on Table 6, model 1 the F-ratio indicates whether the overall regression model is a good fit
 224 for the data. The Table 6 also shows that the independent variables such as COD, TN and TP, are
 225 statistically significant to predict the dependent variable biomass production, $F(3, 2) = 205.969$,
 226 in piggery wastewater. The statistical prediction of dependent variable, that is biomass production
 227 of various algal species was determined with help of CO₂ as an independent variable and shown
 228 as $F(1, 4) = 1833.243$.

229 Table 6. Statistical significance of regression analysis

Table 6. Statistical significance of regression analysis					
Model	Sum of Squares	df	Mean Square	F	p-value
1 Regression	27021,152	3	9007,051	43,730	0,022
Residual	411,939	2	205,969		
Total		5			
	27433,091				
a. Dependent Variable: Biomass					
b. Predictors: (Constant), TP, TN, COD					
2 Regression	,007	1	,007	,009	,928
Residual	5,378	7	,228		
Total	5,385	8			
a. Dependent Variable: Biomass					
b. Predictors: (Constant), CO ₂					

230

231

232 Based on Table 7, the general form of the equation to predict biomass production from COD, TN,

233 TP, is:

234 $Predicted\ biomass\ production = -920,535 + (0,041 \times COD) + (9,102 \times TN) +$

235 $(3,910 \times TP).$ (6)

236 Where: y is biomass production (mg L⁻¹ d⁻¹) and predictors are COD, TN, TP concentration (mg

237 L⁻¹) respectively.

238 Unstandardized coefficients represents how much the biomass production varies with an
 239 independent variable COD /TN/TP when all other independent variables are held constant. The
 240 statistical significance of each independent variables shown in the “p-value” column is presented
 241 in Table 7. The equation to predict the biomass production from CO₂ fixation rate is:

242 Predicted biomass production = 0, 829 - (0,065×CO₂) (7).

243 Where: y is biomass production (g L⁻¹d⁻¹) and x is CO₂ fixation rate (g L⁻¹d⁻¹)

244

245

246 Table 7, Illustrations estimated model coefficients

Table 7. Illustrations estimated model coefficients					
Model	Unstandardized Coefficients		Standardized Coefficients Beta	t	p-value
	B	Std. Error			
Constant	-920,535	334,849		-2,749	0,111
COD	0,041	0,31	0,633	1,338	0,313
TN	9,102	2,603	0,682	3,496	0,073
TP	3,910	4,350	0,335	0,899	0,464
a. Dependent Variable: Biomass					
Constant	,829	,458		1,809	,113
CO ₂	-,065	,694	-,035	-,093	,928

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3.5. Relationship between algal biomass production and nutrient removal

Biomass production using wastewater sources are currently under intensive investigation. These studies shows that microalgae have some potential to biomass production, and pollutant removal and can act as source of energy coupled with wastewater treatment. The effect of biomass production and nutrient removal in various wastewater resources are shown in Table 8. Yen et al. (2014) reported about the growth of *Chlorella sp.35* in highly concentrated piggery waste water rich in phosphorus and nitrogen [25]. The *Chlorella sp.35* algal culture resulted in 60 – 95.8%, 22 – 68% and 34 – 73.8% removal of ammonia, total phosphorus and COD of piggery wastewater respectively. Hongyang et al., (2011) observed *Chlorella pyrenoidosa* cultivated in soybean processing wastewater resulted in an average biomass production of 0.64 g L⁻¹d⁻¹ and also lead to COD, total nitrogen and total phosphorus removal of 77.8 ± 5.7%, 88.8 ± 1.0%, and 70.3 ± 11.4% respectively in fed-batch process [19]. Wang et al., (2012) investigated the *Chlorella pyrenoidosa* biomass production in diluted piggery wastewater [23]. The *Chlorella pyrenoidosa* algal culture resulted in 55.4 %, 74.6 % and 77.7 % removal of COD, total nitrogen, total phosphorus, respectively from piggery wastewater sample with 1000 mg/L COD concentration. Ding et al. (2015) discussed about the removal of ammonia, phosphorus and chemical oxygen demand in dairy farm waste water with help of microalgae cultivation [26]. The 20% dairy farm waste water sample yields 0.86g/L dry weight in 6 days resulted in 83, 92, and 90 removal percentage of ammonia, phosphorus, COD respectively. Gouveia et al. (2016) reported the performance of three different microalgae such as *Chlorella vulgaris* (Cv), *Scenedesmus obliquus* (Sc) and *Consortium C* (Cons C) for wastewater remediation [27]. The maximum removal was attained by Cv, Sc and

272 ConsC were 84, 95 and 98% for total nitrogen, 95, 92 and 100% for phosphorus and 36, 63 and
 273 64% for COD, respectively.

