The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2020

Volume 11, Pages 174-179

IConTES 2020: International Conference on Technology, Engineering and Science

Optimization of Syngas Feed for Improved Bioethanol Production with Clostridium Ragsdalei

Mine GUNGORMUSLER
Izmir University of Economics

Tugba KESKIN GUNDOGDU
Izmir Democracy University

Abstract: In recent years air pollution has been seriously affecting human health. One of the main contributors to this problem is the formation of syngas from industrial processes. This gas consists of hazardous components including CO, CO_X, NO_X. The fermentation of these C1 gases to produce bioethanol is one of the novel solutions towards a cleaner environment. Considering, the foreseen exhaustion of fossil fuels in 50 years, the production of bioethanol appears as a valuable solution towards this emerging need for alternative energy sources. In this context, in the present study, *Clostridium ragsdalei* was used to evaluate the effects of incrementing volumes (5, 10, 15, 20, 25 mL) of syngas feed on growth and ethanol production by using two different media namely basal ATCC media and Differential Reinforced Clostridial Media (DRCM). The highest yield achieved with 20 mL of syngas was 600 mg/L with the commonly used ATCC media. On the other hand, while this media resulted in higher ethanol yields, the utilization of its counterpart media (DRCM) gave interesting results with the production of acetate reaching almost 3000 mg/L. These results demonstrated the effectiveness of ATCC media with the optimized volume of syngas feed to produce bioethanol.

Keywords: Bioethanol, Syngas, C1 gases, *Clostridium ragsdalei*, Acetate

Introduction

Energy demand all over the world has been increasing because of the rising population and industrialization. Accordingly, environmental pollution and the risks of human health are increasing (Miranda et al. 2020). Fossil fuels are still the main source of energy demand however they are depleting in 50 years. The use of fossil fuel results in release of toxic gases into the atmosphere, thus create air pollution. These problems have directed the researchers to discover alternative, clean, non toxic energy sources or to find a way to use these pollutants as energy sources (Dürre & Eikmanns, 2015). Biofuels are the most popular energy sources to replace conventional resources. Biogas, biohydrogen and bioethanol are the most studied biofuels. Amongst these clean energy technologies, bioethanol has an advantage of ease of usage with mixing to gasoline as 10%to 85%. The use of bioethanol resulted in reducing the air pollution by 5-10% in Brazil, Sweden and Canada (Liberato et al. 2019).

The ethanol production technologies have focused on utilization of sugars, starch, etc. These first generation fuels are competing with the main source of food and this situation creates an important debate (Adıgüzel, 2013). For this reason, second generation fuels have attracted the attention due the the fact that they are produced from lignocellulosic wastes that are obtained from agricultural or domestic activities. The disadvantage of these processes are the high pre-treatment costs and formation of by-products such as lignin. (Phillips et al., 2017). A novel solution has gained attention to use C1 gases (CO, CO_2) in order to produce bioethanol with specific microorganisms that can follow the Wood-Ljungdahlii pathway. This group of microorganisms (mainly *Clostiridium* species) can use CO and CO_2 as substrates for bioethanol production (Liberato et al., 2019). Syngas is the mixture of N_2, CO_2, H_2 and others are the main source of air pollution and it is released from high impact industries such as mining and steel factories (Oelgeschläger & Rother, 2008). Wood-Ljungdahl pathway (acetyl-CoA pathway) is the metabolic pathway to convert C1 gases into acetate and bioethanol (Schiel-Bengelsdorf & Dürre, 2012). The mostly known microorganisms that can convert C1 gases

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

into ethanol are *Clostridium ljungdahlii*, *Clostridium autoethanogenum, Clostridium autoethanogenum, Butybacterium methylotrophicum* (also known as *Eubacterium limosum*), *Alkalibaculum bacchi* and *Clostridium ragsdalei* (also known as *Clostridium sp*. strain P11 (Abubackar et al., 2015; Martin et al., 2016; Schiel-Bengelsdorf & Dürre, 2012; Wilkins and Atiyeh, 2011). The potential of conversion of these gases into bioethanol depends on the type of microorganism and the operational conditions. One of the most important factors in the production via this pathway is the medium composition that directly effects the final product in Wood-Ljungdalii pathway (Abubackar et al. 2015). *Clostridium ragsdalei* is one of the most studied type of microorganism for syngas fermentation. Reseachers have mainly focused on the optimization of medium composition which is still under development (Younesi et al. 2005, Kundiyana et al. 2011).

