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# 1-butanol production from polluted industrialised District using *Synechococcus elongatus* as alternative to gasoline: Comparison with Clean Regions

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**Abstract** In this study it was aimed to produce 1-butanol from $CO_2$ trapping from the polluted industrial regions using *S. elongatus*. The air from two polluted industrial districts (Aliağa and Urla) was trapped with an air pump and it was purified by passing the gas from two bacteria namely *Nitrosomonas sp*. and *Azotobacter sp*. located and grown in two separate adsorbent pads sealed with glass reactors. By consuming of $O_2$ and $N_2$ gases the remaining gas is $CO_2$ and it was used to produce 1-butanol by Cyanobacteria. The effects of $CO_2$ concentrations, $O_2$ level, $NO_3$-$N^{-1}$, $SO_4^{-3}$, pH, temperature and retention time, on the 1- butanol yield were investigated. 1- butanol and $CO_2$ concentrations were measured in GC-MS. The enzymes catalyzing the 1-butanol production (acetyl-CoA acetyltransferase (AtoB), 3-hydroxybutyryl-CoA dehydrogenase (Hbd), crotonase (Crt), trans-2-enoyl-CoA reductase (Ter) and bifunctional aldehyde alcohol dehydrogenase (AdhE2)) were measured in a spectrophotometer. The Cyanobacteria was growth in B-11 media and the biochemical test were performed for the characterization of *S. elongatus*. Nowadays, the cost of 1 liter gasoline is 1.60 Euro while the cost of the 1-butanol produced is only 0.66 Euro. The maximum 1- butanol yield was 98% when the A to B and 3-Hbd concentrations were around $0.7X10^{-1}$ and $2X10^{-1}$ mg/l in Aliağa industrial district while low 1-butanol yield (66%) was obtained in non polluted Urla Region at pH=7.1, after 20 days of incubation at 30 $^oC$.

**Keywords** 1-butanol, biofuel, Cyanobacteria, *S. elongatus*

## 1. Introduction

Although carbon dioxide in air was in minor level (between 0 – 0.03 %) it's concentration elavated significantly in municipal metropollutant areas and industrial regions. The carbon dioxide concentration in the atmosphere increase 2-3 ppm in every year as a result of burning in fosil fuels. Sometimes the carbon dioxide levels in industrail regions can reach to 600-700 ppm [1]. 80–85 % of atmospheric carbon dioxide produced by fosil fuels (petrolium, coal and natural gas), 15-20 % of the atmospheric carbon dioxide orignated from the respiration of humans and some organisms and from the microorganisms transforming the organic compounds. The pollution increase contionusly as the energy source was utilized. The global studies concern to reduce the carbon dioxide level to the minimum limit (450 ppm) by reducing the $CO_2$ emissions to 50-80 % in year 2050. 1-butanol is a primary alcohol with a four carbon structure and wıth a chemical formula of $C_4H_9OH$. Its molecular weight İs 74.1216 g/mol, it is colorless and have the same density as water. 1-Butanol is considered as a potential fuel substitute to displace gasoline. The energy density of 1-butanol (27 MJ/L) is higher than that of ethanol (21 MJ/L) and closer to that of gasoline (32 MJ/L). In addition, its low hygroscopicity and compatibility with current infrastructure make 1-butanol an attractive fuel substitute [2]. 1-butanol can be produced by synthesis the butyryl-CoA from acetyl-CoA in a

pathway. 1-butanol formation pathway consists of five enzymes namely acetyl-CoA acetyltransferase (AtoB), 3-hydroxybutyryl-CoA dehydrogenase (Hbd), crotonase (Crt), trans-2-enoyl-CoA reductase (Ter) and bifunctional aldehyde alcohol dehydrogenase (AdhE2) [3]. The development of new technologies for production of alternative fuel became necessary to circumvent finite petroleum resources, associate rising costs, and environmental concerns due to rising fossil fuel $CO_2$ emissions. Several alternatives have been proposed to develop a sustainable industrial society and reduce greenhouse emissions. The idea of biological conversion of $CO_2$ to fuel and chemicals is receiving increased attention. In particular, the direct conversion of $CO_2$ with solar energy to biofuel by photosynthetic microorganisms such as microalgae and cyanobacteria has several advantages compared to traditional biofuel production from plant biomass. Several emerging technologies are being implemented in order to overcome the problem of greenhouse gas (GHG) pollution and replace fossil fuels by promoting viable production of liquid fuels such as fatty acid esters (biodiesel) Conversion of $CO_2$ for the synthesis of chemicals by photosynthetic organisms is an attractive target for establishing independence from fossil reserves. Direct conversion of $CO_2$ to bio-based fuels and chemicals has emerged as a significant thrust to address the energy and environmental concerns caused by over-reliance on fossil fuels and the increasing level of atmospheric $CO_2$. Cyanobacteria are photoautotrophic bacteria that utilize sunlight as their energy source and $CO_2$ as their carbon source. Because they have adapted or can acclimate to diverse growth environments, they can serve as "photo-bioreactors" for the production of renewable biofuels and green chemicals [4]. Here we report the photosynthetic production of 1-butanol which is an energy-dense fuel molecule, from $CO_2$ in the genetically engineered cyanobacterium *Synechococcus elongatus*.

In this study it was aimed to produce 1-butanol from $CO_2$ trapped from two different regions using *S.elongatus*. The air from was trapped from a non-polluted region (Urla-İzmir Turkey) and from a polluted industrial district (Aliağa-İzmir Turkey). The effects of increasing $CO_2$, $O_2$, $NO_3$-$N^{-1}$, $SO_4^{-3}$ concentrations, temperature, retention time and pH levels on the 1- butanol production from $CO_2$ by *S.elongatus* were investigated. 1- butanol and $CO_2$ concentrations were measured in GC-MS. The enzymes catalyzing the 1- butanol production (acetyl-CoA acetyltransferase (AtoB), 3-hydroxybutyryl-CoA dehydrogenase (Hbd), crotonase (Crt), trans-2-enoyl-CoA reductase (Ter) and bifunctional aldehyde alcohol dehydrogenase (AdhE2) were monitored. The addition of plasmid on 1-butanol production was investigated. A cost analysis was performed.