274

275 Table 8. Effect of algal biomass production on removal of COD, TN, and TP from wastewater.

Table 8. Effect of algal biomass production on removal of COD, TN, and TP from wastewater.

Biomass production (g L ⁻¹)	COD % removal	TN % removal	TP % removal	References
0,19	73,8	68	95,8	[7]
0,64	75,8	88,8	70,3	[8]
0,3	55,4	74,6	77,7	[9]
0,86	90	83	92	[10]
0,1	36	84	95	[11]
0,4	63	95	92	[11]
0,9	64	98	100	[11]

276

277 The percentage removal of COD, TN, and TP in various wastewater resources using different algal
 278 species was shown in Table 9. Based on the regression analysis, R^2 value (also called the
 279 coefficient of determination), which is the proportion of variance in the dependent variables such
 280 as percentage removal of COD, TN and TP that can be explained by the independent variable
 281 biomass. (Technically, accounted by the regression model represented in table 9). Our independent
 282 variable biomass explain 41.5% , 32.7 % and doesn't have any effect respectively with the
 283 variability of our dependent variable such as COD removal, TN removal and TP removal
 284 respectively.

285

286 Table 9. Regression analysis of biomass production versus percentage removal of COD, TN and
287 TP

Table 9. Regression analysis of biomass production versus percentage removal of COD, TN and TP

Model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
COD	,644 ^a	,415	,298	14,331
TN	,571 ^a	,327	,192	9,596
TP	,015 ^a	,000	-,200	11,819

a. Predictors: (Constant), Biomass

288

289

290 *3.5. Phosphorus and nitrogen removal using various phototrophic systems and energy*
291 *potential of phototrophic technologies*

292 Based upon experimental data by Shoener et al., (2014), the major phototrophic technologies
293 used for algal biomass production include high rate algal ponds (HRAT), photobioreactor (PBR),
294 stirred tank reactor (STR) and algal turf scrubber (ATS). Table 10 presents that the best nitrogen
295 and phosphorus removal were obtained by PBR technology. It also indicates that PBR consumed
296 highest energy when mixing which was done by gas sparging. The rate of gas sparging depends
297 on algal species and their tendency to aggregate. ATS is passive system and it does not require
298 any energy where as HRAP require very less amount of energy for paddlewheels per hectare [21].

299 Table 10, Average percent of phosphorus and nitrogen removal, and ranges of energy
 300 consumption (kJ m^{-3}) using different phototrophic technologies [21].

Table 10. Average percent of phosphorus and nitrogen removal, and ranges of energy consumption (kJ m^{-3}) using different phototrophic technologies [12]

Technology	Average percent removal of nitrogen	Average percent removal of phosphorus	Mixing	Pumping	Harvesting
HRAP	67.1	52.1	3.2- 9.6	-	34-170
PBR	78.5	93.2	6300- 13000	55-58	-
Stirred tank	62.3	78.2	770-3100	28-31	-
ATS	70.5	78.6	-	-	-

301

302 *3.6. Physical parameters effect growth of algal species*

303 The growth of conditions of algal species depends on light energy and temperature of wastewater
 304 system (Table 11). The algal growth can be inhibited as a result of too intense light known as
 305 photoinhibition. The photoinhibition value depends on algal species and growing conditions. The
 306 temperature also influence grazing activity, growth rate and species composition of algal
 307 communities [12].

