

Effect of Plasmid Addition on the Production of 1-Butanol from CO₂ in Isolated *S.Elongatus* from the Sea Sediment of Konak - Izmir

*SPONZA D.T.¹, DOGAN C.², GUNEY G³

^{1,3} Dokuz Eylul University Engineering Faculty Environmental Engineering Department Tinaztepe Campus
Buca, Izmir, TURKEY

² Ege University Graduate School of Natural and Applied Science Fundamental and Industrial Microbiology
Department Bornova, Izmir, TURKEY

*corresponding author: SPONZA D.T

e-mails : delya.sponza@deu.edu.tr, cansu_dogan33@hotmail.com; gokce.pehliyaner@deu.edu.tr

Abstract: For a sustainable future it is essential to close the global carbon cycle. Oxidised forms of carbon, in particular CO₂, can be used to synthesise energy-rich organic molecules. Engineered cyanobacteria have attracted attention as catalysts for the direct conversion of CO₂ into reduced fuel compounds. 1-Butanol is a promising gasoline replacement compared to the more commonly used ethanol due to several advantages. Specifically, 1-butanol is less corrosive and has a higher energy density than ethanol. In this study, *Synechococcus elongatus* was isolated from the sea sediment in Konak and the effects of CO₂ isolated from the same region on the production of 1-butanol was investigated. Addition of plasmid slightly increased the CO₂ utilisation rates. The effects of environmental factors (NO₃-N, SO₄-S, NaCl, H₂, O₂) on the ratio of 1-butanol production to CO₂ removal were investigated. Under optimized conditions *S.elongatus* metabolized the CO₂ according to Monod kinetic (K_s = 1.5 mg L⁻¹ and μ = 0.21 day⁻¹). Under high NO₃-N, SO₄-S, NaCl, and O₂ concentrations 1-butanol produced with un-competitive inhibition with a high K_i value of 2.3 mg L⁻¹ indicating the low inhibition in *S.elongatus* with plasmid. The competitive inhibition constant (K_i) is low (0.70 mg L⁻¹) exhibiting the high competitive inhibition at high concentration for the operational conditions given above

Keywords: 1-butanol, carbondioxide, cyanobacteria, Monod, plasmid.

I. Introduction

Cyanobacteria have great potential as a platform for biofuel production because of their fast growth, ability to fix carbon dioxide gas, and their genetic tractability. The Cyanobacteria allows for the use of CO₂ at higher concentrations than that of ambient air (Lan and Liao, 2011) and could potentially allow for the use of concentrated CO₂ emissions from waste industrial sources. 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 CO₂ emissions (Kruse and Lindblad, 2012). Several alternatives have been proposed to develop a sustainable industrial society and reduce greenhouse emissions. The idea of biological conversion of CO₂ to fuel and chemicals is receiving increased attention. In particular, the direct conversion of CO₂ with solar energy to biofuel by photosynthetic microorganisms such as microalgae and cyanobacteria has several advantages compared to traditional biofuel production from plants (Lan and Liao, 2014). The direct conversion of solar

energy into liquid fuel using photosynthetic microorganisms is an attractive alternative to fossil fuels. There are several advantages to using organisms such as microalgae and cyanobacteria: their readily available genetic tools and sequenced genomes; their higher growth rate compared to plants; and their ability to thrive in areas that cannot support agriculture. Utilization of these organisms can provide a way of resolving the potential conflict between the use of land for food or for biofuel production (Oliver et al., 2011). Compared to ethanol, isobutanol is a better candidate for gasoline replacement due to its low hygroscopicity, higher energy density and high compatibility with current infrastructure. Recently it has been reported that production of 1-butanol in cyanobacteria from CO₂ was achieved by transferring a modified CoA-dependent pathway into the autotrophic organism (Kusakabe et al., 2003). Since this CoA-dependent pathway was derived from strict anaerobic bacteria, it was a challenging task to express such a pathway in cyanobacteria, which produce oxygen during photosynthesis. To synthesize 1-butanol, 5 genes were integrated into the *S.elongatus* PCC7942 genome: *hbd*, *crt*, and *adhE2* (Lan and Liao, 2011). Butanol can be used directly in any gasoline engine