## 2.Material and Methods

### *2.1 Microorganisms isolated and used in this study*

In this study, Photosynthetic cyanobacteria *S. elongatus* was İsolated from Urla and Aliağa Bays in İzmir Turkey.

### *2.2 Culture Medium and Conditions*

All *S. elongatus* cultures were grown on BG-11 (1.5 g $NaNO_3$, 0.036 g $CaCl_2$, 0.006 g ferric ammonium citrate, 0.001 g EDTA, 0.04 g $K_2HPO_4$, 0.075 g $MgSO_4$, 0.02 g $Na_2CO_3$, 1000 × trace mineral (2.86 g $H_3BO_3$, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.22 g $ZnSO_4{\cdot}7H_2O$, 0.390 g $Na_2MoO_4{\cdot}2H_2O$, 0.0790 g $CuSO_4{\cdot}5H_2O$, 0.0494 g $Co(NO_3)_2{\cdot}6H_2O$)[5]. The pH of the liquid was adjusted to 7.1 by adding 1 mM $NaHCO_3$ and 100 mM KOH to the BG-11 medium. All *S. elongatus* strains were cultured in BG-11 medium in a shaking incubator at 120 rpm. Cultures were grown under 60 W light condition at 30 °C. Under laboratory conditions to the 250 ml sterile glass bottles containing 100 ml of BG-11 liquid medium 100 ml of isolated *S. elongatus* culture was added. To the each culture purified $CO_2$ gas trapped from the Aliağa and Urla regions were given between hours 10.00-18.00 in a day five times at a flow rate of 0.083 $m^3$/sec during 30 days.

### *2.3 Purification of $CO_2$ from the trapped air*

The atmospheric gas trapped by vacuum pumps from the Urla And Aliağa atmosphere regions and it is were stored in the polyethylene air bags (Figures 1 and 2). The atmospheric gas was passed from an absorbant ped containing *Azotobacter* (Figure 3) In this step the $N_2$ gas present in the air samples was eliminated since this bacteria used $N_2$

gas for growth. The *Azotobacter sp.* medium consisted from 08% $K_2HPO_4$; 0.02% $KH_2PO_4$, 0.02% $MgSO_4$ 0.01% $CaSO_4$; 0.0015% $FeSO_4/7H_2O$; 0.00025% g $NaMoO_3$ and 0.5% sucrose. Then the remaining gas was passed from an absorbant ped containing *Nitrosomonas* sp. bacteria. This bacteria used the $O_2$ in the air (Figure 3). As *Nitrosomonas* medium Medium A, Medium B, Medium A-Z and A-2 Trace medium were used. A medium consisting of $(NH_4)2SO_4$ 0.1 g; $K_2HPO_4$ 0.1 g; NaCl 0.2 g; $MgSO_4.7H_2O$ 0.05 g; $FeCl_3$ trace; $CaCO_3$ 1.0 g and tap water 100 ml. Medium B was made up as follows: NaCl 0.3 g; $MgS0_4.7H_20$ 0.14 g; $FeS0_4.7H_20$ 0.03 g AND $(NH_4)_2SO_4$ 0.66 g. They are dissolved in 90 ml distilled water. The 'A-2' trace element mixture contains $LiSO_4$ 0.01 g; $CuSO_4$ 0.02 g; $ZnSO_4$ 0.02 g; $H_3BO_4$ 0.22 g; $Al_2(SO_4)$ 0.02 g; $SnCl_2$ 0.01 g; $MnCl_2$ 0.14 g; $NiCl_2$ 0.02 g; $CoSO_4$ 0.02 g; $TiCl_4$ (15% solution) 0.13 ml; Kl 0.01 g; KBr 0.01 g and distilled water, 360 ml. As a result the air was purified from $N_2$ and $O_2$ and contained %99.9 $CO_2$ with the exception of some nox gases.

*2.4 The Identification of S.elongatus PCC 7942*

In order to identify the *S.elongatus* cynobacteria isolated from the atmoshere its dimensions were measured under microscope. Then it's growth in urea, ammonia, nitrite and nitrat media tests were performed. Bradford test was performed to determine the protein content.

*2.5 Measurement of 1-butanol*

1-butanol was measured in an Agilent GC-MS 7890-A system with flame ionization detector and DB-FFAP capillary column. 1-Butanol standard of 0.001% v/v was used and dilutions were performed. Helium gas was used as the carrier gas. The injector and detector temperatures were maintained at 250 °C. Injection volume was 1 µL. The GC oven temperature was initially held at 40 °C for 4 min and then raised to 250 °C. Column flow rate was 3 ml/min. 1-Butanol typically had a retention time of 2.483 min.

*2.6 $CO_2$ measurement in HPLC*

For $CO_2$ quantification; 1 ml of gas was drawn by 1MR-VLL-GT FL0D syringe. Helium gas was used as the carrier gas. The injector and detector temperatures were maintained at 40 °C. Injection volume was 1 µL. The GC oven temperature was initially held at 40 °C for 4 min then it increased to 250 °C [6].

*2.7 Enzyme Activity Measurenments*

3-hydroxybutyryl-CoA dehydrogenase (Hbd) activity measurement: Hbd activity was measured in a spectrophotometer AQUAMETC by monitoring the decrease in absorbance at 340 nm, which corresponded to consumption of NADH [7]. 3-hydroxybutyryl-CoA dehydrogenase (Hbd) measurement: Hbd activity was measured in a spectrophotometer AQUAMETC by monitoring the decrease in absorbance at 340 nm, which corresponded to consumption of NADH [7]. Bradford protein measurement: By taken into consideration the blue colour production with interaction of Coomasie brillant blue and proteins at a adsorbans of 595 nm as measured in an AQUAMATE (Helios Aquamate) spectrophotometer.

