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Biomass and Bio-butanol Production from *Borodinellopsis texensis CCALA 892* in Synthetic Wastewater: Determination of Biochemical Composition

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Abstract: Microalgae can generally maintain the high amounts of biomass in the wastewater and they can be converted from biomass to bio-butanol. Bio-butanol is a liquid biofuel and it has significant physical and chemical properties. In this study, we carried out bio-butanol production from *Borodinellopsis texensis CCALA 892* grown in various concentrations of the municipal wastewater. Also, we determined biochemical composition ratios of microalgae samples and studied the some antioxidant enzymes such as catalase, superoxide dismutase and ascorbate peroxidase. In the current study, bio-butanol was produced by the acetone-butanol-ethanol (ABE) fermentation method. The microalgae sample grown in 25% of wastewater had the highest biomass productivity among five wastewater samples with 0.114 ± 0.002 g $L^{-1}d^{-1}$. The carbohydrate and protein concentrations of control group increased day by day and their values reached stationary phases at seven days. The sample grown in 25% of wastewater had the highest carbohydrate concentration with 0.30 g L^{-1} and protein concentration with 0.35 g L^{-1} at the stationary phase. The maximum enzyme activities for catalase, superoxide dismutase and ascorbate peroxidase were 15.33 ± 0.88, 8.67 ± 0.67 and 33 ± 1.53 µmole/mg, respectively at 25% of wastewater. In addition, bio-butanol content of *B. texensis CCALA 892* was 3.63 ± 0.21 g L^{-1} and its bio-butanol yield was found as 0.18 ± 0.011 g/g sugar. In the next study, we can examine large scale butanol production.

Sentetik Atıksu İçerisindeki *Borodinellopsis texensis CCALA 892*'den Biyokütle ve Biyo-bütanol Üretimi: Biyokimyasal Kompozisyonun Belirlenmesi

Anahtar Kelimeler
Biyo-bütanol üretimi,
Mikroalgler,
Atıksu**,**
Antioksidan enzimler

Özet: Mikroalgler genelde biyokütlenin yüksek miktarlarını üretebilirler ve biyokütleden biyo-bütanole dönüştürülebilirler. Biyo-bütanol sıvı bir yakıttır ve önemli fiziksel ve kimyasal özelliklere sahiptir. Bu çalışmada, belediye atık suyunun çeşitli konsantrasyonunda büyütülen *Borodinellopsis texensis CCALA 892*'den biyo-bütanol üretimini inceledik. Birde, mikroalg örneklerinin biyokimyasal içeriğinin oranlarını belirleyerek katalaz, süperoksit dismutaz ve askorbat peroksidaz gibi bazı antioksidan enzimleri çalıştık. Yaygın çalışmada, biyo-bütanol aseton-bütanol-etanol (ABE) fermantasyon metodu ile üretildi. Beş atık su örneği içerisinde %25 atıksu içerisinde büyütülen mikroalg örneği 0,114 ± 0,002 g $L^{-1}g^{-1}$ ile en yüksek biyokütle verimine sahipti. Kontrol grubunun karbonhidrat ve protein konsantrasyonları gün ve gün arttı ve değerleri yedi günde durağan faza ulaştı. Durağan fazda, %25 atık su içerisinde büyütülen mikroalg örneği 0,30 g L^{-1} ile en yüksek karbonhidrat konsantrasyonu ve 0,35 g L^{-1} ile de en yüksek protein konsantrasyonuna sahipti. %25 atık su içerisindeki mikroalg örneğinde katalaz, superoksit dismutaz ve askorbat peroksidaz'ın maksimum enzim aktiviteleri sırası ile 15,33 ± 0,88, 8,67 ± 0,67 and 33 ± 1,53 µmole/mg idi. Buna ek olarak, *B. texensis CCALA 892*'nin biyo-bütanol içeriği 3,63 ± 0,21 g L^{-1} ve biyo-bütanol verimi 0,18 ± 0,011 g/g şeker olarak bulundu. Bir sonraki çalışmada, geniş yelpazede bütanol üretimini inceleyebiliriz.

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1. Introduction

Nowadays, energy demand has been increased because of unpredictable growth of the world's population and technological advances [1]. Fossil fuels are still the most important energy sources in the world. But, the greenhouse effects such as carbon dioxide, methane and nitrous oxide and the environmental pollutants (sulfur, carbon monoxide and chlorofluorocarbons) have contributed to the negative effects of fossil fuels. So, scientists have researched new energy sources. Bio-fuels are one of the renewable energy resources and they can diminish the effects of these gaseous [2, 3].

Bio-fuels can be separated to two groups according to states of matter as liquid and gaseous. Bio-butanol is a liquid biofuel and has unique chemical and physical properties for the fuel industry. It is called as "drop-in'' biofuel due to mixed with gasoline up to 85% [4]. Bio-butanol can be produced from plants and crops. These resources have advantages and disadvantages for bio-fuel production. One of their most significant disadvantages is their low growth rates. As a result of this, a smaller amount of bio-fuel is obtained. Unlike, microalgae have fast growth rates and they can be used for the renewable energy resources [5].