without modification and/or substitution, because it has sufficiently similar characteristics to gasoline as a liquid fuel. For example, in 2005, a gasoline car with unmodified engine was fueled with 100% butanol and successfully ran almost 10,000 miles across the USA (Lan and Liao, 2014). This result clearly demonstrates that biobutanol is one of the most powerful alternative fuel. The genetic tractability of cyanobacteria makes them attractive targets for use in metabolic engineering for renewable technology (Oliver et al., 2011). *Synechococcus elongatus* sp. PCC 7942 (*S.elongatus* 7942) has a number of unique sigma factors involved in regulation in response to light and circadian rhythm (Kusakabe et al., 2003). In this study, 1-butanol was produced from the CO₂ trapped from the Konak region in İzmir metropolitan air (Turkey) using *S.elongatus* isolated from Konak sediment in İzmir bay. The air was trapped from Balçova and the CO₂ inside air was used to produce 1-butanol in İzmir Turkey via photosynthesis. The effects of increasing NO₃-N, SO₄-S, NaCl, H₂, O₂ concentrations on the 1-butanol levels and on the yield of 1-butanol production to CO₂ used were investigated. The effects of plasmid addition on the 1-butanol production yields were investigated while the effect of increasing NO₃-N, SO₄-S, NaCl, H₂, O₂ concentration on monod and inhibition kinetics were evaluated.

II. Materials and Methods

2.1. Analytical procedure

Cultures were grown in an orbital shaker at 120 rpm under a continuous fluorescent white light illumination of 34 W L⁻¹. The *S.elongatus* in 100 mL sediment samples were grown in 250 mL erlenmeyer flasks. Closed bottles contained 20 mL of liquid culture inoculated to a starting an MLVSS concentration of 50 mg L⁻¹ was monitored at after 30 h cultivation at 20 °C at a UV power of 5 W m⁻². *Synechocystiselongatus* plasmid PCC7942 was obtained from American Type Culture Collection (ATCC) and was transformed to *S.elongatus* by grown in BG11 medium. The morphology of *S.elongatus* species was observed using a fluorescence microscope (Olympus, Japan). 1-butanol and CO₂ concentrations were measured in an Agilent GC-MS. 3-hydroxybutyryl-CoA dehydrogenase (Hbd), Trans-2-enoyl-CoA reductase (Ter) and Aldehyde/alcohol dehydrogenase (AdhE2) enzyme activities were measured in a spectrophotometer. The air was trapped using air

bags (Figures 1 and 2). The air was purified from N₂ and O₂ gases by passing the air from the *N.europaea* culture produced on nutrient agar at a flow rate of 0.083 m³ min⁻¹. The O₂ from the collected air was removed by passing the rest of the air from the culture of *A.vinelandi*.



Figure 1. Air trapping device and empty nylon bags



Figure 2. Air purification device

III. Results and Discussion

3.1. Enumeration of *S.elongatus* isolated from Konak sediment and *S.elongatus* with PCC 7942 plasmid

S.elongatus cyanobacteria numbers isolated from Konak sediment were as high as *S.elongatus* containing PCC 7942 plasmid using the CO₂ trapped from the Konak-Izmir district air (Table 1).

