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REPORT

ANALYSIS OF VARIOUS TYPES OF WATER SAMPLES USING DIFFERENT QUANTITATIVE METHODOLOGIES FOR STANDARDIZATION PURPOSES IN THE ENVIRONMENTAL CHEMISTRY & MICROBIOLOGY LABORATORY OF SURABAYA INSTITUTE OF STANDARDIZATION AND INDUSTRIAL SERVICES

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INTERNSHIP REPORT

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Analysis of Various Types of Water Samples Using Different Quantitative Methodologies for Standardization Purposes in the Environmental-Chemistry and Microbiology Laboratory of Surabaya Institute of Standardization and Industrial Services

We hereby declare that this internship project is from the student's own work. The internship project has been read and presented to i3L's Examination Committee. The internship project has been found to be satisfactory and accepted as part of the requirements needed to obtain an i3L bachelor's degree.

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ABSTRACT

Water is a vital part of the ecosystem. Not only is it significant for human survival and is essential for human health, it is also the main constituent of the earth's hydrosphere and a crucial element which allows chemical reactions to take place. On the other hand, water is also highly affected by man made pollutants. Illegal and unmonitored waste dumping to water bodies contributes to umbrella issues that affect the human population and the environment. Understanding this, water monitoring has been made a requirement to maintain water quality and to determine possible bioremediation processes in case of contamination in Indonesia. BSPJI is a governmental institution that focuses on the standardization and the certification of, amongst other samples, different kinds of water in Indonesia. The institution is responsible to control and monitor the quality of the samples through various quantitative methodology analyses. The water in question includes drinking water, wastewater, and water obtained from environmental water bodies. This report aims to deliver the results of the tests done for said standardization and certification processes during the internship period. Amongst the many parameters done in the chemistry laboratory of BSPJI, the results of 6 parameters from the environmental-chemistry laboratory and 3 parameters from the microbiology laboratory were taken into account for this report. From the results obtained, most of the samples tested in the institution are within the safe range of SNI's threshold, with only some exceptions such as the wastewater from slaughterhouses and drinking water from a few new companies whose product is not yet certified for nation-wide consumption.

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INTRODUCTION

1.1 Balai Standardisasi dan Pelayanan Jasa Industri (BSPJI) Profile

Balai Standardisasi dan Pelayanan Jasa Industri (BSPJI) Surabaya is a governmental institution under the Ministry of Industry authorized to test, analyze, and give standardization certification for different products (Kementrian Perindustrian, 2020). An institution as such, is also known as an LsPro, which is also responsible to determine the commercial and disposal eligibility of the products as well as to control its quality, to ensure the safety of everyone consuming and coming into contact with the products (Indonesia, 2014).

The institution was first established on March 4th 1947 in Klaten, Central Java with the name "Balai Penyelidikan Kimia" under the Ministry of Prosperity (Kementerian Kemakmuran). Over the years, BSPJI has undergone several changes in both location and name. From Klaten, the standardization institution was first moved to Solo on April 25th 1950 under the name of "Balai Penyelidikan Kimia" before it permanently moved to Surabaya in May 1961. In Surabaya, the institution moved location three times before finally settling at Jl. Jagir Wonokromo 260, Surabaya on November 10th, 1975 under the of name "Balai Penelitian dan Pengembangan Industri Surabaya" in accordance with Keputusan Menteri Perindustrian No. 257/MK/SK/8/1980 on August 26th 1980. This change was done to specify the organizational structure as well as the responsibilities of the institution. On 2006 however, following the separation of the ministry of industry and the ministry of trading (perdagangan), the name of the institution changed for the last time in accordance with Surat Keputusan Menteri Perindustrian No. 49/M-IND/PER/6/2006 to "Balasi Standardisasi dan Pelayanan Jasa Industri Surabaya (BSPJI)".

Apart from being an authorized certification institution which aims to push forward the quality of industrial products in Indonesia, internally BSPJI is also responsible to improve one's competence through numerous training as well as to provide technical support and services to different industry sector sizes. At its core, BSPJI aims to support the development of different industrial sectors in Indonesia as supported by the Ministry of Industry, which includes the improvement of the overall quality of the products commercialized in Indonesia.