Algae can be classified into two groups, macro and microalgae. Microalgae are prokaryotic or eukaryotic microorganisms. They can use the elements such as carbon, nitrogen and phosphorus and metabolize these elements. With this way, microalgae can convert light energy to chemical energy. Then, this energy turns into bio-fuel [6]. Bio-fuel production can be affected by biomass content. It can be used for animal feed, biofuel, food, fertilizer and by-product production. Biomass production depends on environmental factors such as light, pH, the availability of macro and micro components and various types of wastewater [7]. The biomass can be obtained in the various media and microalgae can be cultivated in different wastewaters such as domestic, municipal, swine and industrial wastewater and they can remove some toxic compounds from wastewater [8, 9].

Microalgae have the metabolic compounds like other living cells. They include lipid, carbohydrate and protein. A ratio of them can change from a strain to another one [10]. Generally, lipid and carbohydrate contents of microalgae can increase with the limitation of cultivated microalgae biomass [11]. Lipid, carbohydrate contents and the growth rates of microalgae increase when carbon, nitrogen and phosphorus concentrations decrease [12]. Also, lipid and carbohydrate concentrations of microalgae can fluctuate according to the cultivation conditions [13].

We carried out the changes of antioxidant enzymes in microalgal samples. The main enzymes involved in antioxidant systems of the living cells have been studied and their functions have been explained in the literature [14, 15]. Under the stress conditions, all living cells produce reactive oxygen species (ROS). ROS lead to the cellular damages and change the functions of cells. ROS formation can be followed by antioxidant enzymes [16]. Catalase (CAT) is an antioxidant enzyme. It is responsible for converting H_2O_2 into H_2O and O_2. Superoxide dismutase (SOD) is another antioxidant enzyme and it converts O_2^- to hydrogen peroxide and water [17]. Ascorbate is an antioxidant compounds and it functions as the protective agent in immune system [18]. Ascorbate peroxidase (APX) is an antioxidant enzyme too. In this reaction, ascorbate reacts with hydrogen peroxide and the last products are water and monodehydroascorbate. Actually, the aim of all antioxidant enzymes is to reduce the damage of reactive oxygen species [19].

Borodinellopsis texensis is one of the most important microalgae. It belongs to Chlorococcaceae family and Borodinellopsis genus [20]. In this study, we carried out bio-butanol efficiency of *Borodinellopsis texensis CCALA 892*, which was grown in various concentrations of municipal wastewater and the carbohydrate, protein concentrations and the antioxidant enzyme activities of the microalgal samples were determined.

2. Material and Method

2.1. Cultivation of *Borodinellopsis texensis CCALA 892*

We used *Borodinellopsis texensis CCALA 892* and it was gotten from microalgae collection of Czech Republic. *Borodinellopsis texensis* was grown in TAP (Tris-Acetate-Phosphate) medium with some modifications according to Andersen for the cultivation of microalgae [21]. The culture medium was adjusted to pH 7.2 after the optimization studies related with the investigation of suitable pH value and these media were used for the control experiments.

2.2. Preparation of wastewater and medium

Municipal wastewater was prepared synthetically [22, 23]. It included 400 mg of peptone, 275 mg of meat extract, 70 mg of dipotassium phosphate, 17.5 mg of sodium chloride, 10 mg of calcium chloride and 5 mg of magnesium sulfate. Wastewater samples were sterilized by autoclave at 121 ^{0}C in 15 min before using. Then, various concentrations of wastewater (0%, 25%, 50%, 75% and 100%) were mixed with TAP medium. To ignore bacterial and fungi contaminations, the wastewater samples continuously were checked with cell counter and adjusted to less to 3% of contamination.

2.3. Parameters of photo-bioreactor (PBR)

Microalgae were grown at batch cultures. Microalgae samples were adjusted to 250 mL in 500 mL of flask with constant shaking at 150 rpm at the room temperature under 16:8 ratio of light/dark. We prepared continuous cultures to obtain the high amounts of biomass.

Properties of flat photobioreactor (FPBR) (1 L) were given in previous study in the detail [24]. Air was adjusted to 0.25 $L.min^{-1}$. Light intensity was 160 $\mu mol\ m^{-2}\ s^{-1}$. In our experiments, FPBR was cleaned with 5 mM peroxyacetic acid at 30 min and rinsed twice with distilled water.

2.4. Harvesting of *Borodinellopsis texensis CCALA 892*

Microalgae cells were grown until they reached the late logarithmic growth phase and centrifuged at 3600 g for 10 min at 4 ^{0}C [24]. The pellets were frozen at overnight by freeze-dried. Then, they were used for the further experiments.

2.5. Determination of specific growth rate

Microalgae were monitored at 680nm for the growth of microalgae. Specific growth rates were calculated below.

$$SGR\ (\mu): (Y1 - Y2) / (t_2 - t_1) \qquad (1)$$

Y1: The last biomass concentration;
Y2: The first biomass concentration;
$t_2 - t_1$: The last time – The first time (d). The doubling time equation was used according to Onay and given below [25];
$T_d = 0.693/\mu$.