In this study two different types of medium compositions were used to compare the yields on bioethanol production using *Clostridium ragsdalei*. In addition, the medium composition and the initial syngas concentration were optimized.

Method

Microorganism and the Culture Media

Clostridium ragsdalei ATCC BAA622 was obtained from the American Type Culture Collection (ATCC, USA). The lyophilized cultures were activated by growing in ATCC Media at 30°C for 24 h. In the present study, two types of medium, namely ATCC (recommended by the culture collection) and DRCM (Differential Reinforced Clostridial Broth)were used for inoculation. The cultures were kept at 30°C for 24 h and used as inoculum. Prior to inoculation, the cultures were kept active by transferring into fresh media including fructose every 2 weeks. In order to force faster growth 10% of active inoculum was used in growth media for both types of medium. Medium composition of ATCC for 1 L is as follows; 1 g NH_4Cl, 0.1 g KCl, 0.2 g $MgSO_4x7H_2O$, 0.8 g NaCl, 01.g KH_2PO_4, 0.02 g $CaCl_2x2H_2O$, 1 g yeast extract, 10 mL trace element solution, 0.5 mL Na-resazurin solution (0.1% w/v), 1 g $NaHCO_3$, 5 g D-Fructose, 10 mL vitamin solution, 0.3 g L-Cystein.$HClxH_2O$, 0.3 g Na_2Sx9H_2O. Trace element solution (for 1 L): 1.5 g nitriloacetic acid, 3 g $MgSO_4x7H_2O$, 0.5 g $MnSO_4xH_2O$, 1 g NaCl, 0.1 g $CaCl_2x2$ H_2O, 0.18 g $ZnSO_4x7H_2O$, 0.01 g $CuSO_4x5H_2O$, 0.02 g $KAl(SO_4)2x12H_2O$, 0.01 g H_3BO_3, 0.3 mg $Na_2SeO_3x5H_2O$, 0.4 mg $Na_2WO_4x2H_2O$. Vitamin solution (for 1 L): 2 mg biotin, 2mg folic acid, 10 mg pyrodixne-HCl, 5 mg Thiamine-HCl, 5 mg Riboflavin, 5 mg nicotinic acid, 5 mg D-Ca panthenoate, 0.1 mg Vitamin B12, 5 mg p-aminobenzoic acid, 5 mg lipoic acid DRCM medium was directly supported from Merck Milipore(Germany) as 500 g form. The ingredents of this medium are; Peptone from casein 5.0 Peptone from meat 5.0, meat extract 8.0, yeast extract 1.0, starch 1.0, D(+)glucose , 1.0 L-cysteinium chloride, 0.5 sodium acetate , 5.0 sodium di-sulfite, 0.5 ammonium iron(III) citrate , 0.5 sodium resazurin 0.002.

Batch Reactors

100 mL small scale glass serum bottles with 50 mL working volume were used as batch reactors. The reactors were inoculated with 10% of microorganisms by using actively passaged cultures. After adding the necessary chemicals to the mediums, the reactors were capped with rubber and sealed with Al rings and sterilized at 121°C for 15 min using autoclave (HIRAYAMA, 110 L). Following the sterilization process, the headspace of the reactors were sparged with 99% pure N_2 to washout the available O_2 from the reactors. After growth on sucrose for 24 h, syngas at varying volumes (5 mL, 10 mL, 15 mL, 20 mL and 25 mL) were added to the reactors. The composition of syngas is 60% CO , 10% CO_2, 10% CH_4 ,10%H_2, 10% N_2 (Habas, Turkey). Fermentation was initiated following the syngas addition. The reactors were kept at 30°C for 24 days. All experiments were carried out in triplicates and the results were reported as average values with standard deviations.