Dissolved oxygene was measured in a WTW dissolved oxygene meter. The ligth power was measured by a luminometer (OASIS). Additional pure $CO_2$ was added with an $CO_2$ tube with a purity of 99.9%.

*2.8 Plasmid addition*

2 µg pAM2991 plazmit-DNA was transferred to the *S. elongatus* grown in BG-11 media containing 3.4 X $10^2$ cell /ml in 2 days incubations in dark. pAM2991 plasmid is consisting from neutral region – I (NSI), target vector and Ptrc initial primer. It contains the genes namely asetil-CoA asetiltransferaz (atoB) and aldehid/alkol dehidrojenaz (adhE2) for 1- butanol productions**.**

*2.9 Environmental Factors affecting the number of cyanobacteria, produced 1-butanol concentration and 1-butanol$_{produced}$/$CO_{2utilized}$ yield*

The effect of excess $CO_2$ concentration on 1- butanol yield was researched by passing of $CO_2$ gas with a purity of 99.99% at a flow rate of 0.013 $m^3$/ sec from a $CO_2$ tupe at concentration varying between 10, 20 and 30 mg/l and compared with the 1-butanol production from the trapped $CO_2$ concentration (9.8 mg/l). The effect of dissolved oxygene on the yield of 1-butanol production was investigated. The do concentration was increased from 0.2 mg/l from which is found in the trapped air after purification to 0.4, 0.5, 2 and 4 mg/l. Then, the effect of increasing $NO_3$-N concentrations (0.5 mg/l, 1, 1.5 and 2 mg/l) on the 1- butanol yields was investigated. Effects of increasing $SO_4^{-3}$ concentrations (0.5 mg/l,1, 1.5 and 3 mg/l) and the effects of increasing temperatures (15°C, 30, 40, 50 and 65°C) on 1- butanol productions were studied. Furthermore, the effects of Ph (4, 7, 8 and 9) and retention times ( 25 days, 40, 55 and 75 days) on 1-butanol yields was studied.

The effect of addition of pAM2991 plasmid to each *S.elongatus* culture was evaluated. Plasmid pEL5 was constructed by insertion of atoB and adhE2 enzymes into pAM2991 between the BamHI and NotI sites, under an IPTG-inducible promoter Ptrc. *S. elongatus* strains. The genes were transformed by incubating cells at mid-log phase with 2 µg of plasmid DNA overnight in dark. The culture was then spread on BG-11 plates and 20 µg/ml spectinomycin was added into BG-11 agar plates.

*2.10 Calculation of 1- butanol production yields versus $CO_2$ utilized*

The calculation of the yield for 1-butanol concentration produced from utilized $CO_2$ were done via the formula given in (1), and the yield percentages were calculated via the formula given in (2). In the calculations, the theoretical value of 1-butanol $_{produced}$/$CO_{2used}$ is 0, 25.

$$\text{Yield (Y)} = \frac{\text{1-butanol produced}}{\text{CO 2utilized}} \quad (1)$$

$$\%\ \text{Yield (\% Y)} = \frac{\text{1-butanolproduced/CO2utilized}}{\text{Theoric 1-butanolproduced/CO2utilized}}\ \text{X100} \quad (2)$$

## 3. Results and Discussion

*3.1 Identification of S.elongatus PCC 7942 Cynobacteria*

The identification of *S.elongatus* cynobacteria was done at the end of 30 days (t = 30°C, pH = 7.1, Eh = 120 mV, Light conditions = 60 W, $CO_2$ flow rate = 0,083 $m^3$/sec). The dimensions of the *S.elongatus* cells differed from 1,5 up to 2,0 µm of length with a width of 0,4-6 µm as is reported in literature studies. In the ammonia test the re-production of blurry exhibited that ammonia was used by *S. elongatus* at 28°C which is this a positive result given in the literature (Table 1). In the nitrat test *S.elongatus* produced blur by using nitrat reductase enzymes nitrat medium. For nitrit test *S. elongatus* used the reductase enzyme and utilized the nitrit and converted to nitrate. On the other hand, *S. elongatus* use urea and turns them into ammonia and caused blur in tubes (Table 1). Bradford test was done at the end of 30 days in order to determine the protein amount of *S. elongatus* (Table 1).

**Table 1:** Identification of *S.elongatus* cynobacteria

| Studies | Cyanobacteria Sizes | | Number of Cyanobacteria (colony/ml) | Ammonia Test | Nitrate Test | Nitrite Test | Urea Test | Bradford Test Protein Concen. (µg/ml) | $CO_2$ concen. (mg/l) |
|---|---|---|---|---|---|---|---|---|---|
| | lengths (µm) | widths (µm) | | | | | | | |
| Aliağa | 3 - 18 | 0.6 – 5 | 10000 | Blured - Positive (+) | Blured – Positive (+) | Blured - Positive (+) | Blured - Positive (+) | 78 | 178 |
| Aliağa | 2 - 17 | 0.5 – 5 | 9800 | Blured - Positive (+) | Blured – Positive (+) | Blured - Positive (+) | Blured - Positive (+) | 75 | 177 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Urla | 1 - 16 | 0.4 – 4 | 7000 | Blured - Positive (+) | Blured – Positive (+) | Blured - Positive (+) | Blured - Positive (+) | 57 | 118 |
| Urla | 1 - 15 | 0.4 – 3.8 | 6900 | Blured - Positive (+) | Blured – Positive (+) | Blured - Positive (+) | Blured - Positive (+) | 44 | 117 |

According to the results obtained from the biochemical tests all isolated Cyanobacteria in Aliağa and Urla are *S. elongatus. The* maximum *S. elongatus* cells reached in the Aliağa region which in this region the Measured $CO_2$ concentration are at the higher level. The *S. elongatus* numbers reduced in Urla Region are lower than that Aliağa Region since in this region the $CO_2$ concentrations were at the lowest level. Therefore, from the low $CO_2$ concentration (mole) the produced of 1mol 1-butanol will be lower. In other words, from the $CO_2$ the proced butanol yields will be high. On the other hand the maximum protein concencentration was observed at the *S. elongatus* cells isolated from Aliağa Region.