2.6. Chemical break up of microalgae

We used acid hydrolysis procedure. Firstly, biomass was reacted with 0.5 N H_2SO_4 and heated at 121 ^{0}C at 20 min. Then, they were left to cooling at 25 ^{0}C and carbohydrate content was determined.

2.7.Calculation of macronutrients

Total protein was carried out to Weis and Bradford method [26, 27]. Carbohydrate concentrations were examined according to anthrone method [28]. Glucose and bovine serum albumin were used as the standards for the determination of protein and carbohydrate concentrations.

2.8. Determination of CAT, SOD and APX activities

20 mL of samples was centrifuged at 6000 rpm for 20 min. Then, pellet was taken and dissolved in 0.05 M phosphate buffer. The sample was centrifuged again and CAT, SOD and APX activities were measured. CAT and SOD activities were determined according to nitroblue tetrazolium and thiobarbituric acid methods [29-31]. APX activity was measured according to Nakano and Asada [32].

2.9. Fermentation of *Borodinellopsis texensis CCALA 892* and bio-butanol production

We used *C. acetobutylicum* for the fermentation of microalgae. The medium consisted of sugar (up to 10g) or microalgal samples as the carbon source. Biotin (0.01g) as the vitamin source was used in the media. Monopotassium phosphate arranged the media for the suitable pH (4.75).

Fermented microalgae were used for bio-butanol production. Bio-butanol concentrations were calculated according to Maiti method [33, 34].

3. Results

3.1. Determination of growth curves of *B. texensis CCALA 892* grown in wastewater

We firstly characterized the content of the municipal wastewater (MWW). The results of parameters such as total organic carbon (TOC), total nitrogen (TN) and, and total phosphorous (TP) were given in Table 1. Microalgae can show different growth curves at various environmental factors such as stress factors (light, pH, temperature and macronutrient).

In our study, various proportions (0-25-50-75-100%) of MWW were mixed with TAP medium for the cultivation of *B. texensis CCALA 892*. The medium including entirely municipal wastewater was named as 100% MWW. On the other hand, control group completely consisted of TAP medium and this medium was named as 0% MWW.

Table 1. Municipal wastewater characterization

Parameters	Units	Values
TOC	mg/L	178
TN	mg/L	51
TP	mg/L	16
Turbidity	NTU	4

We plotted the curves of *B. texensis CCALA 892* according to absorbance values or biomass concentrations versus time (days). The absorbance values versus time were shown for *B. texensis CCALA 892* in Figure 1.

The medium with 25% MWW showed the biggest absorbance value (0.85 ± 0.001), reaching the late logarithmic phase at seven days. The media with control, 50% MWW and 100% MWW reached the late logarithmic phase at seven days with OD 0.62 ±0.001, 0.69 ± 0.001, 0.53 ± 0.001 and 0.43 ± 0.001, respectively.

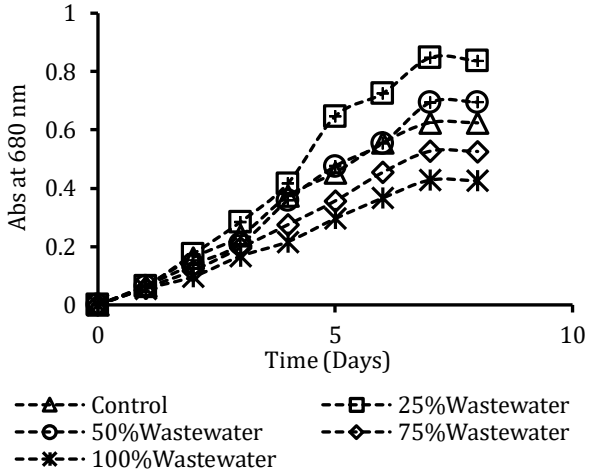

Figure 1. Absorbance values of *B. texensis CCALA 892* grown in wastewater.

Similar to absorbance measurements, biomass concentrations showed the linearity with the cell numbers of *B. texensis CCALA 892*.

The medium with 25% MWW had the maximum biomass with 800 mg L^{-1} among five wastewater samples. In addition to this, 50% MWW displayed higher biomass (744 mg L^{-1}) than that of control group (689 mg L^{-1}) and 75% (600 mg L^{-1}). In contrast, 100% MWW showed the lowest biomass (500 mg L^{-1}). The growth curves of *B. texensis CCALA 892* associated with these results were given in Figure 2.

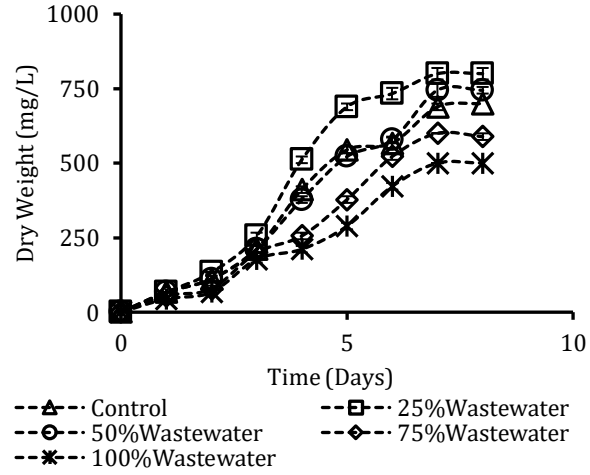

Figure 2. Dry weight concentrations of *B. texensis CCALA 892* grown in wastewater.

