Extraction of exopolysaccharide from *Acinetobacter baumannii* strain VESASC-ASC and its application in sewage treatment

[1]Ankita Kharat, [2]Suman Ganger, [3] Shweta Patil*
[1]Post Graduate Student,[2]Assistant Professor,[3]Assistant Professor (Corresponding Author)
[1]Department of Microbiology,
[1]Vivekanand Education Society's College of Arts, Science and Commerce, Mumbai, India

Abstract: **Domestic sewage contains both organic and inorganic compounds. It also contains products of decomposition like ammonia, hydrogen sulphide, methane and carbon dioxide. The chemical composition, pH, low dissolved oxygen and high load of pathogenic bacteria lead to major water pollution. Around 2100 million litre a day of waste water sewage is released into the creeks of the Arabian Sea. Hence, there is a dire need to use innovative and effective methods which can be used at source to handle the waste water. Bacteria produce diverse biopolymers with varied chemical properties via utilization of simple and complex substrates. These extracellular polymeric substances (EPS) producing organisms indigenously play an important role in waste water treatment. These find immediate applications in sewage treatment and biofloculation. In this study 12 EPS producing bacteria were screened from various sources and selected based on EPS producing capacity on solid media. *Acinetobacter baumannii* strain VESASC-ASC gave a promising EPS production of 0.45 g %. Media was optimized based on physiochemical and operational media parameters for maximum EPS production. The EPS was partially characterized by HPTLC. The extracted EPS showed antibacterial activity against 10 test lab isolates. The EPS producers were found to be promising in treatment of domestic sewage sample. 70%, 99%, 99.7% reduction in COD, BOD and coliform count respectively was noted.**

***Keywords*- Exopoysaccharide, *Acinetobacter baumannii*, sewage treatment, BOD, COD, coliform.**

I. INTRODUCTION

Around 2100 million liter a day of waste water sewage is released into the creeks of the Arabian Sea. The waste that arrives at the plants is pumped 3km into the sea. A global study found that the sea near the Mumbai coast is the most polluted. 25% of the city's waste which comes from the nullahs and slums do not enter the 1915 km sewer network enters the sea directly. (Bharucha N., 2017). Sewage is a type of wastewater that is produced by a community of people. It is characterized by volume or rate of flow, physical condition, chemical and toxic constituents, and its bacteriologic status. It consists mostly of greywater, blackwater; soaps and detergents; and toilet paper.Sewage usually travels from a building's plumbing either into a sewer, which will carry it elsewhere, or into an onsite sewage facility. (Sperling V, 2007). Pollution from domestic sewage adds to the water, organic and inorganic matter which reduces the dissolved oxygen in the water body further which leads to damages to the ecosystems. Moreover this waste water also contains pathogenic microorganisms some of which may be drug resistant.

Flocculation is an essential phenomenon in wastewater treatment. Flocculation can be carried out with chemicals both organic and inorganic. Inorganic chemicals are less expensive but leave lot of metal residues. Organic polymeric flocculants are much promising but are not biodegradable and leave away toxic monomers. Thus there is more attraction towards biopolymers as they are environment friendly. However the amount of these biopolymers produced by microorganisms is less and large dosage would be needed to meet the requirement (Lee et al., 2014).

Bacteria produce diverse biopolymers with varied chemical properties via utilization of simple to complex substrates. Some of these biopolymers include Extracellular polymeric substances (EPS) (Nwodo et. al., 2012). EPS are a complex high-molecular-weight mixture of polymers excreted by microorganisms, produced from cell lysis and adsorbs organic matter from wastewater. They are a major component in microbial aggregates for keeping them together in a three-dimensional matrix such as biofilms. EPS are the construction material of bacterial settlements and either remain attached to the cell's outer surface, or are secreted into its growth medium (Nwodo et al., 2012). Their

characteristics like adsorption abilities, biodegradability and hydrophilicity/hydrophobicity make them suitable in the process of flocculation for waste water treatment. The major components of EPS are carbohydrates, proteins, humic substances and nucleic acids. These crucially affect the properties of microbial aggregates, such as mass transfer, surface characteristics, adsorption ability, stability, etc. (Sheng et al., 2010). The overwhelming diversity of bacterial polysaccharides allows for categorization based on chemical structure, functionality, molecular weight and linkage bonds (Nwodo, et. al., 2012). However, as EPS are very complex, the knowledge regarding EPS is far from complete and much work is still required to fully understand their precise roles in the biological waste treatment process. (Sheng et al., 2010).