*3.2 Purification of trapped air from $O_2$ and $N_2$ gases*

The air with a flow rate of 0.083 $m^3$/sec was passed from an adsorbent containing *N. europaea* for 5 minutes in every half an hour time for 7 days tances of. The number of *N.europaea* was $2x10^2$ /ml and the flow rate of $O_2$ gas in the air was 0.055 $m^3$/sec (Table 2). The $N_2$ gas in the rest of the air was removed by passing the remaining air from an adsorbant containing *Azotobacter sp.* with a flow rate of 0.015 $m^3$/sec containing $1x10^2$/ml *A. vinelandii*. The rest of the air was used by *S.elongatus* PCC 7942 in production of 1-butanol which contains pure $CO_2$ at a flow rate of 0.013 $m^3$/ sec (Table 2).

**Table 2:** Result of purification process that captured in the $CO_2$-rich air in the polluted area

| | **Culture Properties** | | **Cultural Properties of organisms growing in adsorbant pads with the gases trapping from the atmosphere** | |
|---|---|---|---|---|
| | ***N. europaea*** | ***A. vinelandii*** | ***N. europaea*** | ***A. vinelandii*** |
| **Number of *N. europaea* (number/ml)** | $5x10^5$ | - | $2x10^3$ | - |
| **Number of *A. vinelandii* (number/ml)** | - | $3x10^4$ | - | $1x10^3$ |
| **Flow of dissolved $O_2$ ($m^3$/sec)** | 0.04 | - | 0.055 | - |
| **Flow of $N_2$ ($m^3$/sec)** | - | 0.038 | - | 0.015 |

*3.3.$CO_2$ utilization By S. elongatus*

Water, N, P and $CO_2$ play an important role in the growing of photosynthetic cynobacteria [4]. Murray et al., (1982) [8] determined that cynobacteria grow faster in high $CO_2$ concentration while Fu et al. (2007) [9] determined that the more $CO_2$ concentration increase the photoshynthesis in cyanobacteria. In this study it was determined that *S. elongatus* remove more $CO_2$ in Aliağa industry district where the $CO_2$ concetrations are high in this polluted area (Table 3). The $CO_2$ concentrations are low in Urla region since this domain is a seaside in which the tourism activities are high. Therefore the $CO_2$ levels are low and the utilized $CO_2$ is low in Urla which not a polluted area. To produce 1 mol 1-butanol with Calvin-Benson-Bassham cycle, 6 mol $CO_2$ is required. This amount of $CO_2$ is calculated with the numbers of glyceraldehyde-3-phosphate (G3P) and NAD(P)H which are necessary for 1-butanol production. 1 mol G3P availability from 3 mol $CO_2$ is required for 6 mol NADPH. Consequently, 2 mol G3P is required to produce 1 mol 1-butanol. Thus, 6 mol $CO_2$ is required to produce 1 mol of 1-butanol [10]. Cyanobacteria fix carbon to provide the skeletons needed to assimilate N into amino acids and build protein and cellular biomass; fixed carbon can also be used to accumulate carbohydrate storage products (carbohydrate ballasting) in order to make the cell heavier during buoyancy regulation.

**Table 3:** $CO_2$ utilization by *S. elongatus*

| Studies | The initial concentration of $CO_2$ (mg/L) | After 30 days concentration of $CO_2$ (mg/L) | Utilized concentration of $CO_2$ (mg/L) |
|---|---|---|---|
| Aliağa | 178.7 | 25.7 | 153 |
| Aliağa | 178.2 | 38.2 | 140 |
| Urla | 118.5 | 30.5 | 80 |
| Urla | 118.12 | 20.82 | 83.3 |

*3.4. Effects of environmental factors on the 1-Butanol production from $CO_2$*

*3.4. 1. Effect of excess $CO_2$ concentrations on 1- butanol production*

The $CO_2$ concentrations in Aliağa and Urla regions were measured around 178 and 118 mg/l, respectively. As the the $CO_2$ concentrations were increased from 178 mg/L to 188 and to 198 mg/l by addition of pure $CO_2$ gas the 1-butanol productions increased from 153 mg/l to 176 and to 185 mg/l in Aliaga region. As the $CO_2$ concentration were increased from 118 mg/l to 128 and to 138 mg/l by the additions of 10 and 20 mg/l pure $CO_2$ gas. Further increase of pure $CO_2$ concentration to 30 mg/l the 1- butanol production decreased significantly in both regions. This showed that an optimum $CO_2$ level can stimulate the 1- butanol production via photoshynthesis. *S. elongatus* use sunlight as an energy source, and recycle $CO_2$, making them an advantageous platform for the production of 1-butanol. *Synechococcus* is a genus of unicellular marine cyanobacteria that are found in both freshwater and marine water environments. *Synechococcus* exhibits several pathways for carbon metabolism. Its main pathway for carbon metabolism under photoautotrophic conditions involves glycolysis, pentose phosphate shunt, incomplete TCA cycle, and photophosphorylation [11]. Under mixotrophic conditions involving either glucose or acetate, these compounds are incorporated as organic carbon compounds. Yan et al., [12] showed in 2010 that glucose is metabolized through the main pathway and the $CO_2$ released through respiration and fixed by photosynthesis (Calvin cycle). ATP is produced via three metabolic pathways: substrate phosphorylation, oxidative phosphorylation, and photophosphorylation. Substrate phosphorylation is the production of ATP by the donation of a phosphoryl group ($PO_3$) from the metabolites in all reaction pathways. In oxidative phosphorylation, which is carried out using an electron transport chain, ATP is generated using either NADH or FADH with low $O_2$ as the terminal electron acceptor. Photophosphorylation uses light energy to convert ADP to ATP through photosynthetic reactions.