3.2. Growth parameters of *B. Texensis CCALA 892*

The biomass concentrations of the samples were determined according to concentrations of *B. texensis CCALA 892* at stationary phases. The medium with 25% had the maximum biomass concentration with 0.80 g L^{-1}. The media with control, 50%, 75% and 100% MWW had values of 0.69 ± 0.01 g L^{-1}, 0.75 ±0.02 g L^{-1}, 0.60 ± 0.01 g L^{-1} and 0.50 g L^{-1}, respectively.

Biomass productivities of *B. texensis CCALA 892* were examined to their biomass weights at stationary phases. The maximum productivity (25% MWW) was 0.114 ± 0.002 g $L^{-1}d^{-1}$. The medium with 50% MWW had the value of 0.107 ± 0.003 g $L^{-1}d^{-1}$. Biomass productivity of the medium with 75% MWW (0.085 ± 0.001 g $L^{-1}d^{-1}$) was higher than that of 100% MWW (0.071 ± 0.001 g $L^{-1}d^{-1}$). On the other hand, control had productivity with 0.098 g $L^{-1}d^{-1}$.

We also determined the specific growth rates of *B. texensis CCALA 892*. They were calculated according to linear regions of plots. Specific growth rate was 5.11 ± 0.03 d^{-1} for the medium with 25% MWW. The value of control was 4.79 ± 0.02 d^{-1}. In addition, while wastewater concentrations increased, specific growth rates decreased. The medium with 100% MWW showed the lowest specific growth rate with 4.11 ± 0.04 d^{-1} and 75% MWW (4.40 ± 0.02 d^{-1}) had lower value than that of 50% MWW (4.73 ± 0.02 d^{-1}).

Also, we calculated the doubling times of *B. texensis CCALA 892*. The medium with 100% had the highest doubling time (0.169 d). In contrast, the doubling time value of 25% MWW was 0.135 d. Doubling times of 50% MWW, 75% MWW and control were 0.147 d, 0.157 d and 0.145 d, respectively. The specific growth rates were inversely proportional to the rates of doubling times of *B. texensis CCALA 892*. The growth parameters *of B. texensis CCALA 892* were displayed in Table 2.

3.3. Concentrations of macronutrients

The carbohydrate and protein concentrations of *B. texensis CCALA 892* were determined according to dried weight percent (dwt %). The highest carbohydrate concentrations were found in the media with 25% and 50% of MWW. Both of them had similar results. The carbohydrate concentrations of the medium with 25% of MWW and 50% of MWW were 37.1 ± 0.4% and 38.5 ± 0.5% respectively. Carbohydrate concentration almost remained stable when MWW concentration much more increased. The media with 75% (33.4 ± 1.9%) and 100% (33.9 ± 1.5%) of MWW could not change their carbohydrate concentrations remarkably compared to that of the control group (32.4 ± 0.6%).

Likewise, protein concentrations of *B. texensis CCALA 892* could not change with more addition of wastewater and they had similar results. Protein concentrations of control, the media with 25%, 50%, 75% and 100% of MWW were 43.6 ± 1.2%, 43.3 ± 0.9%, 43.4 ± 0.6%, 42.5 ± 1.8% and 42.7 ± 1.8% respectively. The lipid concentrations of *B. texensis CCALA 892* were not studied and the protein and carbohydrate concentrations of *B. texensis CCALA 892* were given in Table 3.

Table 2. Growth parameters of *B. texensis CCALA 892*

Samples (wastewater)	Biomass concentrations (g L^{-1})	Biomass productivity (g $L^{-1}d^{-1}$)	Specific growth rate (μ) (d^{-1})	Doubling time (d)
Control	0.69 ± 0.01	0.098 ± 0.001	4.79 ± 0.02	0.145
25%	0.80 ± 0.02	0.114 ± 0.002	5.11 ± 0.03	0.135
50%	0.75 ± 0.02	0.107 ± 0.003	4.73 ± 0.02	0.147
75%	0.60 ± 0.01	0.085 ± 0.001	4.40 ± 0.02	0.157
100%	0.50 ± 0.01	0.071 ± 0.001	4.11 ± 0.04	0.169

Table 3. Percentages of metabolic substances of *B. texensis CCALA 892*

Samples (wastewater)	Carbohydrate (dwt %)	Protein (dwt %)
Control	32.4 ± 0.6	43.6 ± 1.2
25%	37.1 ± 0.4	43.3 ± 0.9
50%	38.5 ± 0.5	43.4 ± 0.6
75%	33.4 ± 1.9	42.5 ± 1.8
100%	33.9 ± 1.5	42.7 ± 1.8

3.4. Time course calculation of metabolic substances of *B. texensis CCALA 892*

We plotted the metabolic substance concentrations of *B. texensis CCALA 892* in the time course. The carbohydrate and protein concentrations of control group increased day by day. Control group reached the maximum concentration at seven days at the stationary phase. While the carbohydrate and protein concentrations of control group were 0.02 g L^{-1} and 0.03 g L^{-1} at first day, these values reached 0.23 g L^{-1} and 0.29 g L^{-1} at the stationary phase, respectively. The results related with metabolic substances of control group were given in Figure 3.