The activated sludge treatment is a common process for waste water treatment which uses the aerobic indigenous flora present in the wastewater to treat it in large aerated and settling tanks. The waste however has to be carried to the waste treatment site for the treatment process. The best alternative would be to treat the waste water on site (Shchegolkova et al., 2016).

EPS may be overproduced when there is abundance of sugars to become reserves of carbohydrate for subsequent metabolism. Example of EPS are cellulose, alginate, xanthan,dextran (Nwodo et al., 2012).

EPS can be extracted from different plant materials, but it has many disadvantages such as time consumption for production, harvesting of EPS is laborious, difficulties in separation and purification of EPS. EPS can also be synthesized chemically but it is costly and also the type of bonding present naturally will not be produced chemically. The bonding produced synthetically may not remain stable for long time. Hence production of EPS from micro-organisms is a better alternative (Sirajunnisa et al., 2011).

The limitation of the applications of some of these bacterial polysaccharides has been largely due to cost of production relative to their commercial value; however the approach generally employed to address this problem includes; using cheaper substrates, improving product yield by optimizing fermentation conditions, or developing higher yielding strains via mutagenesis, and/or genetic and metabolic manipulations, and optimizing downstream processing. Conversely, the possession of unique properties by the EPS, which may not be found in other traditional (plant and algae) polysaccharides would invariably translate to high-value applications thus, product quality wholly surpasses production cost (Welman, et al., 2003).

Microbial EPS seem to be a promising alternative as they can effectively act as strong reducing as well as stabilizing agent for metal nanoparticle production. There is growing interest in the pharmaceutical industry for the use of natural components such as EPS. Microbial EPS are also used in the pharmaceutical industry to synthesize EPS with antibiotics properties (Clemens, M. E. 2015). Removing of heavy metals from water through mechanical, chemical methods is relatively time consuming and costly. Thus a better alternative to this is to use EPS. These EPS producing micro-organism have the ability to adsorb metals on the surface of EPS.

In this study, EPS from *Acinetobacter baumannii* was extracted and studied. The effect of various media physicochemical parameters on the production of EPS was analyzed. Antibacterial activity and the role of EPS in the sewage treatment was studied with respect to BOD, COD, TSS, total viable count, coliform count.

II. Materials and Methods

Screening and isolation of EPS producers:

Garden soil and raw milk were collected from various locations in a plastic bags. (Zhang T. et al, 2011, Fusconi et al., 2002). 1gm of the sample was homogenized in 2ml of sterile saline (pH 7.0) and plated out on sterile Nutrient agar medium and incubated at 27°C for 24 hours (Sirajunnisa et al., 2012). The presence of EPS was checked from the mucoid appearance of the colonies. These colonies were further purified and maintained on sterile Nutrient agar slants. Further confirmation of the EPS presence was done in sterile P liquid medium and the mucoid colonies (EPS showing) from the above plates were inoculated and kept at 27°C for 1-2 week. (Fusconi et al., 2002)

Extraction of EPS:

EPS was extracted using polar and non-polar solvents and it was measured on weight basis (Kumar, M. A et al., 2011). Selected mucoid cultures (Abs530nm - 0.10) were inoculated in 50 ml of sterile YGM broth and incubated at

27℃ for 1 week. After incubation the broth was centrifuged at 5000 rpm at 4◦C for 30 min.The supernatant was collected and equal volume of chilled solvent was added and the precipitated EPS was spooled out. Extracted EPS was added into pre weighted tube and kept for drying. (Mehta et al., 2014, Muthusamy et al., 2011). Confirmation for the presence of EPS was done using Molisch's test for carbohydrates (Devor, 1950) and protein by Biuret method (Herbertz, 1976).