**Table 4:** Effect of excess $CO_2$ concentrations on 1- butanol production

| Region | $CO_2$ mg/L (178 Aliağa and 118 Urla) | $CO_2$ addition- 10 mg/L | $CO_2$ addition- 20 mg/L | $CO_2$ addition- 30 mg/L |
|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 176 | 185 | 120 |
| Aliağa1-BT (mg/l) | 140 | 159 | 165 | 100 |
| Urla 1-BT (mg/l) | 124.38 | 132 | 167 | 119 |
| Urla1-BT (mg/l) | 103.3 | 121 | 142 | 90 |

*3.4.2. Effect of $NO_3$-N concentrations 1-butanol productions*

The trapped air after purification has no $NO_3$-N concentration. As the $NO_3$-N concentration was incresed from 1 to 2 mg/l the 1- butanol production reached to a maximum (178 and 140 mg/l, respectively in the *S. elongatus* isolated from Aliaga and in Urla regions) in both domains (Table 5).

**Table 5:** Effect of $NO_3$-N concentrations 1-butanol productions

| District | $NO_3$-N conc. 0 mg/l | $NO_3$-N conc. 1 mg/l | $NO_3$-N conc. 2 mg/l | $NO_3$-N conc. 4 mg/l | $NO_3$-N conc. 5 mg/l |
|---|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 167 | 178 | 110 | 90 |
| Aliağa1-BT (mg/l) | 140 | 155 | 167 | 100 | 89 |
| Urla 1-BT (mg/l) | 110.5 | 132 | 140 | 90 | 70 |
| Urla1-BT (mg/l) | 101.3 | 121 | 142 | 90 | 68 |

Further increase of $NO_3$-N concentration decrease significantly the 1- butanol production**.** As $NO_3$-N is also a charged molecule it's transported into the cell via active transport. Cyanobacteria use two different transport systems. Most freshwater species, including *Anabaena*, *Synechocystis* and *Gloebacter*, use the high affinity ATP-binding cassette (ABC) transporter NrtABCD [13]. Most marine species (*Synechococcus* and others) take up $NO_3$ - and $NO_2$- via the major facilitator superfamily transporter NrtP, also a high-affinity transporter [13]. Some species also have a $NO_{2-}$ specific transporter NIT [14]. Nitrate uptake is tightly regulated by the external concentration of $NH_4^+$; when $NH_4^+$ becomes available, cells cease $NO_3$ - uptake and switch to use $NH_4^+$ which is preferred. This process is regulated at the level of $NO_3$- uptake [15]. In addition, $CO_2$-fixation (regulated by irradiance) is required to maintain active $NO_3$ - uptake, a regulatory link that ensures that the product of $NO_3$ - reduction (ammonium) can be incorporated into carbon skeletons [13]. Reduction of $NO_3$ - to $NH_4^+$ is a two-step process catalyzed by the enzymes nitrate reductase (NR) and nitrite reductase (NiR). The power for the reduction reaction, in the form of 2 electrons for NR and 6 electrons for NiR, is provided by Fdred via PSI providing a strong link between the light reactions and $NO_3$- use by the cell [13]. In cyanobacteria, the genes encoding NR, narB, and Nir, nirA, and the $NO_3$ - transporter NrtP, are typically clustered in the same operon. An operon is a unit that tells the cells to transcribe a sequence of genes simultaneously. In cyanobacteria, the transcription of operons associated with metabolism is tightly regulated by the transcription factor NtcA.

*3.4.3. Effect of Dissolved Oxygen on 1-butanol production*

The dissolved oxygen (DO) concentration was measured around 0.01 and 0.03 mg/l in trapped air after purificant in both Urla and Aliağa regions. The addition of do effect on 1-butanol production was investigated in both regions. Increasing of DO concentration to 0.4 and 0.5 mg/l affected slightly the 1- butanol productions in both regions (Table 6). Increasing of do concentrations to 2 and to 4 mg/l sligthly decreased the 1-butanol productions. The optimum do for maximum 1-butanol production was found to be 0.5 mg/l. Samuel et al., (2014) [16] found that **w**ith the increase of dissolved $O_2$ concentration from 0.01 mg/l to 0.05 mg/l, the number of cyanobacteria, produced 1- butanol concentrations and 1-butanol $_{produced}/CO_{2used}$ yields increased. On the conducted studies, the number of cyanobacteria*s* grown under the anaerobic strict conditions decreased compared to low do (0.05 mg/l) concentrations. As *S. elongatus* are naturally adapted to the low concentrations of atmospheric oxygen [17]; however, in the photosystem II, the lack of oxygen blocks plastoquinone that is an electron carrier [2]. In addition to this, high $O_2$ and $CO_2$ levels can inhibit their growth.

**Table 6:** Effect of Dissolved Oxygen on 1-butanol production

| Region | 0.01 -0.03 mg/l in trapped air | 0.4 mg/l | 0.5 mg/l | 2 mg/l | 4 mg/l |
|---|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 156 | 163 | 145 | 120 |
| Aliağa1-BT (mg/l) | 140 | 145 | 153 | 145 | 120 |
| Urla 1-BT (mg/l) | 124.38 | 126 | 138 | 115 | 118 |
| Urla1-BT (mg/l) | 103.3 | 107 | 115 | 98 | 98 |

*3.4.4. Effect of $SO_4^{-2}$ concentration on 1- butanol production*

The trapped and purified air did not contain $SO_4^{-2}$. Addition of $SO_4^{-2}$ concentratio from 0.5 to 1. 00 mg/l increased the 1- butanol production. 3 mg/l $SO_4^{-2}$ decrease the 1- butanol production (Table 7). The optimum $SO_4^{-2}$ concentration for maximum 1- butanol production was found to be 1 mg/l. Yang et al. (2015) [18] found 40% more growth with the addition of 0, 0361 g/L $MgSO_4$ to the BG-11 media and mentioned that $SO_4^{-2}$ is a basic component necessary for the growth of *S. elongatus.* However, they emphasized that increasing of $SO_4$ concentrations did not increase the growth of *S. elongatus* PCC 7942 more than 20%.