Table 1. Enumeration of *S.elongatus* isolated from Konak sediment and *S.elongatus* containing PCC 7942 plasmid under laboratory conditions

Samples/Places	Cyanobacteria Sizes		Number of Cyanobacteria (colony mL ⁻¹)
	lengths (µm)	widths (µm)	
Konak sediment and air	3 - 15	0.4 – 6	11800 <i>S.elongatus</i>
Konak sediment and air	2 - 14	0.4 – 5	11300 <i>S.elongatus</i>
Konak sediment and air	2.2 - 12	0.4 – 5.24	10600 <i>S.elongatus</i>
Konak sediment and air	1.9 - 11	0.3 – 398	11400 <i>S.elongatus</i> with plasmid
Konak sediment and air	1.9 – 14	0.4 – 3.7	11600 <i>S.elongatus</i> with plasmid
Konak sediment and air	2.4 – 15	0.3 – 3.5	11500 <i>S.elongatus</i> with plasmid

3.2. 1- *butanol_{produced}/CO_{2utilized} yields for S.elongatus isolated from Konak sediment and S.elongatus with plasmid in Izmir metropolitan*

The maximum 1-butanol concentration produced from the polluted air containing 0.17 mg L⁻¹ O₂ is 99 mg L⁻¹ with an 1-butanol_{produced}/CO_{2utilized} yield of 88% at 21 °C temperature, using 34W L⁻¹ light intensity, at pH = 6.70 at 100 mV redox potential in the *S.elongatus* isolated from the Konak bay sediment. The O₂ and the CO₂ trapped from the Balçova region air (**Table 2**). The maximum 1-butanol concentration produced from the *S.elongatus* containing plasmid with an air containing 0.17 mg L⁻¹ O₂ is 90.0 mg L⁻¹ with an 1-butanol_{produced}/CO_{2utilized} yield of 89% at 21 °C temperature, at 34W L⁻¹ light intensity, at pH = 6.60 at 100 mV redox potential. It was found that no significant 1-butanol yields was obtained in both *S.elongatus*.

Table 2. 1-butanol_{produced}/CO_{2utilized} yields

Samples/places	1-butanol (mg L ⁻¹)	1-butanol _{produced} /CO _{2consumed}	Cyanobacteriatype
Konak sediment and air	98	0.89	<i>S.elongatus</i> with PCC7942
Konak sediment and air	98	0.87	<i>S.elongatus</i> with PCC7942
Konak sediment and air	98	0.90	<i>S.elongatus</i> with PCC7942
Konak sediment and air	97	0.83	<i>S.elongatus</i> isolated
Konak sediment and air	96	0.81	<i>S.elongatus</i> isolated
Konak sediment and air	97	0.82	<i>S.elongatus</i> isolated

3.3. Enzyme activities

Hbd and Ter enzyme activities are high at both *S.elongatus* species. The CO₂ from the Konak air in Izmir was trapped and purified from the N₂ and O₂. No significant differences in both *S.elongatus* types was obtained (**Table 3**). It was found that the Hbd enzyme activities were high then that Ter enzyme in both *S.elongatus*. The CO₂ trapped from the air was used as carbon source and it was converted to the 1- butanol via photosynthesis process. During metabolic pathway the production of Hbd and Ter enzymes catalyse the formation of 1-butanol from CO₂.

Table 3. Enzyme activity measurements in both *S.elongatus* species

Samples	Hbd ($\mu\text{g mL}^{-1}$)	Ter ($\mu\text{g mL}^{-1}$)	Cyanobacteria type
Konak sediment and air	0.099	0.0032	<i>S.elongatus</i> PCC7942
Konak sediment and air	0.098	0.0030	<i>S.elongatus</i> PCC7942
Konak sediment and air	0.096	0.0028	<i>S.elongatus</i> PCC7942
Konak sediment and air	0.089	0.0031	<i>S.elongatus</i> isolated
Konak sediment and air	0.086	0.0024	<i>S.elongatus</i> isolated
Konak sediment and air	0.0089	0.0026	<i>S.elongatus</i> isolated

3.4. Effects of different $\text{NO}_3\text{-N}$, $\text{SO}_4\text{-S}$, NaCl , H_2 and O_2 concentrations on 1-butanol productions