Analytical Methods

Bacterial growth was monitored via optical density (OD) measurements at 660 nm with a UV spectrophotometer (Thermo Fisher Scientific, MA, United States). Ethanol and acetic acid concentrations in the fermentation broth were analyzed using a High Pressure Liquid Chromatography (HPLC) instrument (Thermo Fisher Scientific) equipped with a Refractive Index Detector (RID). The initial temperature of the HyperREZXP Carbohydrate +H 8 nm column (Thermo Fisher Scientific) was 50°C and 10 mM H_2SO_4 was used as the mobile phase. 1 mL of liquid samples were collected under sterile conditions for each analyte sampling point using

disposable syringes. The samples were centrifuged at 8944 g for 10 min using a microcentrifuge (Thermo Fisher Scientific) and filtered through PTFE syringe filters with a pore size of 0.20 μm.

Results and Discussion

The effects of two different media have been observed in the production of bioethanol from the model synthesis gas mixture. The effects of the utilization of defined DRCM and ATCC medica were comparatively evaluated. The compositions of the two mediums were reported in methods section. These two mediums reported positive effects on growth of *Clostiridium ragsdalei* and their ethanol production performances were compared using same medium compositions.

Optimization of the Amount of Syngas Feed Using DRCM Medium

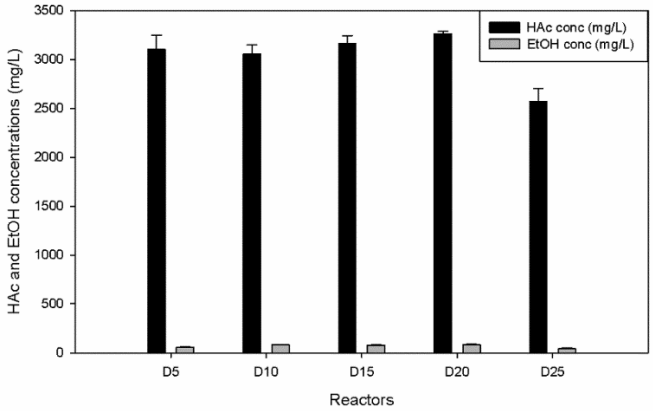

Figure 1. The concentrations of ethanol and acetate produced from syngas using DRCM medium by *Clostridium ragsdalei*

Figure 1 shows the acetic acid and ethanol concentrations observed when DRCM media was used. Considering the results, it can be clearly seen that the acetic acid production values in the reactors are much higher than ethanol production. As a result of 5, 10, 15 and 20 syngas feeds, high acetate productions varying between 3055-3262 mg/L were observed in all reactors. 2567 mg / L acetate production was observed in 25 mL syngas feed. Lower values observed with 25 mL is thought to be due to the toxic effect of the CO gas present in the syngas. When a comparison is made in terms of ethanol production, it is seen that the values are obtained as a maximum of 84 mg/L. Looking at the Wood-Ljungdahlii pathway, it is seen that acetate and ethanol production are related in metabolite production. In cases with high acetate production, lower ethanol production values were reported due to these two metabolites being produced simaltenouslty (Monir et al. 2020).