Identification of the isolates:

Based on morphology, biochemical and molecular biology methods. Gram staining was carried out on the selected isolate (Hucker, G. J., & Conn, H. J. ,1923). Further identification of the isolate was done based on biochemicals on VITEK analysis at Sunflower laboratory, Mumbai. This was followed by 16S rRNAtechnique (Saffron Life Science, Navsari - Gujarat)

Effect of physicochemical and operational parameters for efficient production of EPS:

1ml of the culture suspension of the selected isolate (Abs530nm - 0.10) was inoculated and incubated at 27℃ for 1 week. After 1 week of incubation EPS was extracted. P media, basal salt mineral media, B media, Yeast Glucose Malt (YGM) media were tested for maximum amount of EPS production (Muthusamy et al., 2011, Zhang, T. et al., 2011). The effect of temperature and pH was studied at 21℃, 27℃, 37℃, 55℃. (Wang et al., 2011) and pH 3, 5, 6, 7, 8 and 9 (Zhang et al., 2011). The time duration for maximum EPS was identified by incubating the medium at different time intervals i.e. from 2, 3, 4, 5, 6, 7 days to 2 weeks. (Sirajunnisa et al., 2012). Effect of agitation was checked by incubating the medium at static and shaker (120 rpm) conditions. (Kim, S. W. et al 2013). With respect to the chemical parameters, effect of carbon and nitrogen was studied. Carbon sources at a 1% concentration (glucose, maltose, sucrose, lactose and glycerol), nitrogen sources (organic - 1% peptone, 1% yeast extract, 1% meat extract, 1% beef extract, Inorganic - 0.5% ammonium sulphate, 0.5% ammonium nitrate, 0.5% sodium nitrate, 0.5% ammonium chloride) and was added to YGM media respectively and incubated.(Wang et al., 2011).

Characterization of exopolysaccharide by High Performance Thin Layer Chromatography (HPTLC):

To check the components of EPS, HPTLC (HPTLC analysis server vision CAT-server-PH, 2.5.18053.1) was performed (Anchrom Lab, Mulund - Mumbai.) Stationary phase used was Silica gel 60 F 254 and the plate was impregnated with 0.3N NaH_2PO_4. The sample of EPS was treated with acetonitrile and 2, 4, 6, 8 and 10 µl of the sample was used for loading along with standard carbohydrates (1% - arabinose, fructose, galactose, glucose, mannose, ribose, rhamnose, starch and xylose).

Antimicrobial property of EPS:

The extracted EPS was checked for antimicrobial activity by using agar cup method (Li, et al., 2014). Test culture suspensions (Abs530nm - 0.10) of *Salmonella typhi, Streptococcus pyogenes A, Streptococcus pyogenes B, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheria, Enterococcus fecalis , Escherichia coli, Candida albicans* were used. 50 µl of extracted EPS was added in the well. Plates were incubated at 37℃for overnight and zone of inhibition were measured (Mahendran et al., 2013).

Sewage water treatment:

For this study sewage sample from open nullah in Chembur area was collected in sterile sampling bottles and used for analysis within 6 hours. The following experiments were set up,

Set I - 250 ml sewage sample + 5 ml culture of EPS producing culture (Abs530nm - 0.10)

Set II- 250 ml of sewage sample + 5ml of extracted EPS suspension

Control -250 ml of sewage sample

These bottles were kept for incubation at 27°C for 7 days. After 7 days of incubation BOD, COD, total viable count, Coliform count of sewage sample was performed using BIS 10500 Standard (APHA-AWWA-WPCF, 1997)

III. RESULTS:

Screening, Isolation and Identification of EPS producer:

From the samples analyzed, 16 isolates with mucoid appearance and EPS producing were selected. Out of these the culture C produced maximum EPS (10.3 g%). The selected isolate was gram negative short rods and identified as *Acinetobacter baumannii* strain VESASC-ASC (GenBank accession number MH703073).

Extraction and confirmation of EPS: Chilled acetone gave maximum extraction of the EPS. The Molisch's test gave red color ring and biuret gave slight blue colour confirming the presence of carbohydrate and less amount of protein in EPS. **Characterization of exopolysaccharide by HPTLC:**

The EPS showed the presence of glucose (Fig. 1) as the released component carbohydrate when compared with standard glucose.

