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Morphological characterization of gram-positive and gram-negative bacteria from treated latex processing wastewater

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Abstract

A preliminary morphological screening and isolation of bacterial colony from latex industrial wastewater was carried out. Bacteria colonies from latex processing wastewater were isolated from a local latex processing industry. It was found that 17 bacterial isolates had been purified grown on nutrient agar under 35 ^{o}C. The colonies were then purified and morphologically indicated via Gram staining and motility test. After morphological observation, it was identified that out of 17 isolates, 9 isolates were Gram positive and 8 isolates were Gram negative. There are 11 out of 17 colonies were rod-shaped bacterial colonies, while the other 6 colonies were cocci-shaped bacteria. There were 11 colonies of gliding bacteria, three colonies were non-motile bacteria and the other three colonies were flagellated bacteria. This study investigates the potential occurrence of viable growth in treated latex processing wastewater. The bacterial colonies were classified base on their morphological properties shown. This study has classified several genera such as Staphylococcus, Escherichia, Thiobacillus, Arthrobacter and other Genus. The growth curve of 17 isolates studied and the chemical oxygen demand were determined.

Keywords :
Latex processing wastewater, chemical oxygen demand, Gram staining, bacterial motility

1 Introduction

Malaysia is among the largest rubber producer in the world after Thailand, Indonesia, Vietnam, India and China. In South-east Asia, about 70 to 80% of produced raw rubber in the world supply comes mainly from Thailand, Malaysia and Indonesia (Andersen et al., 2005, Mohammadi et al., 2010). Latex industry produces a massive amount of wastewater daily, mainly coming from the manufacturing process. Latex processing wastewater (LPW) contains high level of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), solids, nitrate and heavy metal which affects the water quality. Illegal discharge of untreated wastewater into the rivers and streams cause various environmental pollution issues. Previously, latex processing industry produced 20 tons of latex and 410000 liters of effluent per day in average (Mohammadi et al., 2010). In natural rubber processing plants, a ton of Standard Malaysian Rubber Block produced with 20.5 m^3 effluents which containing 21.3 kg of BOD (Sharib and Halog, 2017). Latex processing effluent generally included wastewater, small amounts of uncoagulated latex, carbohydrates, carotenoids and serum (Mohammadi et al., 2010) which contain large quantities of protein, sugar, organic and inorganic salt.

In terms of inorganics, sulfuric acid utilized in coagulation process of skim latex produce high concentration of sulphate in and later discharged as effluent. The sulphate then will produce hydrogen sulphate (H_2S), during biological anaerobic treatment process. Zinc oxide are used in the vulcanization latex. As a result, it will produce high concentration of zinc in the effluent. Long term exposure of zinc will cause nausea and anaemia. Genetic of aquatic life also will be affected and changed due to the unknown chemicals. Therefore, untreated latex wastewater is not allowed discharge to rivers and streams. Therefore, implementation of proper wastewater management whether biologically or chemically treated is required in order to minimize the pollution and future environmental arising issues (Fakhri et al., 2021, Hena et al., 2021, Liu et al., 2021, Song and Liu, 2021, Sorinolu et al., 2021, Yoo et al., 2020, Zhang et al., 2020).

Wastewater treatment using biological processes is widely used in rubber industries in Malaysia. Pond technology is the most common method being used. Although pond technology can remove 95% of biological oxygen demand (BOD) from LPW ineffective design and optimum operation, pond technology needed a large area, requires high operating cost and long hydraulic retention time (90 – 120 days) (Ibrahim et al., 1980). Electrochemical method also had been introduced in Malaysia to treat the rubber wastewater. But this method is not successful due to the concomitant energy utilization.

Previously, Atagana et al. (1999) found that effluents from latex processing support microbial growth. The concentrated latex wastewater was identified. The efficiency for organic removal by isolated bacterial was evaluated. Another study showed that,

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after 40 hours of cultivation, 34% of chemical oxygen demand (COD) was removed by Rubrivivax gelatinosus and Thiobacillus sp. (Mohammadi et al., 2010). The purple non-sulphur photosynthetic bacteria (PNSB) isolated from rubber sheet wastewater in Thailand can reduce the chemical oxygen demand and biological oxygen demand of wastewater to 90% under suitable conditions. Therefore, this study aims to investigate the potential occurrence of viable growth in treated LPW via morphological properties classification base on basic microbiological approach. The effectiveness of a biological wastewater treatment is highly dependent on the viable microorganism community as wastewater nutrient removal involves dynamic and complex biochemical metabolisms either under aerobic or anaerobic conditions.