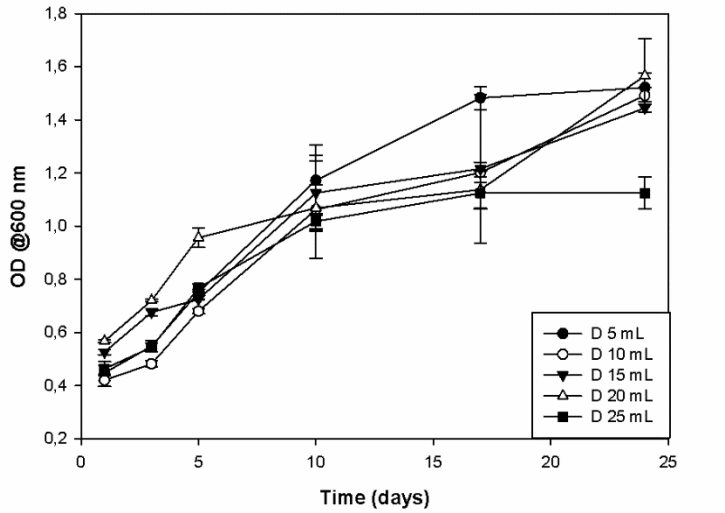

Figure 2. OD values - using DRCM medium by *Clostridium ragsdalei*

Figure 2 shows the bacterial growth values in bioethanol production from the synthesis using DRCM media. When the values observed until the 5th day are compared, it is seen that the fastest growth and thus the highest concentration of bacteria are obtained from the reactors containing 20 mL syngas. The lowest bacterial concentrations were observed in reactors containing 10 and 25 mL syngas. In the following days of the process, it is seen that the reactor containing 20 mL of syngas switched to the stationary phase and the growth of bacteria continued in the reactors containing lower amounts of synthesis gas. In the reactor containing 10 mL syngas a slow growth is observed on the acclimation days and transition to the exponential phase occurred after the 5th day. The bacterial growth continued rapidly until day 20 in the reactor containing 10 mL syngas and then slowed down. A similar trend was observed in the reactor containing 15 mL syngas as compared to the reactor containing 10 mL syngas. Increasing acetate concentrations in the reactor containing 20 mL synthesis gas are thought to cause the pH to decrease faster (Gunay et al. 2020). The fact that the lowest bacterial concentrations were observed in reactors containing 25 mL syngas supports our idea that the increased synthesis volume begins to cause toxic effects.

Optimization of the Amount of Syngas Feed Using ATCC Medium

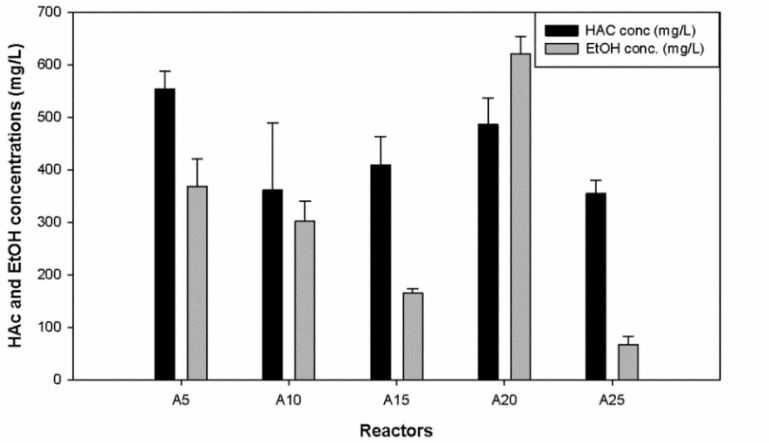

Figure 3. The concentrations of ethanol and acetate from syngas using ATCC medium by *Clostridium ragsdalei*

Figure 3 shows that varying amounts of syngas feed have different effects on acetate and ethanol productions. It is depicted that the syngas volumes of 5, 10 and 15 mL resulted in 362, 302 and 165 mg/L ethanol production, respectively. Furthermore, increasing syngas volume had a negative effect on ethanol production. The highest ethanol production was observed as 620 mg/L in 20 mL syngas feed. On the other hand, a low value of 67 mg/L was observed in 25 mL of syngas feed. It is believed that the amount of CO in the syngas has a toxic effect. While ethanol production decreased in low volume syngas addition.

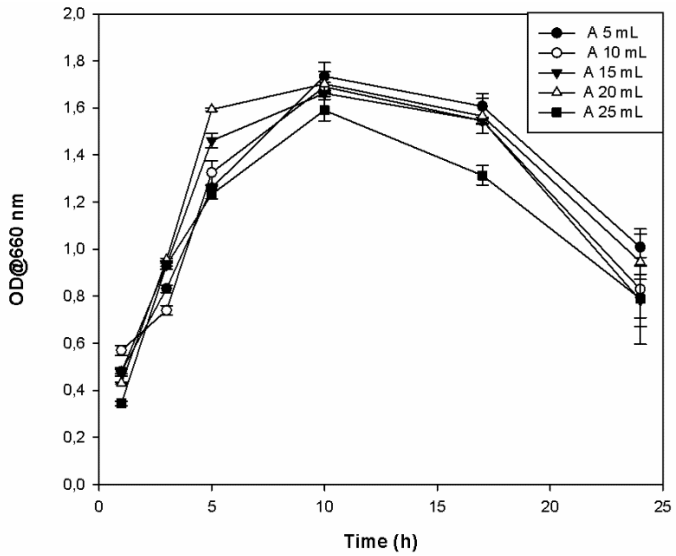

Figure 4. OD values - using ATCC medium by *Clostridium ragsdalei*

The acetate concentrations depicted that the highest acetate production was obtained with 5 mL syngas feed. Due to the possibility that the process parameters will be shifted from the production of acetate to ethanol production with changes to be made in pH or process type (continuous or batch fed) systems, potentially, the high production potential in 5 mL syngas feed stands out. The high ethanol value obtained with 20 mL syngas can be explained by the rapid onset of bacterial growth and the rapid decrease of the pH value with this increasing acetate concentration and the direction of the process to ethanol.