308 Table 11. Influence of various physical parameters on growth of algal species in different cultural
 309 medium

Table 11. Influence of various physical parameters on growth of algal species in different cultural medium

Culture medium	Algal species	Light ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Photoperiod	Temperature ($^{\circ}\text{C}$)	Productivity ($\text{g m}^{-2} \text{d}^{-1}$)
Raw and anaerobically digested dairy manure	Algal consortia	40-140	16:8	22	5
Anaerobically digested dairy manure	Algal consortia	270-390	23:1	19-24	5-23
Dairy manure	<i>Chlorella</i> sp.	110-120	24	20	0.58-2.57
Swine manure	Algal consortia	240-633	23:1	23-26	7.1-9.6
Centrate and raw municipal wastewater	Mixed culture	72-104	16:8	18-27	0.5-3.1
Municipal wastewater	<i>S.obliquus</i> and <i>C. vulgaris</i>	100	24	23-27	7
Municipal and synthetic wastewater	Mixed culture	230	24	22	2.1-7.7
Modified BG11	Mixed culture	15,30,60,120	16:8	20,30	0.02-2.9

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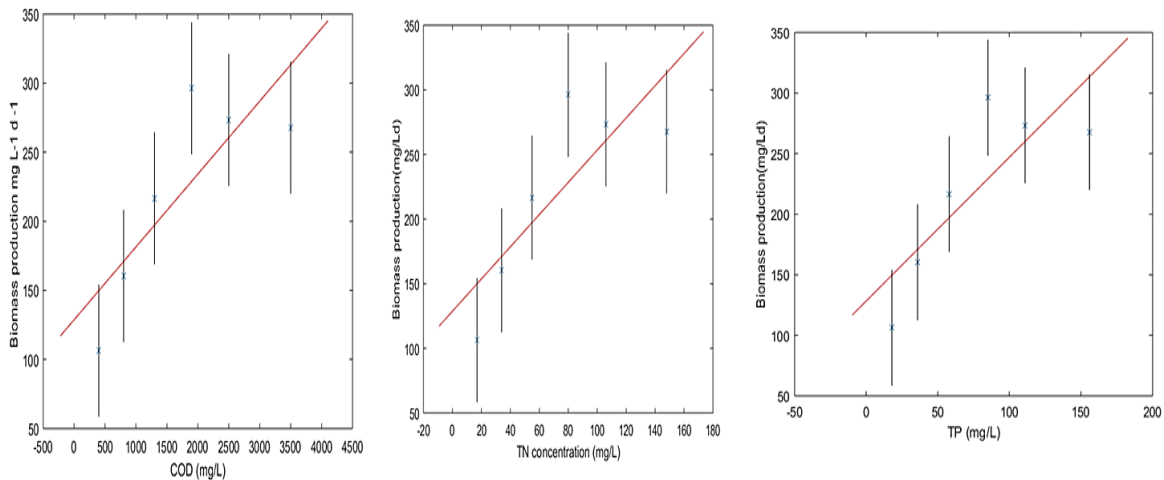
314

3.7. Uncertainties in linear fit using Monte Carlo simulation

315

316

317 The uncertainty in biomass production (Y) after performing linear fit with uncertainties in x and
318 y, using a Monte Carlo method is shown in Fig 5. Based on Monte Carlo method, the estimated
319 error on Y is: 47.78 and Linear fit function: $Y = (0.053 \pm 0.019) * X + (128.64 \pm 37.74)$, where
320 X is COD concentration. For X= TN concentration, based on Monte Carlo method, estimated error
321 on Y is: 47.95 and Linear fit function: $Y = (1.25 \pm 0.442) * X + (128.71 \pm 37.89)$. Furthermore,
322 the estimated error on Y is: 47.75 and Linear fit function: $Y = (1.19 \pm 0.419) * X + (128.25 \pm$
323 $37.82)$, where X is TP concentration based on Monte Carlo method.