2 Materials and methods

2.1 Description of real-rubber wastewater treatment plant

Rubber wastewater processing involves various stages which comprises of physical, chemical and biological treatment. Wastewater samples were collected at the equalization tank, chemical treatment process and lastly in final discharge. The wastewater was characterized for physicochemical properties.

2.2 Sample preparation

Samples of wastewater were collected specifically from activated sludge from latex processing effluent. Sample were collected using sterile screw-capped bottle. Sample was stored at a temperature of 4 ^{o}C prior to further analysis.

2.3 Inoculation and purification

The entire samples were serially diluted to 10-10 prior inoculation. Isolation and purification of bacterial cultures were performed following microbiological standard procedure by using nutrient agar and 0.85% sodium chloride. Pure cultures were then stored in nutrient agar plate and store at refrigerant at 4 ^{o}C until needed.

2.4 Motility

A drop of ultrapure water was placed on the centre of the slide. A single isolated colony was taken up from nutrient agar surface then transferred into the water drop and spread by sterilized wire loop. A slide was covered on the drop of water and the motility of bacteria was identified under microscope.

2.5 Fixed smear preparation

A drop of ultrapure water was placed on the centre of the slide. A single isolated colony was taken up from nutrient agar surface then transferred into the water drop and spread the emulsion evenly on the surface of the glass slide to give a relatively thin and smooth layer by sterilized wire loop. The slide was quickly passed through flame of Bunsen burner three times.

2.6 Gram staining

A slide with fixed smear flooded with several drops of crystal violet for 20 seconds, the slide then washed with water to remove excess crystal violet. Gram's iodine was added to cover the smear for 30 seconds. Poured off the iodine and washed through with acetone. De-colorization has occurred when the solvent flows colourlessly from the slide. Acetone then was wash gently with running water tap water. Safranin then covered the smear for 30 seconds. The safranin then was gently washed for a few seconds; the smear then was blotted and dried at room temperature. Fixed slides then were examined under microscope.

2.7 Bacterial colony classification

Preliminary study of isolates was done by using standard biochemical tests including Gram staining to observe the bacterial morphology properties and motility test for motility behaviour. All the results were then carried out with references to Bergey's Manual of Systematic Bacteriology for classification. Morphology characteristics were based on the characteristic features of bacterial growth in culture. Whole colony, pigmentation, margin and elevation of colony of the bacteria were examined after 24 hours incubated on nutrient agar.

2.8 Analytical Analysis

The wastewater collected were characterized in terms of temperature, dissolved oxygen (DO), Total suspended solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), heavy metal, ammonia nitrogen, pH, and turbidity according to the standard methods (APHA, 2005). The temperature is measured by using APHA method and turbidity is measured by using the 2100 N turbid meter. While total suspended solid is measured by using HACH method 8006. For the dissolved oxygen, the reading is measured by using YSI model 5000 (S/N: 08F 101429). While the concentrations of heavy metal and ammonia nitrogen was measured using HACH method.

3 Result and discussion

3.1 Isolation and identification of bacteria

Biological treatment commonly may not be suitable for rubber wastewater treatment. Often, coagulation and flocculation are applied for rubber wastewater treatment. However, this study shows that certain bacteria are able to grow aerobically in treated rubber wastewater indicates their tolerance to rubber wastewater. Seventeen bacterial strains were isolated from collected effluent sample. Out of the 17 isolates purified, 9 were Gram positive and another 8 were Gram negative. Results of microscopic observation and Gram staining were summarized in Table 1.