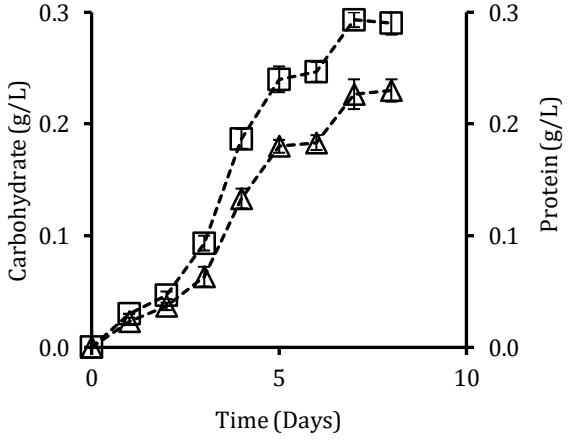

Figure 3. Carbohydrate and protein concentrations of *B. texensis CCALA 892* grown in control groups.

The medium with 25% of MWW showed similar results (0.02 g L^{-1} and 0.03 g L^{-1}) at first day but at the stationary phase, its carbohydrate and protein concentrations (0.30 and 0.35 g L^{-1}) increased notably. The medium with 50% of MWW had nearly same results (0.29 g L^{-1} and 0.32 g L^{-1}) for the carbohydrate and protein concentrations. In contrast, the carbohydrate and protein concentrations of 75% of MWW decreased drastically with 0.20 g L^{-1} and 0.25 g L^{-1}, respectively. The medium with 100% of MWW displayed the lowest carbohydrate (0.15 g L^{-1}) and protein (0.21 g L^{-1}) concentrations**.** The results were given in Figure 4.

3.5. CAT, SOD and APX enzyme activities of *B. texensis CCALA 892*

We also studied the antioxidant enzymes for the explanation of the stress effects on microalgae in the wastewater.

The microalgae samples grown at 25% of MWW and Control had the lowest CAT activities with about 10μmole/mg. In contrast, the highest CAT activity was 15.33μmole/mg at the medium with 100% of MWW. The CAT activities of the microalgae samples grown at the medium with 50% and 75% of MWW were 12.33 μmole/mg and 14.67 μmole/mg, respectively. In addition, SOD activities of microalgae samples were carried out.

Borodinellopsis texensis which was cultured in 100% of MWW sample gave the highest SOD activity with 8.67 μmole/mg. While wastewater concentrations decreased, SOD activities declined. Control and the medium with 25% of MWW had the lowest SOD activities with about 5 μmole/mg. The microalgae samples grown at 75% of MWW showed higher SOD activity with 8.33 μmole/mg than that of 50% of MWW with 6.67 μmole/mg.

Similarly, the medium with 100% of MWW had the maximum APX activity with 33 μmole/mg. The minimum APX activity was 14.67 μmole/mg and this value belonged to Control samples. The APX activities of the media with 25%, 50% and 75% of MWW were 15.67 μmole/mg, 22 μmole/mg and 27.67 μmole/mg, respectively.

The enzyme activities of CAT, SOD and APX were illustrated in Figure 5. In conclusion, low concentrations of wastewater caused negative effects on antioxidant enzymes. By contrast, high concentrations of wastewater enhanced the activities of antioxidant enzymes.