It was observed from Figure 4 that all reactors had the same trend in terms of bacterial growth. In all reactors with varying volumes of syngas feed, bacterial growth slowed down after a fast lag phase followed by an exponential phase and a short stationary phase. On day 10, the highest bacteria concentrations were observed in all reactors. The daily results showed that, ethanol production was not observed after the 10th day. Figure 4 shows that the lowest bacterial growth was observed in the reactor containing 25 mL of syngas and the highest bacteria concentrations in the reactors containing 5 and 20 mL syngas feed.

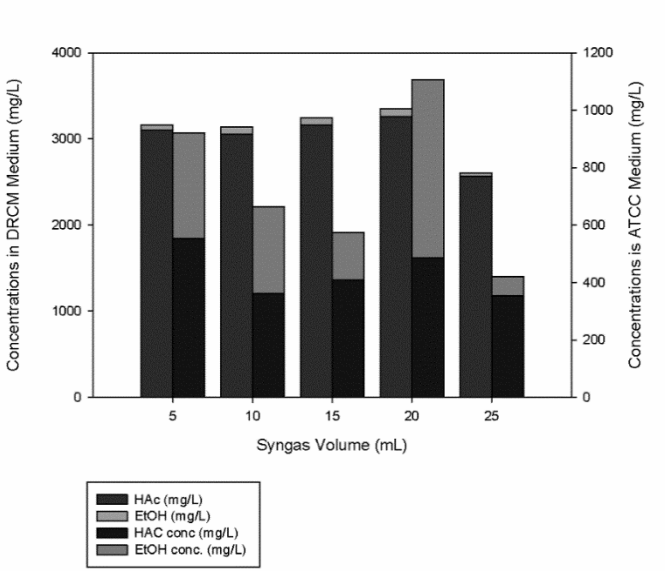

Figure 5. Comparison of all results

Several studies have been performed on ethanol production from syngas using different types of media (Maddipati et al. 2011). The main aim of these studies were to optimize the fermentation medium to reach the highest ethanol yields. The results shown on Figure 5 also showed that the composition of the medium greatly effects the routes on Wood-Ljungdahlii pathway. DRCM medium directed the fermentation route to acetate production and ATCC is more favorable for ethanol production. However as it can be clearly seen from the metabolic pathway, the ethanol and acetate production are inversely related and the higher acetate values shows the potential of higher ethanol values. DRCM medium has high potential to produce ethanol since the acetate concentrations reached up to 3 g/L. Using ATCC medium resulted in higher ethanol production up to 0.7 g/L. In another study, *Alchalibacterium bacchii* CP15 resulted in 1.7 g/L ethanol production and 0.8 g/L acetate production (Liu et al. 2012). These results showed that the lower acetate values resulted in higher ethanol production values. Similarly to our study, Gao et al (2013) reported 1.1.g/L ethanol and 2.1 g/L acetate production using *Clostridium ragsdalei*. Kundiyana et al (2011) also performed a study using *Clostridium ragsdalei* and reported 4.75 g/L acetate production in which the medium composition is similar with DRCM medium. As a result, it can be reported that the medium composition directly effects the metabolic route of *Clostridium ragsdalei* which has high potential to produce acetate, thus, it must be directed to ethanol production by changing the operational conditions.

Conclusion

Bioethanol can successfully be produced from syngas *by Clostridium ragsdalei*. The medium composition direct effects the metabolic pathway of the microorganism. DRCM medium directed the Wood-Ljungdahlii pathway towards acetate production reaching up to 3500 mg/L. The highest bioethanol production from ATCC medium was 622 mg/L at 20 mL syngas feed. The highest addition of syngas (25 mL) resulted in lower bioethanol production. It is known that acetate can be converted to ethanol by changes in its metabolic pathway. Acetate

produced by DRCM medium could be a good source for higher bioethanol production values by mitigation in the metabolic pathway.