Collectively, a mixture of certain genus in consortium are effective in nutrient removal significantly in ammonium and phosphate (Pillai and Girish, 2014). Purple nonsulfur bacteria have shown significant roles in H_2S elimination in rubber wastewater (Kornochalert et al., 2014). It was found that certain major genera are significant to rubber processing wastewater. Sulfate-reducing bacteria possessed high growth rate in rubber processing wastewater (Tanikawa et al., 2016). Promnuan et al. (2020) investigated the potential of *Desulfovibrio* sp., *Desulfitibacter* sp., *Dethiosulfatibacter* sp., and *Clostridium* sp. in biohythane production and sulfate removal under anaerobic condition. *Clostridium* sp., *Citrobacter* sp., *Klebsiella* sp., and *Enterobacter* sp.were found possessed optimum growth and capable of producing biohydrogen in rubber latex processing wastewater (Nguyen et al., 2018). Some genera have shown outstanding removal rate of pollutants. Tanikawa et al. (2019) have discovered key microbes for nitrogen removal in rubber wastewater degradation with highest occurrence of rubber-degrading bacterium *Gordonia* sp. with 28.6% dominance rate. These bacteria were found to achieve high removal efficiency of residual rubber by both coagulation and biological degradation (Tanikawa et al., 2020). Pseudomonas sp. is known as indigenous bacteria in rubber wastewater. Recently, *Pseudomonas* sp. and *Stenotrophomonas* sp. demonstrated ability to degrade hazardous chemical compound such as 2-mercaptobenzothiazole (MBT) (Krainara et al., 2020).

Table 1 Summary of morphological characteristics and presumed genus

No	Colony pigmentation, form, margin, and elevation	Bacteria shape, arrangements, motility and Gram stained	Expected genus
Gram-positive cultures			
1	Cream, Circular Entire, Flat	Rod, Coccobacillus, Gliding, Gram Positive	ND
2	Cream, Circular Entire, Raised	Coccus, Clusters Nonmotile, Gram Positive	*Aerococcus, Gemella, Micrococcus, Pediococcus, Saccharococcus, Staphylcoccus, Stomatococcus, Propionibacterium*
3	White, Circular Entire, Flat	Coccus, Chains	*Nonmotile, Gram Positive Aerococcus, Brachybacterium, Brevibacterium, Caseobacter, Lactococcus, Leuconostoc, Melissococcus, Saccharococcus, Streptococcus, Trichococcus*
4	Cream, Circular Entire, Raised	Coccus, Pairs Nonmotile, Gram Positive	*Agromyces, Aerococcus, Enterococcus, Gemella, Lactococcus, Leuconostoc, Micrococcus, Pediococcus, Staphylococcus, Stomatococcus, Streptococcus, Trichococcus, Propionibacterium*
5	Cream, Circular Entire, Pulvinate	Rod, Pairs Flagella, Gram Positive	*Arthrobacter, Amphibicillus, Bacillus, Clostridium, Caryophanon,Eubacterium, Sporalactobacillus, Kurthia, Lactobacillus, Listeria, Microbacterium, Rarobacter*
6	White, Circular Entire, Convex	Coccus, Clusters Flagella, Gram Positive	*Enterococcus, Kurthia, Listeria, Jonesia, Marinococcus, Planococcus, Pimelobacter, Vagococcus*
7	White, Circular	Erose, Raised Rod, Pairs Gliding, Gram Positive	*Actinomycetes, Eubacterium, Flexibacter*
8	White, Concentric Undulate, Crateriform	Rod, Chains Gliding, Gram Negative	Beggiatoa, Cedecea, Sporocytophaga,Thiobacillus, Thiothrix
9	White, Circular	Irregular, Raised Rod, Chains Gliding, Gram Negative	*Beggiatoa, Cedecea, Thiobacillus, Sporocytophaga, Thiothrix*
Gram-negative cultures			
1	Cream, Circular Entire, Convex	Rod, Chains, Gliding, Gram Negative	*Beggiatoa, Capnocytophaga, Cedecea, Chitinophaga; Cytophaga, Flexibacter, Desulfotomaculum, Lysobacter, Sporocytophaga, Thiothrix, Thiobacillus*
2	Orange, Circular Entire, Raised	Coccus, Chains Gliding, Gram Negative Agitococcus	
3	Blue – Green, Circular Lobate, Flat	Coccus, Clusters Gliding, Gram Negative	*Achromatium, Metallogenium , Thiosphaera, Vitreoscilla*
4	Yellow, Circular Entire, Convex	Rod, Pair Gliding, Gram Negative	*Beggiatoa, Capnocytophaga, Cedecea , Cytophaga, Herpetosiphon, Microscilla, Sphingobacterium, Sporocytophaga, Thiobacillus, Thiothrix*
5	Cream, Puniform Erosa, Hilly	Rod, Clusters Gliding, Gram Negative	*Beggiatoa, Cedecea, Flexibacter, Lysobacter, Thiobacillus, Thiothrix, Sporocytophaga*
6	Cream, Circular Entire, Convex	Rod, Pairs Flagella, Gram Negative	*Acidiphilium, Budvicia, Cedecea, Buttiauxella, Citrobacter, Serratia, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Leclercia, Enterobacteriaceae, Hafnia, Kluyvera, Morganella Pantoea, Nitrosomonas Protues, Providencia, Salmonella, Xenorhabdus, Yokenella, Oscillospira, Thermothrix, Thiobacillus, Thermothrix*
7	Yellow, Circular Entire, Raised	Rod, Chains Gliding, Gram Negative	*Beggiatoa, Cedecea, Capnocytophaga, Cytophaga, Chitinophaga, Desulfotomaculum, Flexibacter, Herpetosiphon, Microscilla, Sporocytophaga, Thiobacillus, Thiothrix*
8	White, Circular Entire, Convex	Rod, Chains Gliding, Gram Negative	*Beggiatoa, Thiothrix, Thiobacillus, Sporocytophaga*