**Table 7:** Effect of $SO_4^{-2}$ concentration on 1- butanol production

| | **0 mg/l** | **0.5 mg/l** | **1 mg/l** | **1.5 mg/l** | **3 mg/l** |
|---|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 158 | 164 | 163 | 120 |
| Aliağa1-BT (mg/l) | 140 | 146 | 158 | 157 | 130 |
| Urla 1-BT (mg/l) | 124.38 | 129 | 143 | 120 | 100 |
| Urla 1-BT (mg/l) | 104 | 110 | 143 | 100 | 98 |

*3.4.5. Effect of pH on 1- butanol production*

For maximum 1-butanol productions the optimum pH was found to be around 7.0. Acidic and alkaline conditions decrease the photosynthesis of $CO_2$ to 1- butanol in both *S. elongatus* types from Aliağa and Urla regions (Table 8). At basic pH the growth of cyanobacteria is limited and the produced 1-butanol concentrations and 1-butanol$_{produced}$/$CO_{2used}$ yields decreased. Rippka et al. (1979) [5] found that maximum growth in BG-11 medium for *S. elongatus* PCC 7942 was observed at a pH of 7.1.

**Table 8:** Effect of pH on 1- butanol production

| | **pH** | **pH** | **pH** | **pH** |
|---|---|---|---|---|
| | **pH= 7.1** | **pH=4.0** | **pH=9.0** | **pH=13** |
| Aliağa – 1-BT (mg/l) | 153 | 45 | 38 | 134 |
| Aliağa1-BT (mg/l) | 140 | 40 | 36 | 100 |
| Urla 1-BT (mg/l) | 124.38 | 34 | 26 | 98 |
| Urla1-BT (mg/l) | 103.3 | 29 | 20 | 78 |

*3.4.6. Effect of temperature on 1- butanol productions*

At low temperature the *S. elongatus* was not effectively grew and could not produce effectively 1- butanol since at low temperatures the matabolic activities decreased (Table 9). At high temperatures the protein content of S. elongatus and the enxymes exhibiting inhibitions resulting in decrease in enzyme activities (Table 9). The optimum teperature for maximum 1-butanol production was found to be 40°C. Water temperatures have increased globally over the last few decades as a result of global warming [19].This indicates that a doubling in cyanobacterial growth rates occurs with an increase in temperature from 20-27°C. From incresing S. elongatus cells more 1- butanol will be produced. Kajiwara et al., (1997) [20] found that the growth ratio of *S. elongatus* generally continues to increase with the increasing temperature until it is reached to an optimum temperature. The temperature highly affects its trapping of $CO_2$, holding the nutrients and cellular chemical components in *S. elongatus* [21]. For the maximum growth *S. elongatus* cyanobacteria, has been always cultivated at a stable temperature of 30°C.

**Table 9:** Effect of temperature on 1- butanol productions

| | 30 °C | 40 °C | 15 °C | 50°C |
|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 154 | 143 | 125 |
| Aliağa1-BT (mg/l) | 140 | 141 | 128 | 123 |
| Urla 1-BT (mg/l) | 124.38 | 126 | 118 | 114 |
| Urla1-BT (mg/l) | 103.3 | 105 | 98 | 76 |

*3.4.7. Effect of retention-incubation time on 1-butanol productions*

The optimum 1- butanol production was obtained at an incubation time of 30 days (Table 10). İncreasing of retention-incubation times of *S. elongatus* culture did not affect the 1-butanol production. The enzymes catalyzing the photoshtnthesis are active at 30°C. Further increasing of temperature did not activated the enzimes catalyzing the 1-butanol production from $CO_2$. By extending the retention period from 15 days to 30 days, an incensement of the number of *S. elongatus* PCC 7942, in 1- butanol concentrations and 1-butanol $_{produced}/CO_{2used}$ yields has been observed by Armbrust et al., (2014) [10]. With the retention period is extended to 45 days, the number of *S. elongatus* PCC 7942, 1- butanol concentration and 1-butanol $_{produced}/CO_{2used}$ yields decreased [10]. The first period for 15 days is the acclimation period of *S. elongatus* while after 45 days was going to the stationary phase. Because residence time is determined by the flushing rate, the direct effect of increased residence time is to decrease the loss rate of cyanobacteria [22]. Studies that report on the effect of residence time suggest that cyanobacterial abundance, cell size and toxin concentration are positively related to increased residence time [22, 23].

**Table 10:** Effect of retention time on 1- butanol productions

| | 30 days | 40 days | 55 days | 70 days |
|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 153 | 145 | 60 |
| Aliağa1-BT (mg/l) | 140 | 140 | 130 | 50 |
| Urla 1-BT (mg/l) | 124,38 | 124,38 | 119 | 45 |
| Urla1-BT (mg/l) | 103,3 | 103,3 | 96 | 30 |

*3.5. Enzymes catalyzing the 1-butanol production from $CO_2$*

The enzymes controling and governing the photosyntesis of $CO_2$ to produce 1- butanol are HdD, Ter, AdhE2, AtoB and Crt. Among these enzymes Ter and Crt were catalyzing crotonyl-CoA and 3-hydroxybutyryl-CoA, respectively and they are measures at low concentrations (Table 11).