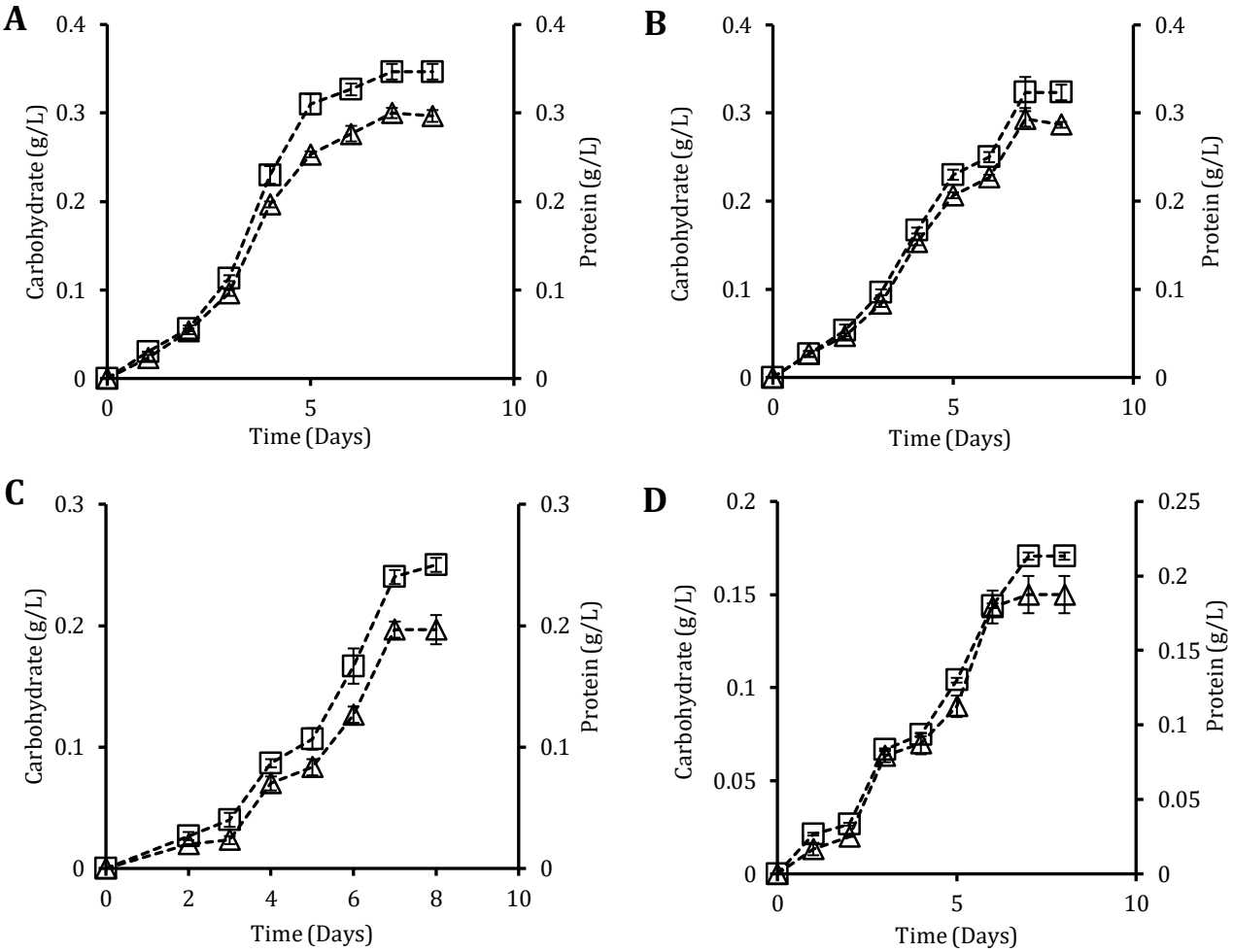

Figure 4. Carbohydrate and protein concentrations of *B. texensis CCALA* A) 25% B) 50 % C) 75 % D) % 100.