Table 2 Results of characterization of wastewater from rubber processing system

Parameters	Units	Rubber wastewater processes/ concentrations		
		Equalization	Chemical treated water	Final discharge
pH	-	7.6	6.7	6.0
Temperature	^{o}C	33.4	32.8	31.8
Dissolved Oxygen (DO)	mg/L	6.74	0.14	8.32
Turbidity	NTU	399	8.54	7.07
Total Suspended Solid	mg/L	327	17	4
Biological Oxygen Demand (BOD)	mg/L	583	15.24	8.06
Chemical Oxygen Demand (COD)	mg/L	577	230	18
Ammonia Nitrogen	mg/L	6.2	5.7	22.2
Zinc	mg/L	1.5	0.26	1.84
Manganese	mg/L	0.48	0.079	0.16
Iron	mg/L	2.93	0.482	0.473
Nickel	mg/L	0.08	0.019	0.007

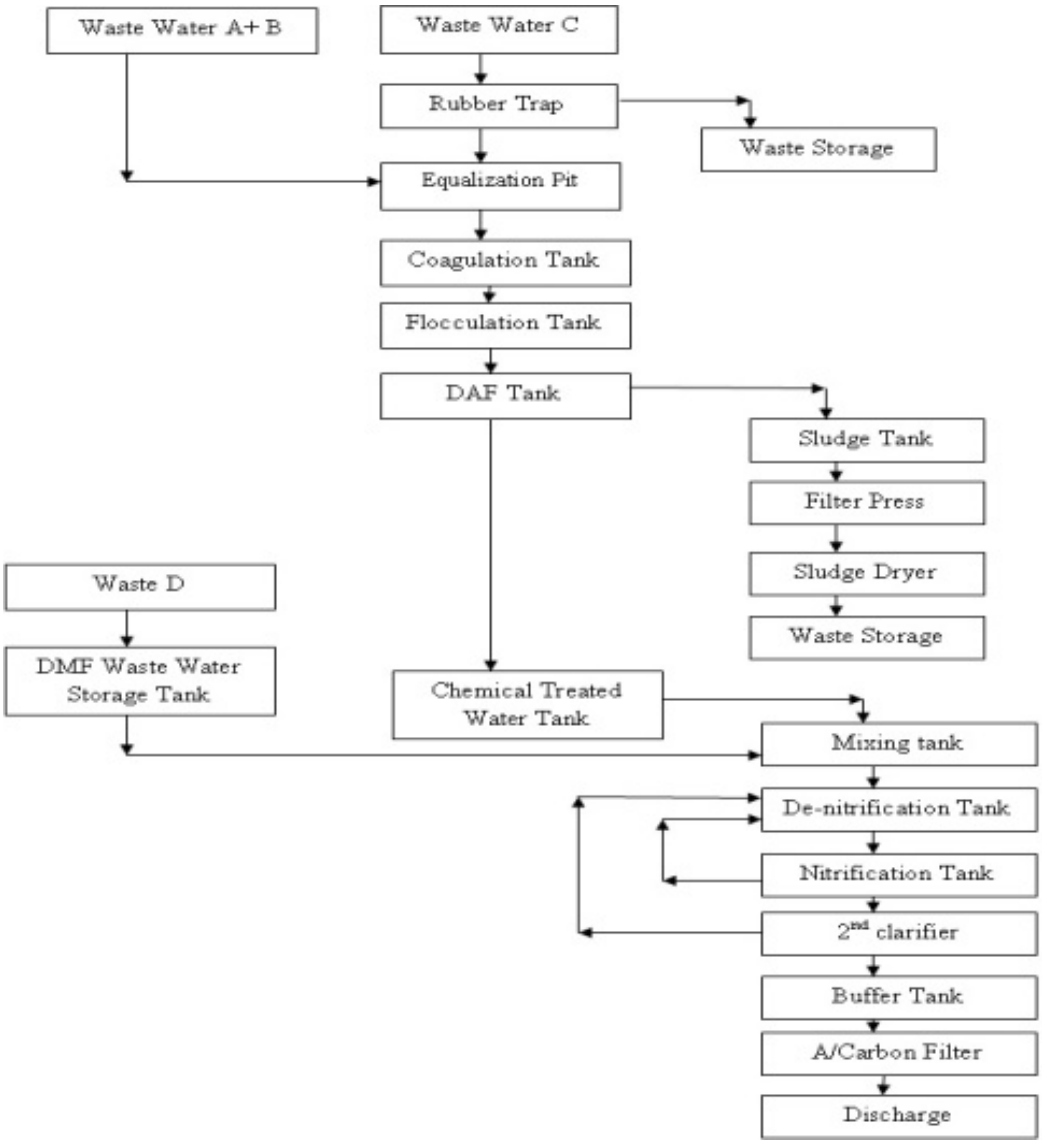

Figure 1 The real-rubber wastewater treatment processes

Rubber wastewater is readily complex with the presence of various chemical compounds used in the processing line. The ability of microorganisms to grow and treat the wastewater is utmost important to ensure minimal environmental impact of the treated wastewater discharged. Recent evidence suggests that there are significant roles of indigenous microorganism in rubber wastewater treatment. Factors found to be influencing their growth, abundance and biodegradability of nutrients and specific compounds in rubber wastewater also have been explored in several studies. This indicates a need to understand the various perceptions of microbial contributions particularly in rubber wastewater. The studies presented thus far provide evidence that there is vast potential in exploration of the biodiversity of microorganisms in rubber wastewater further in depth and to understand their biochemical mechanisms in biodegradation.

3.2 Real-wastewater characteristics

The physicochemical characteristics of collected rubber wastewater were determined. The results are shown in Table 2. The results showed the performance of the rubber wastewater treatment processes according to the real-treatment plants set-up. The variation of the wastewater characteristics exhibits the processes occurred in plants subsequent to the manufacturing production segments which involves orderly processes as shown in