Acknowledgements or Notes

This experimental work was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK-CAYDAG) [project no 118Y305]. The authors gratefully thank Prof. Dr. Nuri Azbar for his technical support.

References

Abubackar, H.N., Veiga, M.C.& Kennes, C., (2015). Carbon monoxide fermentation to ethanol by *Clostridium autoethanogenum* in a bioreactor with no accumulation of acetic acid. *Bioresour. Technol. 186*, 122–127.

Adıgüzel, O. (2013). Lignoselülozik materyallerden biyoetanol üretimi için kullanılan ön-muamele ve hidroliz yöntemleri. *SAU J. Sci 17*, 381–397.

Dürre, P.& Eikmanns, B.J. (2015). C1-carbon sources for chemical and fuel production by microbial gas fermentation. *Curr. Opin. Biotechnol. 35*, 63–72.

Gao, J., Atiyeh, H.K., Phillips, J.R., Wilkins, M.R. & Huhnke, R.L. (2013). Development of low cost medium for ethanol production from syngas by *Clostridium ragsdalei. Bioresour. Technol. 147*, 508–515.

Gunay, B., Azbar, N.& Keskin, T. (2020). The effect of corn syrup and whey on the conversion process of CO to ethanol using *Clostridium ljungdahlii. Chemosphere 261*, 127734.

Kundiyana, D.K., Huhnke, R.L. & Wilkins, M.R. (2011). Effect of nutrient limitation and two-stage continuous fermentor design on productivities during "*Clostridium ragsdalei*" syngas fermentation. *Bioresour. Technol. 102*, 6058–6064.

Liberato, V., Benevenuti, C., Coelho, F., Botelho, A., Amaral, P., Pereira, N., Ferreira, T. (2019). Chemicals Production in a Biorefinery Context. *Catalysts 1*, 1–37.

Liu, K., Atiyeh, H.K., Tanner, R.S., Wilkins, M.R. & Huhnke, R.L. (2012). Fermentative production of ethanol from syngas using novel moderately alkaliphilic strains of *Alkalibaculum bacchi. Bioresour. Technol. 104*, 336–341.

Maddipati, P., Atiyeh, H.K., Bellmer, D.D. & Huhnke, R.L.(2011). Ethanol production from syngas by Clostridium strain P11 using corn steep liquor as a nutrient replacement to yeast extract. *Bioresour. Technol. 102*, 6494–6501.

Martin, M.E., Richter, H., Saha, S. & Angenent, L.T. (2016). Traits of selected *Clostridium* strains for syngas fermentation to ethanol. *Biotechnol. Bioeng. 113*, 531–539.

Miranda, J.C. de C., Ponce, G.H.S.F., Arellano-Garcia, H., Maciel Filho, R. & Wolf Maciel, M.R.(2020). Process design and evaluation of syngas-to-ethanol conversion plants. *J. Clean. Prod. 269*, 122078.

Monir, M.U., Yousuf, A. & Aziz, A.A., (2020). Syngas fermentation to bioethanol, in: Lignocellulosic Biomass to Liquid Biofuels. Elsevier, 195–216.

Oelgeschläger, E. & Rother, M., (2008). Carbon monoxide-dependent energy metabolism in anaerobic bacteria and archaea. *Arch. Microbiol. 190*, 257–269.

Phillips, J.R., Huhnke, R.L. & Atiyeh, H.K. (2017). Syngas fermentation: A microbial conversion process of gaseous substrates to various products. *Fermentation 3*(2), 28.

Schiel-Bengelsdorf, B. & Dürre, P. (2012). Pathway engineering and synthetic biology using acetogens. *FEBS Lett. 586*, 2191–2198.

Wilkins, M.R. & Atiyeh, H.K. (2011). Microbial production of ethanol from carbon monoxide. *Curr. Opin. Biotechnol. 22(3), 326-330.*

Younesi, H., Najafpour, G., Mohamed, A. R. (2005) Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, *Clostridium ljungdahlii, Biochem. Eng. J., 27,* 110-119.

Author Information

Mine Gungormusler
Izmir University
Izmir University of Economics Department of Genetics and Bioengineering, , 35330, Izmir, Turkey
Contact e-mail: *mine.gungormusler@ieu.edu.tr*

Tugba Keskin Gundogdu
Izmir Democracy University
Department of Environmental Protection Technologies, , 35140, Izmir, Turkey