**Table 11:** Enzym activities during 1 butanol production from $CO_2$

| **Enzymes** | ***S. elongatus (µg/l)*** |
|---|---|
| Hbd | $2 \times 10^{-1}$ |
| Ter | $3.2 \times 10^{-1}$ |
| AdhE2 | $2.3 \times 10^{-1}$ |
| Atob | 0.7 X10 -1 |
| Crt | 2.8 x 10-1 |

*3.6. Yield Measurements (1-butanol$_{productions}$ / $CO_{2utilized}$)*

The maximum yield obtained in this study was 96% and 95% in the *S. elongatus* isolated from the Aliağa Region (Table 11). Lan and Liao (2012) [24] and Shen et al., (2011) [25] found 68-70 % 1-butanol yields by producing 30-42 mg/L 1-butanol from $CO_2$ using *S. elongatus* PCC 7942 cyanobacteria. In this study, the produced 1- butanol concentrations were around 120-135 in the S. elongates cyanobacteria isolated from Aliağa region. Among the studies carried out, the lowest 1- butanol yields was obtained in the *S. elongatus species* isolated from Urla district 1-butanol $_{produced}/CO_{2used.}$

**Table 11:** 1-butanol yields from utilized $CO_2$

| Studies | Utilized concentration of $CO_2$ (mg/L) | Concentration of 1-butanol (mg/L) | Yield | % Yield |
|---|---|---|---|---|
| Aliağa | 153 | 133.5 | 0.167 | 96.6 |
| Aliağa | 140 | 122.83 | 0.14 | 95 |
| Urla | 124.38 | 80.73 | 0.11 | 60 |
| Urla | 103.3 | 81.5 | 0.09 | 59 |

*3.7. Effect of plasmid addition on 1-butanol production*

Plasmid transformation; has been done by carrying the atoB and adhE2 genes in pEL5 plasmid to the genes of *S. elongatus* PCC 7942 via using the recombinant DNA techniques. The pEL5 plasmid DNA has been done with the recombination of *S. elongatus* PCC 7942's neutral zone. Plasmid pEL5 consists of the genes a toB and adhE2. These plasmids have been used because they include atoB and adhE2 which is necessary to produce 1-butanol from $CO_2$ via *S. elongatus* PCC 7942. The genes atoB and adhE2 in plasmid pEL5 have been transferred into *S. elongatus* PCC 7942 DNA via BamH1 and Notl. areas in pAM2991. pEL5 genotype is an adhE2 that is resistant to spectinomycin ($Spec^R$), intended for NSI, Ptrc: atoB [2]. After pAM2991 plasmid was transferred to S. elongatus a recombination was recorded. PCR photos for adhE2 (a) and atoB (b) gens primers were given in Figure 2 (a) exhibited the acetyl-CoA acetyltransferase (atoB) while (b) showed the aldehyd/alchol dehydrogenaz (adhE2).

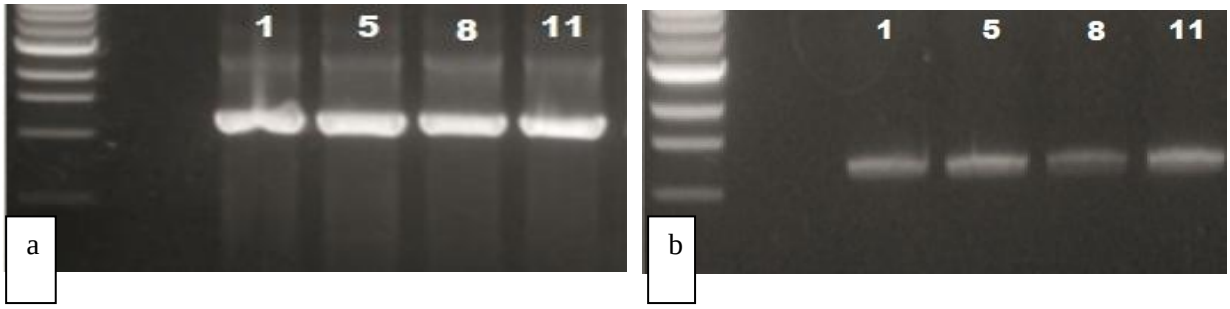


*Figure 2: PCR photos AdhE2 (a) and atoB (b) genes primers: (a) acetyl-CoA acetyltranferase (atoB), (b) akdehyde/alchol dehydrogenaz (adhE2).*

As shown in Table 12, the addition of plasmid to the *S. elongatus* increased significantly 1- butanol production, the number of *S. elongatus*, 1-butanol yields and the atob and AdHE2 enzymes activities compared to *S. elongatus* without plasmid**.**

**Table 12:** Effect of plasmid addition on 1- butanol production

| | Without plasmid | With plasm | *S. elongatus* number/ml with plasm | 1-butan. yield (%) with plasm | atoB (µg/l) with plasm | adhE2 (µg/l) with plasm |
|---|---|---|---|---|---|---|
| Aliağa – 1-BT (mg/l) | 153 | 189 | 15000 | 93 | $12x\ 10^{-1}$ | $9x10^{-1}$ |
| Aliağa1-BT (mg/l) | 140 | 162 | 14000 | 90 | $12x10^{-1}$ | $8x10^{-1}$ |
| Urla 1-BT (mg/l) | 124.38 | 134 | 12000 | 84 | $9x10^{-1}$ | $6x10^{-1}$ |

**4. Cost analysis**

The isolation of *S. elongatus*, labour, chemical costs; purıfication and electricity costs were recorded as 1.06 Euro to produce 1 liter 1- butanol from 180 mg/l $CO_2$ trapping from the Tablo 13. In order to produce 10.000 m3 1- butanol from 1000 g/l $CO_2$, the cost was calculated as 0.13 Euro. With the addition of plasmid the total cost to produce 10.000 $m^3$ 1-butanol is 0.68 Euro. The produced 1 liter of 1-butanol cost was compared with the gasoline. The produced 1-butanol cost is approximately 3 times lower than 1 liter conventional gasoline.