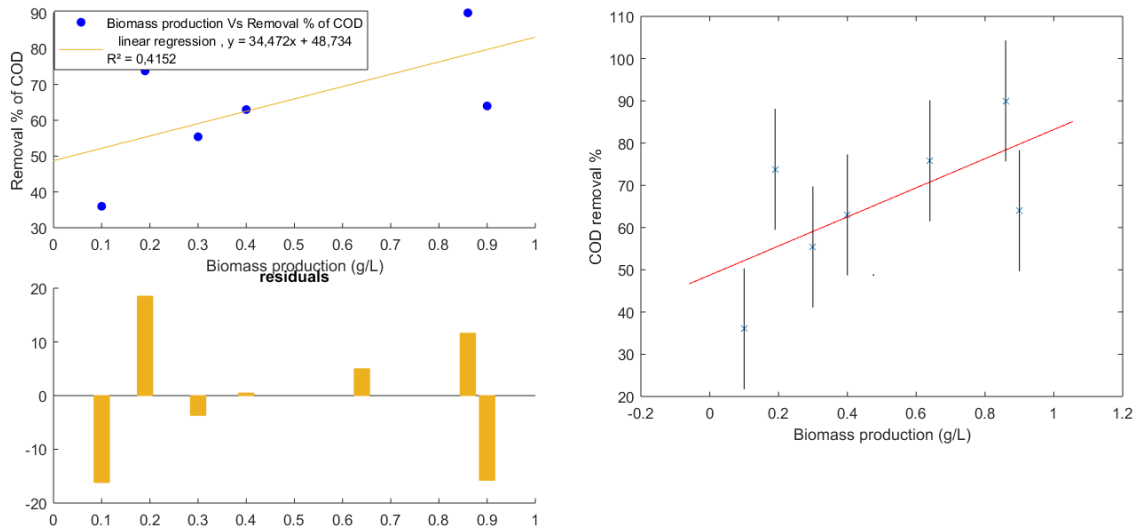
324

325 Fig5. Uncertainty in biomass productions of *Chlorella zofingiensis* mg L⁻¹ d⁻¹ with respect to
326 COD (mg L⁻¹) TN (mg L⁻¹) and TP (mg L⁻¹) in piggery wastewater effluent

327

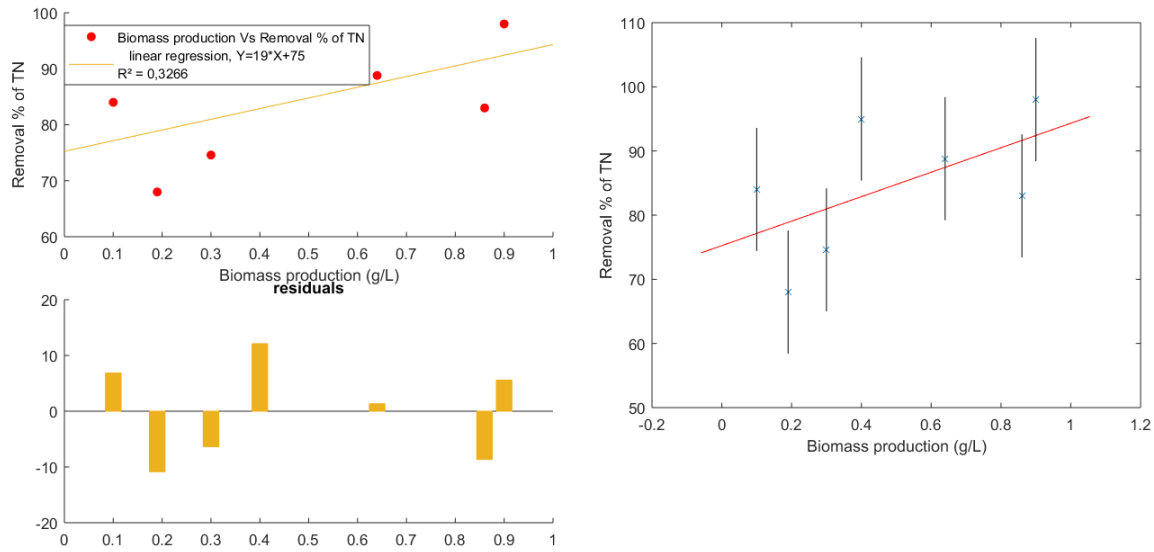
328 Fig 6 displays linear regression analysis of percentage removal of COD by various algal biomass
329 in different wastewater effluent data represented in Table 8 and illustrates the estimated error of Y
330 (percentage removal of COD) as follows: 14.33, Linear fit function: $Y = (34.47 \pm 18.295) * X +$

331 (48.734+/- 10.385), where X is algal biomass concentration in different kinds of wastewater
 332 effluents.