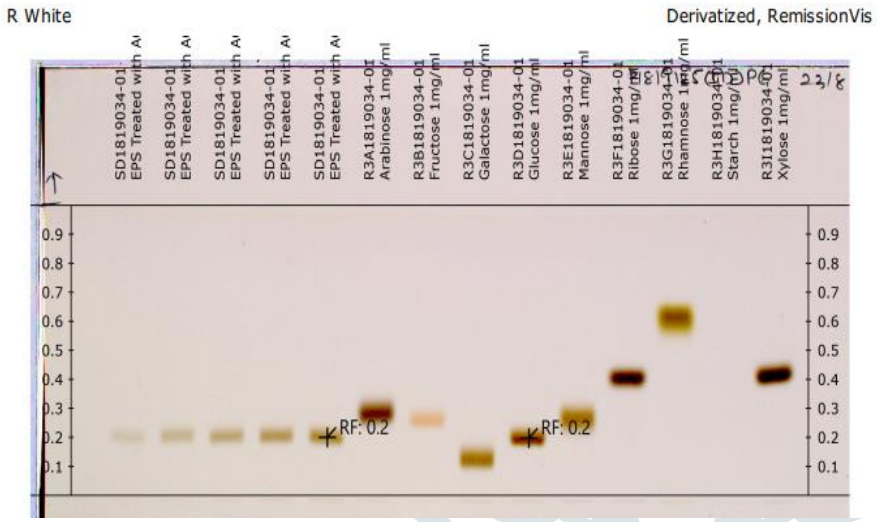

Figure 1 Left to right Rf value of the extracted EPS (Spot 1 to 5) matches with spot 9 that of glucose

Effect of physicochemical and operational parameters for efficient production of EPS:

The table 1 indicates that the EPS is affected by physical and chemical media parameters. Maximum EPS production began at the day 8 and continued up to day 12 i.e. when the culture entered the stationary phase. At this time the EPS was completely released from the cell. Maximum EPS production did not occur in acidic condition probably due to the acid stress on the cell. Glucose when supplemented in the medium gave maximum EPS since this maintained a high C:N ratio. Inorganic nitrogen sources did not help in production of EPS, but organic sources increased the production. This modified medium would increase the production of EPS.

Table 1.Optimized conditions for EPS Production by culture C

Parameter	Maximum production	EPS (g/100ml)

Choice of media	YGM	0.9
Incubation temperature	35-37°C	0.5
pH of medium	7-8	0.6
Duration of EPS production	8-12 days	0.8
Incubation Condition	Static	0.56
Carbon source	Glucose	0.93
Nitrogen sources	Beef extract	0.12

Antimicrobial property of EPS:

The EPS showed antibacterial property (Tab. 2 and Fig. 2) against many gram positive and negative organisms but it did not show antifungal property.

Table 2. Zone of Inhibition (mm) by EPS measured for various test organism

Test Organism	Zone of inhibition (mm)			
	X_1	X_2	X_3	Avg. X
Salmonella typhi	14	14	14	14
Streptococus pyogenes A	14	14.5	14.5	14.33
Streptococus pyogenes B	14.5	14.5	14.5	14.5
Pseudomonas aeruginosa	14.5	14.5	14	14.33
Proteus mirabilis	16	16	16	16
Staphylococcus aureus	15	15	14.5	14.83
Bacillus subtilis	15	15	15.5	15.16
Corynebacterium diphtheria	15.5	15.5	15	15.33
Enterococcus fecalis	13	14	14	13.66
Escherichia coli	16	16	16	16
Candida albicans	0	0	0	0

Figure 2. Zone of Inhibition as observed by Agar cup method for the EPS on test organism

Sewage water treatment:

The results (Tab. 3) were taken as before and after treatment with EPS or EPS producing culture. After treatment with EPS of *Acinetobacter baumannii* strain VESASC-ASC, there was flocculation and sedimentation of the dissolved matter, showing that the EPS is able to flocculate and hence clarify the waste water.