Figure 1. At site, the pH was optimized throughout the processes to maintain the treatment requirement. Temperature were not controlled by the operator as the plant were let operated at ambient temperature. The DO started steadily at 6.74, declined to 0.14 and raised to 8.32 mg/L respectively. Similarly, the BOD and COD were also reduced significantly to from 583 and 577 mg/L to 8.06 and 18 mg/L. The biomass showed reduction and it is indicated by the turbidity where it was reduced significantly from 399 to 7.07 NTU. Heavy metals i.e zinc, manganese, iron and nickel were analysed accordingly.

4 Conclusions

Seventeen isolates were successfully isolated from rubber-based wastewater and their morphological properties were determined. Nine Gram-positive cultures and 8 Gram-negative cultures were isolated. The number of isolated cultures accounted 53% of Gram-positive and 47% of Gram-negative cultures. Morphological observation allows rapid determination of bacteria in rubber wastewater to indicate viable cell growth. Based on these preliminary results, it can be concluded that some microorganisms always existed in the activated sludge collected from rubber wastewater. This concludes that rubber wastewater consists of high microbial diversity. However, the microbial abundance and bacterial identification shall be further validated using molecular technique.

Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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References

Andersen, L.S., Bregnhoj, H., Lonholdt, J. 2005. Water and wastewater management in the tropics. London: IWA Publishing.

Atagana, H.I., Ejechi, B.O., Ayilumo, A.M., 1999. Fungi Associated with Degradation of Wastes from Rubber Processing Industry. Environ. Monit. Assess. 55, 401-408.

Fakhri, H., Shahi, A., Ovez, S., Aydin, S., 2021. Bioaugmentation with immobilized endophytic Penicillium restrictum to improve quorum quenching activity for biofouling control in an aerobic hollow-fiber membrane bioreactor treating antibiotic-containing wastewater. Ecotoxicol. Environ. Saf. 210, 111831.