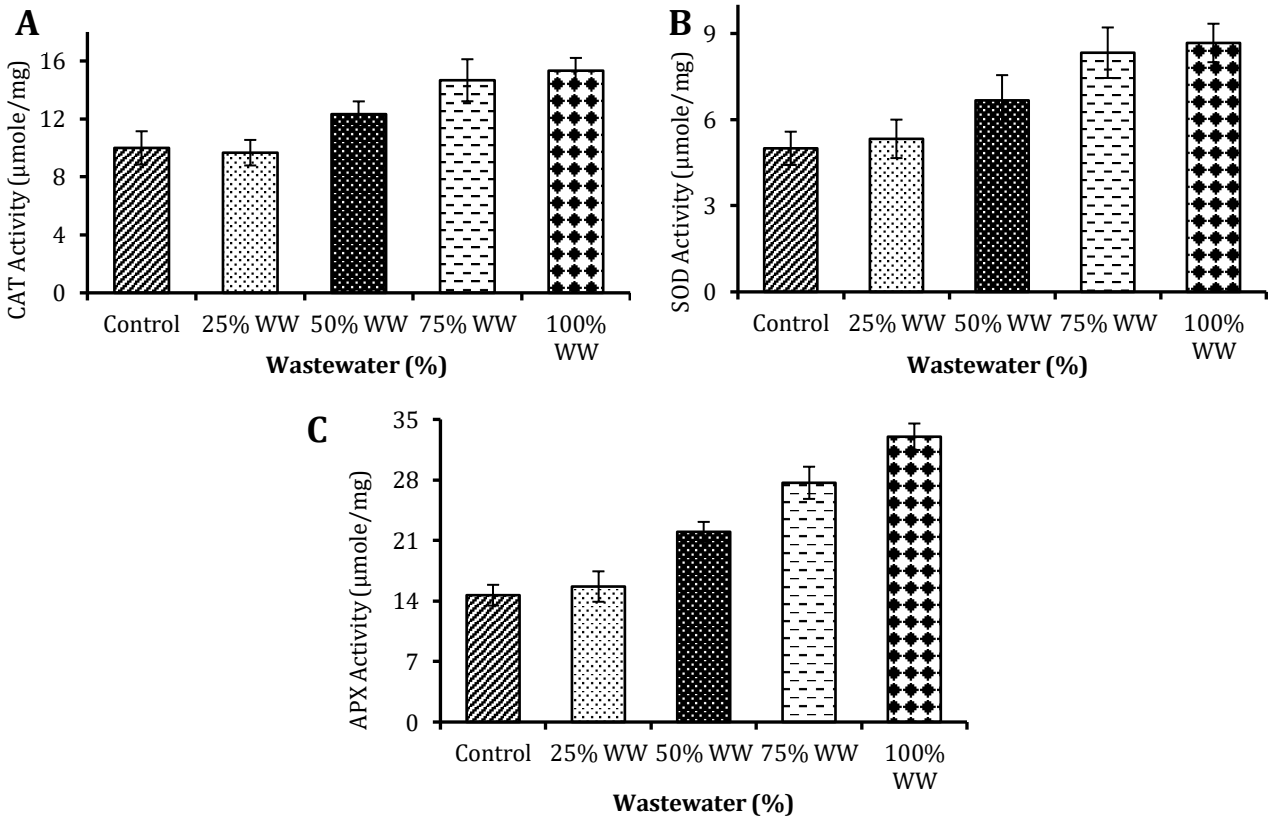

Figure 5. CAT, SOD and APX activities of *B. texensis CCALA.*

3.6. Bio-butanol content and yield of *B. texensis CCALA 892*

C. acetobutylicum was used for bio-butanol fermentation. Microalgal biomass was reacted with 0.5 N H_2SO_4 and heated at 121 ^{0}C. Then, biomass was left to cooling at 25 ^{0}C and centrifuged at 3600 g. After hydrolysis, initial sugar concentrations (1, 5, 10 and 20g) were adjusted and glucose was used as the standard for this study.

The medium with 25% of MWW was used bio-butanol production because it had the maximum carbohydrate productivity and carbohydrate content with 0.114 g $L^{-1}d^{-1}$ and 37.1 ± 0.4%.

Bio-butanol content of *B. texensis CCALA 892* at the medium with 25% of MWW was 3.63 ± 0.21 g L^{-1} and bio-butanol yield was found as 0.18 ± 0.011 g/g of sugar. Bio-butanol parameters of *B. texensis CCALA 892* were displayed in Table 4.

Table 4. Biobutanol parameters of *B. texensis CCALA 892* grown in 25% wastewater

Wastewater Content (%)	Initial sugar concentration (g/L)	Biobutanol content (g/L)	Biobutanol yield(g/g sugar)
25	20	3.63	0.18

4. Discussion and Conclusion

B. texensis CCALA 892 was selected for this study, because, Neofotis et al. investigated this species in detail. In their study, *B. texensis* was isolated from roadside saline soil and it included high amounts of carotenoid content [35]. This situation showed that *B. texensis* can grow fast in the extreme conditions such as wastewater because of the availability of *B. texensis* in soil. Also, we carried out *B. texensis CCALA 892* in our previous study. It was grown in Bold basal medium (BBM) in the various wavelength lights in column photobioreactor. The highest biomass concentration was near 284 mg L^{-1} in blue light. This value was suitable compared to other microalgae taxon such as *Coelastrella*, *Desmodesmus* and *Ankistrodesmus* for the cultivation of *B. texensis* [36]. One of the reasons we chose this strain is that there are very few articles related to it in the literature and this issue needs to be investigated in wastewater.

In the current study, the wastewater was sterilized and mixed with various concentrations of Tris-Acetate-Phosphate (TAP) medium.

The contamination ratio in the medium was less than 3%. This situation provided a suitable environment for the cultivation of *B. texensis CCALA 892*. 0-25-50-75 and 100% of MWW led to the changes related with the growth of *B. texensis CCALA 892*. These changes in light, pH, temperature and macronutrient can affect the growth of microalgae positively or negatively [37].

The medium with 25% of MWW had the maximum biomass and productivity with 0.80 g L^{-1} and 0.114 g $L^{-1}d^{-1}$, respectively. These results had parallel with the literature. We observed that microalgae used the macronutrients such as carbon, phosphorus, nitrogen and sulphur in wastewater and macronutrients completely depleted at the late logarithmic phase. As a result of this, the biochemical behaviors of microalgae positively caused the increase of biomass content. [33,38]. The medium with 100% of MWW displayed the lowest biomass and productivity with 0.50 g L^{-1} and 0.071 g $L^{-1}d^{-1}$, respectively.

The growth parameters of microalgae change significantly in the various wastewaters [39, 40]. Studies showed that the municipal and agricultural wastewaters could lead to changes the growth parameters of microalgae [41-46]. Fan et al. studied *Spirulina platensis* and *Scenedesmus obliquus* in the domestic wastewater. They carried out the light effects on biomass and lipid yields of microalgae. *Scenedesmus obliquus* had higher biomass concentration with 356 mg L^{-1} than that of *Spirulina platensis* at 8000 lux. In addition, *Scenedesmus obliquus* had higher lipid percentage with 36.8% mg L^{-1} [7]. Aketo et al. investigated lipid contents of *P. kessleri NKG021201* and *C. saccharophilum NKH13* in municipal wastewater. *P. kessleri* was grown enough in municipal wastewater and *P. kessleri NKG021201* had high lipid content with 56 mg $L^{-1}d^{-1}$ [47]. In another study, *Coelastrella sp.* was grown in swine wastewater. It was cultivated at different concentrations of zinc. Zinc concentration affected biomass concentration and reduced biomass concentration of *Coelastrella sp.* It led to the decrease of nitrogen volatilization [48]. *Chlorella sorokiniana* was grown in the mixture of municipal and piggery wastewater. Biomass concentration of *Chlorella sorokiniana* increased in the mixture of municipal and piggery wastewater [49]. As described in the literature, stress factors can change biomass concentrations like our study.