Table 3. Sewage water treatment

	Control (Before Treatment)	Set I (sewage sample + *Acinetobacter baumannii* strain VESASC-ASC	Set II (sewage sample + EPS of *Acinetobacter baumannii* strain VESASC-ASC
Chemical oxygen demand (BOD)	441.6 mg/L	128 (Reduction – 70 %)	320 (Reduction – 27 %)
Biological oxygen demand (BOD)	22,000 mg/L	161.1 (Reduction – 99.26 %)	283 (Reduction – 98.7 %)
Total coliform count (MPN INDEX per 100ml)	More than 1600	Nil	Nil
Total viable count (cfu/ml)	$3x10^6$cfu/ml	8×10^3 (Reduction – 99.7 %)	1×10^4 (Reduction – 99.66 %)

Results indicate that the experimental design for both sets I and II gave reduction in the various parameters tested for the sewage water. But set I (sewage sample + *Acinetobacter baumannii* strain VESASC-ASC) where the organism was allowed to grow in the sewage gave more promising results than the set II where the extracted EPS was added into the waste. Thus EPS of *Acinetobacter baumannii* strain VESASC-ASC gave reduction for the sewage sample with respect to COD, BOD, Total viable count, coliform count. Thus this EPS producer can be further explored for waste water treatment.

IV. Discussion:

In the present work, we studied the exopolysaccharide synthesized by *Acinetobacter baumannii* strain VESASC-ASC, and determined its potential biotechnological applications. The majority of EPS with biomedical properties contain sulphate groups and evidence suggests that this is a critical feature as far as these properties are concerned. EPS is biologically inert in their native state, their potent biomedical capacity resulting from chemical over-sulphation. Proteins may play an essential role in the emulsifying capacity of some polysaccharides. Rapid industrialization and increasing urbanization are contaminating our environment by discharging heavy metals and toxic metabolites in the effluents, with all the consequent damage to health and the environment. Remediation of the situation with currently used physical-chemical techniques is expensive and can cause even further environmental harm.

Wastewater treatment with biopolymer at 500 mgL^{-1} showed reduction in biochemical oxygen demand (38–80%), chemical oxygen demand (37–79%), and suspended solids (41–68%). Hence, biotechnological approaches have received a great deal of attention in recent years as an alternative approach to the problem of pollution. The need for safe, effective, economical methods for wastewater treatment has directed attention to EPS produced by algae, bacteria, fungi and yeasts. The strong chelating property of these polymers offers the possibility of their being used as

a biosorbent in water treatment and to clean polluted environments. Apart from the above mentioned applications, the EPS from this study can also be used to design heavy metal removal systems.

V. ACKNOWLEDGMENT

This research was partially supported by Minor Research Grant, University of Mumbai. Authors are thankful to Vivekanand Education Society's College of Arts, Science and Commerce – Central Instrumentation Facility (funded by DST-FIST grants) for their infrastructure and the teaching and non-teaching staff of the microbiology department for their constant support.

REFERENCES

[1] Andersson, S., Dalhammar, G., &Kuttuva Rajarao, G. (2011). Influence of microbial interactions and EPS/polysaccharide composition on nutrient removal activity in biofilms formed by strains found in wastewater treatment systems. Microbiological Research, 166(6), 449-457. doi:10.1016/j.micres.2010.08.005

[2] Andhare, P., Chauhan, K., Dave, M., & Pathak, H. (2014). Microbial Exopolysaccharides: Advances in Applications and Future Prospects. In Biotechnology Volume 3: Microbial Biotechnology (1st ed., pp. 1-25). Houston: Studium Press LLC. doi: 10.13140/RG.2.1.3518.4484.

[3] American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Facility (WPCF). (1997). Standard Methods for examination of water and Wastewater, 21st ed., APHA, Washington DC, USA.

[4] Azeredo, J., & Oliveira, R. (1996). A new method for precipitating bacterial exopolysaccharides. Biotechnology Techniques, 10(5), 341-344. doi:10.1007/bf00173251

[5] Bartual, S. G., Seifert, H., Hippler, C., Luzon, M. A., Wisplinghoff, H., & Rodriguez-Valera, F. (2007). Development of a Multilocus Sequence Typing Scheme for Characterization of Clinical Isolates of *Acinetobacter baumannii*. Journal of Clinical Microbiology, 45(6), 4382-4390. doi:10.1128/jcm.00807-07

[6] Bharucha, N. (2017, May 15). Mumbai dumps 2,100mn litres of human waste in sea daily - Times of India. Retrieved from https://timesofindia.indiatimes.com/city/mumbai/mumbai-dumps-2100mn-litres-of-human-waste-in-sea-daily/articleshow/58676358.cms

[7] Clemens, M. E. (2015). *Dietary Fiber: Production Challenges, Food Sources and Health Benefits*. Hauppauge, NY: Nova Science Pub.