The 1-butanol productions were slightly higher in *S.elongatus* containing plasmid than that in *S.elongatus* isolated from Konak sediment in Izmir. The results showed that NaCl , $\text{NO}_3\text{-N}$ and $\text{SO}_4\text{-S}$ affect negatively the 1-butanol production at high concentrations (4 and 5 mg L^{-1}) in *S.elongatus* without plasmid (Table 4). Salt contained both osmotic pressure and ionic stress. NaCl cause fouling in the membrane water channels and the cells were disrupted with osmotic pressure and the electron transporting system inactivated causing decrease in photosynthesis of CO_2 to 1-butanol. High $\text{NO}_3\text{-N}$ and $\text{SO}_4\text{-S}$ concentrations decrease the photosynthesis and decrease the 1-butanol production from CO_2 . The *S.elongatus* containing plasmid is more resistant to high NaCl , $\text{NO}_3\text{-N}$ and $\text{SO}_4\text{-S}$ concentrations. The maximum specific growth rate (μ_{max}) of *S.elongatus* with plasmid is high compared to *S.elongatus* isolated one. The *S.elongatus* with plasmid did not exhibited death at high NaCl , $\text{NO}_3\text{-N}$ and $\text{SO}_4\text{-S}$ concentrations.

Table 4. Effect of some environmental conditions on 1-butanol productions and Monod kinetic

Samples	1-butanol product ion (mg)	$\text{NO}_3\text{-N}$ (mg L^{-1})	$\text{SO}_4\text{-S}$ (mg L^{-1})	K_i (mg L^{-1})	NaCl (mg L^{-1})	H_2 (mg L^{-1})	O_2 (mg L^{-1})	μ_{max} (h^{-1})	X_{max} (mg L^{-1})
Konak	87	1	1	-	1	1	1	0.03	100
Konak	87	2	2	-	2	2	2	0.04	100
Konak	70	3	3	2.1	3	3	3	0.02	50
Konak	60	4	4	1.2	4	4	4	0.02	50
Konak	56	5	5	0.7	5	5	5	0.01	30
With	89	1	1	-	1	1	1	0.04	100
With	89	2	2	-	2	2	2	0.04	100
With	89	3	3	-	3	3	3	0.04	100
With	88	4	4	-	4	4	4	0.04	100
With	80	5	5	2.3	5	5	5	0.03	80

The inhibition level is low in *S.elongatus* with plasmid indicating a high inhibition constant ($K_i = 2.3 \text{ mg L}^{-1}$) at high inorganic concentrations. The K_i is high in *S.elongatus* isolated from Konak sediment with high competitive inhibition.

3.5. Cost analysis

The total cost for 1-butanol production from 1 m^3 CO_2 using *S.elongatus* isolated from the Balcova sea is only 0.90 Euro while the total cost is 1.00 Euro for *S.elongatus* PCC 7942 (Table 5).

Table 5. Cost analysis for 1-butanol production in both *S.elongatus* strains

	1-butanol production
Isolation cost for <i>S.elongatus</i>	0.20 Euro
Purchasing of <i>S.elongatus</i> PCC 7942	0.30 Euro
BG – 11 media	1 liter: 0.20 Euro
Electricity	60 W light power: 0.50 Euro Illumination for 35 days
CO ₂ purification	0.00 Euro
1 liter 1-butanol cost in <i>S.elongatus</i> isolated from Konak sediment	Total cost: 0.90 Euro L ⁻¹
1 liter 1-butanol cost in <i>S.elongatus</i> with PCC 7942 plasmid	Total cost: 1.00 Euro L ⁻¹

IV. Conclusions

In this study it was found that 1-butanol as an alternative of gasoline can be effectively produced from the CO₂ present in air of Konak located in Izmir Turkey using *S.elongatus* isolated from Izmir Bay sediment and with *S.elongatus* with PCC 7942 plasmid. Throughout the *S.elongatus* containing plasmid exhibited slightly higher 1-butanol productions is more resistant to high NO₃-N, SO₄-S

and NaCl concentrations than *S.elongatus* without plasmid. The cost of the 1-butanol is cheaper than the 1 liter of commercial gasoline and the CO₂ emissions in Izmir metropolitan can be reduced in this way.

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