In addition to kinetic parameters, we studied the protein and carbohydrate concentrations of *B. texensis CCALA 892*. The media with 25% and 50% of MWW had the maximum carbohydrate concentrations with nearly 38% (dwt %). This result was logical because 25% and 50% of MWW brought about the stress effect for *B. texensis CCALA 892*. This resulted in the accumulation of much more carbohydrate concentration in microalgae. The media with 75%, 100% of MWW and control showed approximately similar results with 33% (dwt %). This situation occurred because of higher amounts of MWW concentrations. High amounts of wastewater led to depletion or the limitation of

macronutrients. Unavailability of macronutrients resulted in lower carbohydrate concentrations [50, 51, 52]. We did not carried out the lipid content for *B. texensis CCALA 892* in this study. However, the lipid content *B. texensis CCALA 892* probably increased at high amounts of wastewater. Metabolic parameters such as lipid and protein can reach 80% under stress conditions for microalgae [53, 54]. In further study, it can be researched. To prove the formation of stress factors, we carried out antioxidant enzyme activities.

CAT, SOD and APX activities can explain the nature of microalgae. These enzymes are antioxidant enzymes and the concentrations of them can change under the stress conditions. In the literature, *Coelastrella sp.* was studied for superoxide dismutase activity under the zinc stress conditions in swine wastewater. When zinc concentration increased, superoxide dismutase activity enhanced and its maximum SOD activity was 63.4 U/mg at 8 mg L^{-1} of zinc [48]. High amounts of zinc caused the formation of stress and this situation resulted in the increased activity of SOD. Yang et al. carried out the toxicities of polyethylene, polyamide and polystyrene on *Chlorella pyrenoidosa*. Polyethylene, polyamide and polystyrene are micro plastics. Micro plastics lead to reduce the growth of *Chlorella pyrenoidosa*. CAT and SOD activities increased when the concentrations of polyethylene, polyamide and polystyrene reached 50 mg L^{-1}. CAT and SOD activities had the maximum values at 100 mg L^{-1} of three micro plastics [55]. In their study, high concentrations of micro plastics caused swelling of microalgal cells and stress factor occurred. This condition leaded to the increase of CAT and SOD activity. In another study, *Skeletonema costatum* was searched for the toxicities of triclosan and polyvinyl chloride 800. Triclosan and polyvinyl chloride 800 had significant effects on the reduced growth of *Skeletonema costatum*. The maximum SOD activities of triclosan and polyvinyl chloride 800 were around 20 and 80 U/mg at 0.1 g L^{-1} of triclosan and polyvinyl chloride 800, respectively [56]. Similarly, *Chlorella vulgaris* YH703 was studied for the effect of salinity stress. They added 30mM of sodium chloride in the BG-11 medium. Salt concentration was then increased in the BG-11 medium and 500 mM of sodium chloride was added in the medium. Thus, a salt stress was created in the medium. High concentration of sodium chloride caused the enhanced APX activity. APX activity was 174.69 activity % at 500 mM of sodium chloride [57]. In our study, the highest CAT, SOD and APX activities were 15.33µmole/mg, 8.67 µmole/mg and 33 µmole/mg at the medium with 100% of MWW. In parallel with the literature, when the concentration of MWW increased, the formation of stress factor occurred. As a result of this, CAT, SOD and APX activities increased.

We also carried out bio-butanol efficiency of *B. texensis CCALA 892*. Bio-butanol content of *B. texensis CCALA 892* was 3.63 ± 0.21 g L^{-1} and bio-butanol yield was 0.18 ± 0.011 g/g of sugar. There are not enough studies related with bio-butanol in the literature. In our previous study, we researched *Chlorella zofingiensis CCALA 944* in the municipal wastewater. Bio-butanol content was 0.084 g bio-butanol/ g of biomass at 80 µM of indol-3-acetic acid [31].

In another study, *Chlorella vulgaris JSC-6* was grown in basal medium and they researched for the bio-butanol efficiency of *Chlorella vulgaris JSC-6*. The maximum bio-butanol content was 1.36 g L^{-1} at 20 g of total sugar concentration [58]. This result was half of our result. *Chlamydomonas reinhardtii CCAP 11/32C* was examined for bio-butanol production in TAP medium. Bio-butanol content was 12.67 g L^{-1} [59]. *Neochloris aquatica CL-M1* was grown in swine wastewater and bio-butanol content was 12 g L^{-1}. Cheng et al. indicated that the maximum butanol yield was 0.2 g/g sugar for *Chlorella sorokiniana CY1* [60]. This result was parallel to our result.

We showed that *B. texensis CCALA 892* had similar bio-butanol efficiency and it can be used for genetic modification studies and large scale butanol production after handling contamination problems.

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