[8] Devor, A. W. (1950). Carbohydrate Tests Using Sulfonated α-Naphthol. *Journal of the American Chemical Society*, *72*(5), 2008-2012. doi:10.1021/ja01161a037

[9] Fusconi, R., &Godinho, M. J. (2002). Screening for exopolysaccharide-producing bacteria from sub-tropical polluted groundwater. Brazilian Journal of Biology, 62(2), 363-369. doi:10.1590/s1519-69842002000200020

[10] Ghosh, P. K., &Maiti, T. K. (2016). Structure of Extracellular Polysaccharides (EPS) Produced by Rhizobia and their Functions in Legume–Bacteria Symbiosis: — A Review. Achievements in the Life Sciences, 10(2), 136-143. doi:10.1016/j.als.2016.11.003

[11] Gugliandolo, C., Spanò, A., Maugeri, T., Poli, A., Arena, A., & Nicolaus, B. (2015). Role of Bacterial Exopolysaccharides as Agents in Counteracting Immune Disorders Induced by Herpes Virus. Microorganisms, 3(3), 464-483. doi:10.3390/microorganisms3030464

[12] Herbertz, G. (1976). A modified biuret method for the determination of proteins. *Das Medizinische Laboratorium*, *29*(11), 281-284.

[13] Hucker, G. J., & Conn, H. J. (1923). Methods of Gram staining.

[14] Kumar, M. A., Anandapandian, K. T., & Parthiban, K. (2011). Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. *Brazilian Archives of Biology and Technology*, *54*(2), 259-265. doi:10.1590/s1516-89132011000200006

[15] Kim, S. W., Hwang, H. J., Xu, C. P., Choi, J. W., & Yun, J. W. (2003). Effect of aeration and agitation on the production of mycelial biomass and exopolysaccharides in an enthomopathogenic fungus Paecilomyces sinclairii. *Letters in applied microbiology*, *36*(5), 321-326.

[16] Lee, C. S., Robinson, J., & Chong, M. F. (2014). A review on application of flocculants in wastewater treatment. Process Safety and Environmental Protection, 92(6), 489-508. doi:10.1016/j.psep.2014.04.010

[17] Li, S., Huang, R., Shah, N. P., Tao, X., Xiong, Y., & Wei, H. (2014). Antioxidant and antibacterial activities of exopolysaccharides from Bifidobacterium bifidum WBIN03 and Lactobacillus plantarum R315. *Journal of Dairy Science*, *97*(12), 7334-7343. doi:10.3168/jds.2014-7912

[18] Nwodo, U., Green, E., & Okoh, A. (2012). Bacterial Exopolysaccharides: Functionality and Prospects. *International Journal of Molecular Sciences*, *13*(12), 14002-14015. doi:10.3390/ijms131114002

[19] Okada, M., Sumitomo, H., Hirasawa, T., Ihara, K., & Tada, Y. (1986). Chemical Synthesis of Polysaccharides VI. Synthesis of (1→6) α-DL-Glucopyranan (DL-Dextran) by the Ring-Opening Polymerization Method. Polymer Journal, 18(8), 601-611. doi:10.1295/polymj.18.60

[20] Patil, S. V., Patil, C. D., Salunke, B. K., Salunkhe, R. B., Bathe, G. A., &Patil, D. M. (2010). Studies on Characterization of Bioflocculant Exopolysaccharide of Azotobacterindicus and Its Potential for Wastewater Treatment. Applied Biochemistry and Biotechnology, 163(4), 463-472. doi:10.1007/s12010-010-9054-5

[21] Priyanka, N., & Archana, T. (2011). Biodegradability of Polythene and Plastic By The Help of Microorganism: A Way for Brighter Future. Journal of Environmental & Analytical Toxicology, 01(02). doi:10.4172/2161-0525.1000111

[22] Sanalibaba, P., &Cakmak, G. A. (2016). Exopolysaccharides Production by Lactic Acid Bacteria. Applied Microbiology: open access, 2(2). doi:10.4172/2471-9315.1000115

[23] Saravanan, C., & Shetty, P. K. (2016). Isolation and characterization of exopolysaccharide from Leuconostoc lactis KC117496 isolated from idli batter. International Journal of Biological Macromolecules, 90, 100-106. doi:10.1016/j.ijbiomac.2015.02.007