333
 334 Fig 6. Linear regression analysis of percentage removal of COD by various algal biomass and its
 335 uncertainty

336
 337 The linear regression analysis of percentage removal of TN by various algal biomass in different
 338 wastewater effluent data reported in Table 8 is represented in Fig 7. It also illustrates estimated
 339 error on Y (percentage removal of TN) is: 9.5962, Linear fit function: $Y = (19.078 \pm 12.251) * X + (75.247 \pm 6.954)$, where X indicates different algal biomass

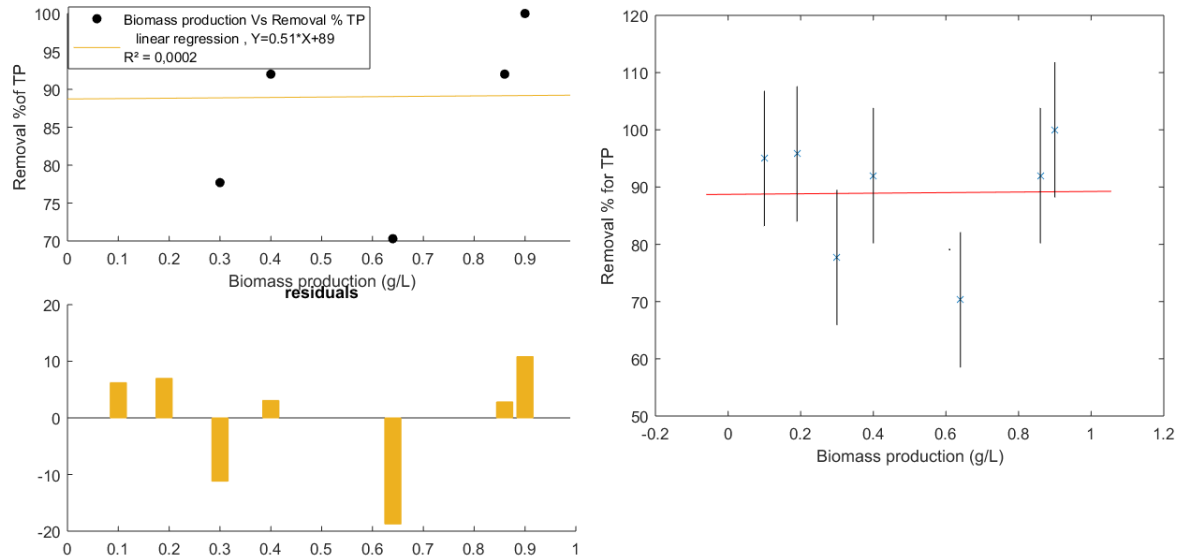


341

342 Fig 7. Linear regression analysis of percentage removal of TN by various algal biomass and its
 343 uncertainty

344

345 The Fig 8 shows linear regression analysis of percentage removal of TP by various algal biomass
 346 in different wastewater effluent data represented in Table 8. Estimated error on Y (percentage
 347 removal in TP concentration) is: 11.8191 and linear fit function: $Y = (0.507 \pm 15.089) * X +$
 348 (88.726 ± 8.565) with respect to X, algal biomass



349

350 Fig 8. Linear regression analysis of percentage removal of TP by various algal biomass and its
 351 uncertainty

352 4. Conclusions

353

354 Regression equations between algae biomass production and different wastewater variables were
 355 developed during algal biomass production from different types of wastewater. Lipid productivity
 356 contributes 93.7% of the variability to the dependent variable of biomass production. Other
 357 independent variables such as COD, TN, TP and CO₂ can explain 66.7%, 66.5 %, 66.7% and 48%
 358 of the variability of our dependent variable, biomass production. In supplementary to that biomass
 359 production has 41.5%, 32.7 % effect on the variability of COD removal, TN removal. The general
 360 form of the equation to forecast biomass production from COD, TN and TP concentration is:

361 $Predicted\ biomass\ production = -920,535 + (0,041 \times COD) + (9,102 \times TN) +$
 362 $(3,910 \times TP).$

363 The uncertainty of regression equation has been quantified using Monte Carlo method. The
364 efficiency of main phototrophic technologies for removal of nitrogen and phosphorus along with
365 energy potential of phototrophic systems were discussed in this research review. The influence of
366 physical parameters on algal biomass was also investigated.

367

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