**Table 13:** Cost Analysis for 1- butanol production from $CO_2$

| | |
|---|---|
| İsolation cost for *S. elongatus* | 0.10 euro |
| Labour cost | 0.06 euro |
| Electricity | 60 W light power: 0.2 euro<br>Illumination for 35 days |
| $CO_2$ purification | 0.3 euro |
| Total cost without plasmid | Total cost : 0.66 Euro/L |
| Addition of plasmid (0.4 euro) | Total cost: 1.06 euro /L |
| Total cost without plasmid | 0.13 EURO / 10.000 $m^3$ 1- butanol |
| Addition of plasmid (0.4 euro) | Total cost: 0.68 euro/10.000 $m^3$ 1-butanol |

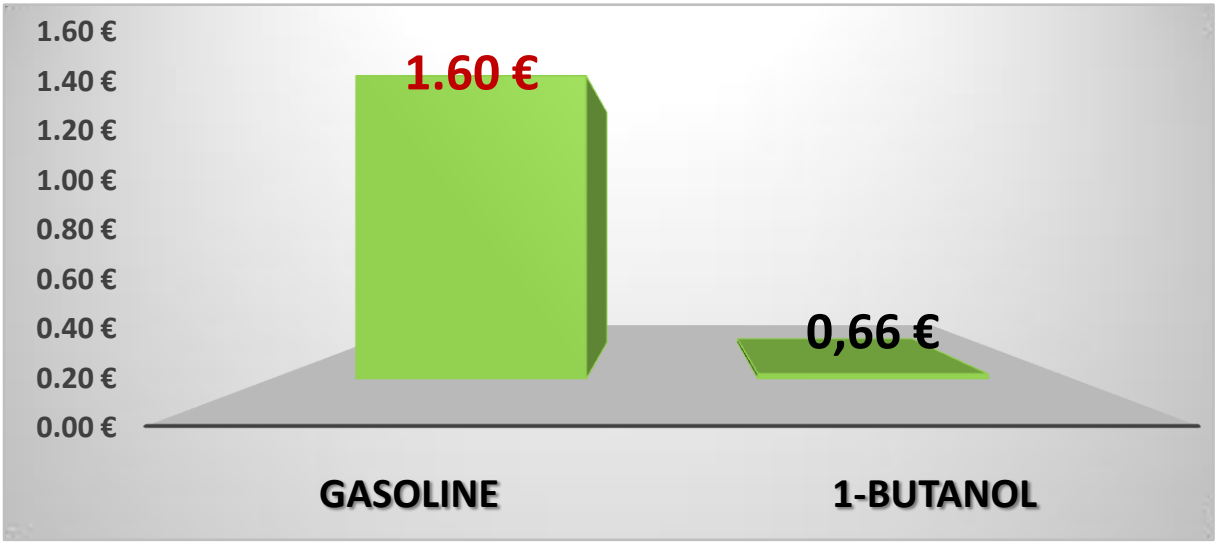


*Figure 3: Comparison of 1- butanol and gasoline costs*

**5. Discussion**

Rising global energy demands and pressing environtal issues, interest is growing in the production of fuels icals from renewable resources. Petroleum consumption reached 42.1 quadrillion BTU in the United States in 2015, of which a large majority (71%) was liquid fuel in the transportation sector. Petroleum and natural gas account for 99% of the feed-stocks for chemicals, such as plastics, fertilizers, and pharmaceuticals in the chemical industry. Considering rapidly increasing world population and exhaustion of fossil fuels, the development of sustainable processes for energy and carbon capture to produce fuels and chemicals is crucial for human society. Energy and carbon capture by cyanobacteria is also directed toward mitigating increasing atmospheric $CO_2$ concentrations. According to the US Energy Information Administration, world energy-related $CO_2$ emissions in 2006 were 29 billion metric tons, which is an increase of 35% from 1990. Accelerating accumulation of atmospheric $CO_2$ is not only a result of increased emissions from world growth and intensifying carbon use, but also from a possible attenuation in the efficiency of the world's natural carbon sinks. As a result, atmospheric levels of $CO_2$ have inreased by ~25% over the past 150 years and it has become increasingly important to develop new technologies to

reduce $CO_2$ emissions. Many creative solutions have been proposed and argued for carbon capture, each with varied environmental side-effects and costs. Sequestration by photosynthetic microorganisms in which $CO_2$ is biologically converted to valuable chemicals is an important addition to the toolbox for overall capture of $CO_2$. Photosynthetic microorganisms, including cyanobacteria, are currently being engineered for platforms to convert solar energy to biochemicals renewably. These microorganisms possess many advantages over traditional terrestrial plants with regard to biochemical production. For example, the photosynthetic efficiency of photosynthetic microorganisms is higher than plants, and photosynthetic microorganisms can be cultivated in locations that do not compete with traditional agricultural crops. Cyanobacteria are collectively responsible for almost 50% of global photosynthesis and are found in a wide range of environments. Cyanobacteria have the biochemical machinery required to fix $CO_2$, but lack the critical components to generate fuels and chemicals efficiently. An increased understanding of cellular systems enables us to construct novel systems using synthetic biology, assembling the components and control systems into new combinations.

At present, the cost of of developed biofuel, is still several times lower than that fossil fuels. Future developments in strain selection and engineering, bioreactor design and processing technology then may pave the way for the production of fuels, using the direct conversion approach that can economically compete with their fossil counterparts.

## 6. Conclusion

The eco-friendly 1-butanol; is an economic alternative fuel, It can be produced by the waste $CO_2$ from the atmosphere which cause pollution, it is suitable to use as heat, force and alternative motor fuel. From a pollutant a renewable energy (biofuel) was produced.

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