[24] Sellami, M., Oszako, T., Miled, N., & Ben Rebah, F. (2015). Industrial wastewater as raw material for exopolysaccharide production by Rhizobium leguminosarum. Brazilian Journal of Microbiology, 46(2), 407-413. doi:10.1590/s1517-838246220140153

[25] Shchegolkova, N. M., Krasnov, G. S., Belova, A. A., Dmitriev, A. A., Kharitonov, S. L., Klimina, K. M., Kudryavtseva, A. V. (2016). Microbial Community Structure of Activated Sludge in Treatment Plants with Different Wastewater Compositions. Frontiers in Microbiology, 7(90). doi:10.3389/fmicb.2016.00090

[26] Sheng, G., Yu, H., & Li, X. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnology Advances, 28(6), 882-894. doi:10.1016/j.biotechadv.2010.08.001Singh, G., Singh, A., & Bhatt, K. (2016). Biodegradation of polythenes by bacteria isolated from soil. International Journal of Research and Development in Pharmacy & Life Sciences, 5(2), 2056-2062.

[27] Singh, P., Saini, P., Puranik, V., Gupta, S., & Dubey, S. (2017). Optimization and characterization of exopolysaccharides produced by lactobacillus strains isolated from cabbage and cucumber. Journal of Microbiology and Biotechnology Research, 6(6), 27-35.

[28] Sirajunnisa, A., Vijayagopal, V., &Viruthagiri, T. (2012). Effect of synthetic carbon substrates and cane molasses, an agro waste, on exopolysaccharide production by P. fluorescens. International Journal of Science and Engineering Applications, 1, 60-66. doi:10.7753/ijsea0101.1010

[29] Sperling, M. V. (2007). Wastewater Characteristics, Treatment and Disposal. IWA Publishing.

[30] Takahashi, E., Ledauphin, J., Goux, D., &Orvain, F. (2009). Optimising extraction of extracellular polymeric substances (EPS) from benthic diatoms: comparison of the efficiency of six EPS extraction methods. Marine and Freshwater Research, 60(12), 1201. doi:10.1071/mf08258

[31] Tiku, D. K., Kumar, A., Chaturvedi, R., Makhijani, S. D., Manoharan, A., & Kumar, R. (2010). Holistic bioremediation of pulp mill effluents using autochthonous bacteria. International Biodeterioration& Biodegradation, 64(3), 173-183. doi:10.1016/j.ibiod.2010.01.001

[32] Venkatesh, P., Balraj, M., Ayyanna, R., Ankaiah, D., & Arul, V. (2016). Physicochemical and biosorption properties of novel exopolysaccharide produced by Enterococcus faecalis. LWT - Food Science and Technology, 68, 606-614. doi:10.1016/j.lwt.2016.01.005

[33] Verhoef, R., De Waard, P., Schols, H. A., Rättö, M., Siika-aho, M., &Voragen, A. G. (2002). Structural elucidation of the EPS of slime producing Brevundimonasvesicularis sp. isolated from a paper machine. Carbohydrate Research, 337(20), 1821-1831. doi:10.1016/s0008-6215(02)00280-x

[34] Vu, B., Chen, M., Crawford, R., &Ivanova, E. (2009). Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. Molecules, 14(7), 2535-2554. doi:10.3390/molecules14072535

[35] Wang, Z. R., Sheng, J. P., Tian, X. L., Wu, T. T., Liu, W. Z., & Shen, L. (2011). Optimization of the production of exopolysaccharides by Bacillus thuringiensis 27 in sand biological soil crusts and its bioflocculant activity. African Journal of Microbiology Research, 5(16), 2359-2366.

[36] Welman, A. D., & Maddox, I. S. (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in biotechnology*, *21*(6), 269-274.

[37] Yan S, N. K., & RD, T. (2016). EPS producing microorganisms from municipal wastewater activated sludge. Journal of Petroleum & Environmental Biotechnology, 07(01), 2-13. doi:10.4172/2157-7463.1000255

[38] Zhang, T., Zhang, C., Li, S., Zhang, Y., & Yang, Z. (2011). Growth and exopolysaccharide production by Streptococcus thermophilus ST1 in skim milk. Brazilian Journal of Microbiology, 42(4), 1470-1478. doi:10.1590/s1517-83822011000400033