Hena, S., Gutierrez, L., Croué, J.-P., 2021. Removal of pharmaceutical and personal care products (PPCPs) from wastewater using microalgae: A review. J. Hazard. Mater. 403, 124041.

Ibrahim, A., Sethu, S., Karim, M.Z., Esa, Z. 1980. Anaerobic/facultative ponding system for treatment of latex concentrate effluent. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=US201302826289.

Kornochalert, N., Kantachote, D., Chaiprapat, S., Techkarnjanaruk, S., 2014. Bioaugmentation of latex rubber sheet wastewater treatment with stimulated indigenous purple nonsulfur bacteria by fermented pineapple extract. Electron. J. Biotechnol. 17, 174-182.

Krainara, S., Suraraksa, B., Prommeenate, P., Thayanukul, P., Luepromchai, E., 2020. Enrichment and characterization of bacterial consortia for degrading 2-mercaptobenzothiazole in rubber industrial wastewater. J. Hazard. Mater. 400, 123291.

Liu, J., Sun, F., Zhang, P., Zhou, Y., 2021. Integrated powdered activated carbon and quorum quenching strategy for biofouling control in industrial wastewater membrane bioreactor. J. Clean. Prod. 279, 123551.

Mohammadi, M., Man, H.C., Hassan, M.A., Yee, P.L., 2010. Treatment of wastewater from rubber industry in Malaysia. Afr. J. Biotechnol. 9, 6233-6243.

Nguyen, H.-L.H., Le, T.D., Thi, B.-D.N., Nguyen, T.-A.D., 2018. Biohydrogen fermentation from rubber latex processing wastewater pretreated by aluminium sulphate flocculation. Int. J. Environ. Waste Manag. 21, 141-154.

Pillai, H., Girish, K., 2014. Rubber processing industry effluent treatment using a bacterial consortium. Int. J. Curr. Microbiol. Appl. Sci. 3, 775-782.

Promnuan, K., Higuchi, T., Imai, T., Kongjan, P., Reungsang, A., O-Thong, S., 2020. Simultaneous biohythane production and sulfate removal from rubber sheet wastewater by two-stage anaerobic digestion. Int. J. Hydrog. Energy. 45, 263-274.

Sharib, S., Halog, A., 2017. Enhancing value chains by applying industrial symbiosis concept to the Rubber City in Kedah, Malaysia. J. Clean. Prod. 141, 1095-1108.

Song, H., Liu, J., 2021. Forward osmosis membrane bioreactor using Bacillus and membrane distillation hybrid system for treating dairy wastewater. Environ. Technol. 42, 1943-1954.

Sorinolu, A.J., Tyagi, N., Kumar, A., Munir, M., 2021. Antibiotic resistance development and human health risks during wastewater reuse and biosolids application in agriculture. Chemosphere. 265, 129032

Tanikawa, D., Kataoka, T., Sonaka, H., Hirakata, Y., Hatamoto, M., Yamaguchi, T., 2020. Evaluation of key factors for residual rubber coagulation in natural rubber processing wastewater. J. Water Process. Eng. 33, 101041.

Tanikawa, D., Syutsubo, K., Hatamoto, M., Fukuda, M., Takahashi, M., Choeisai, P.K., Yamaguchi, T., 2016. Treatment of natural rubber processing wastewater using a combination system of a two-stage up-flow anaerobic sludge blanket and down-flow hanging sponge system. Water Sci. Technol. 73, 1777-1784.

Tanikawa, D., Yamashita, S., Kataoka, T., Sonaka, H., Hirakata, Y., Hatamoto, M., Yamaguchi, T., 2019. Non-aerated single-stage nitrogen removal using a down-flow hanging sponge reactor as post-treatment for nitrogen-rich wastewater treatment. Chemosphere. 233, 645-651.

Yoo, K., Yoo, H., Lee, J., Choi, E.J., Park, J., 2020. Exploring the antibiotic resistome in activated sludge and anaerobic digestion sludge in an urban wastewater treatment plant via metagenomic analysis. J. Microbiol. 58, 123-130.

Zhang, C., Wang, X., Ma, Z., Luan, Z., Wang, Y., Wang, Z., Wang, L., 2020. Removal of phenolic substances from wastewater by algae. A review. Environ. Chem. Lett. 18, 377-392.