The services provided by BSPJI includes sample testing, analysis, and certification. For testing, the samples viable/accepted are as follows:

1. The product/outcome of the metal industry such as ferro (steel, concrete, steel/cast iron, and zinc-coated steel sheet) and non-ferrous metals (metal seeds, bronze, alloys, and aluminum), as well as building materials.
2. Chemical produces and minerals namely fertilizers, baking soda, basic cleaning agents, mineral acid, essential oil, edible oil, rock phosphate, kaolin, ironsand, and silica sand.
3. Various water samples including processed-water, boiler water, wastewater, industrial wastewater, clean water, bottled drinking water (AMDK), demineralized water, and raw water obtained from environmental water bodies (such as from rivers and wells).
4. The product/outcome of the food and beverage industry such as soft drinks, finger food, animal feed, iodized salt consumption, and others.

In terms of analysis, the institution focuses on using different quantitative methods to measure, process, and test the samples. The results of said analyses will then be compared to SNI (Indonesia's National Standard) to see if the samples are eligible for certification. If the outcome of the tests are within the threshold and safe-range of SNI, certification will be issued. On the other hand, if the sample is deemed not safe enough for consumption/the environment, the certification will not be issued. Nonetheless, the results of the tests will still be given back to the customers (sample-giver), so that preventive and/or recovery measures could be taken in accordance with the results obtained. A follow-up testing is usually required for samples that aren't in the range of the threshold requirements from SNI, to make sure that necessary steps have been taken to fix the issue.

1.2 BSPJI Organizational Structure

As BSPJI is a research and standardization institution under the Ministry of Industry, the organizational structure is set under Peraturan Menteri Perindustrian tentang Organisasi dan Tata Kerja Balai Besar Riset dan Standardisasi Industri number **49/M-IND/PER/6/2006** which states that the responsibilities of the members of the institution are as follows:

1. Head of the Institution

The head of BSPJI is responsible to set and uphold the principles of coordination, integration, and synchronization inside the institution as well as to lead, coordinate, and give guidance on the implementation of the different analyses from his/her subordinates.

2. Sub-Administration Section

The members of the sub-administration section are responsible to do all the tasks inclusive to personnel affairs, finance, inventory of state property, correspondence, equipment, archives, housekeeping, coordination and planning of programmes in the institution, preparation of evaluation materials, as well as library management.

3. Program and Competency Development Section

The members of the program and competency development section are responsible for the preparation of the materials needed for program preparation and competency development in the institution, solely for the purpose of research and development.

4. Standardization and Certification Section

The members of the standardization and certification section are in-charge of the preparation of all the materials needed for the formulation and application of the standards, as well as for the analysis and certification for raw materials, auxiliary materials, different analytical processes, equipment/machineries, as well as product results.

5. Technical Services Development Section

The members of the technical services development section are responsible for the preparation of the materials regarding marketing, cooperation, collaborations, and promotions. Additionally, they are also in-charge of publicly-available information (such as ones displayed on websites and student thesis), dissemination, and the utilization of research and development results.

1.3 BSPJI Main duties, Goals, Vision, & Mission

According to PERMEN-Perindustrian Republik Indonesia Nomor: 49/M-IND/PER/6/2006 about the Organizational Structure and Work Responsibilities of BSPJI, the main duties, goals, vision, and missions of the institution are as follows:

1.3.1 Main duties & Functions

The main responsibilities of BSPJI is to carry out research, development, cooperation, standardization, testing, certification, calibration, and competency development activities in fields related to the analysis (such as in the electronics and telematics, chemistry, physics, and calibration sector) all in accordance with the technical policies set and upheld by the head of the institution. In doing so, BSPJI should carry functions including:

1. The implementation of research and development in the industrial technology sector for raw materials, auxiliary materials, processes, equipment/machinery, product results, and the prevention of industrial pollution.
2. Program and competency development in research/R&D areas.
3. Formulation and application of standards, analysis, as well as certification in raw materials, auxiliary materials, processes, equipment/machinery, product results, and the prevention of industrial pollution.
4. Marketing, cooperation, promotion, informational services, circulation and utilization of developmental research results.
5. Implementation of programs for personnel affairs, finance, public relations, logistics, archives, housekeeping, coordination of material and program planning and evaluation, as well as library management.

1.3.2 Goals

The main goals of the institution are as follows:

1. Increasing technological coherence in the fields of electronics and telematics, physical, calibration, and chemical machinery and equipment.

2. Increasing the ability to provide JPT (Jabatan Pimpinan Tinggi; Governmental Position) to help support and sustain the growth and development of the national industry.

3. Improving the ability of human resources in the development and knowledge of the technologies that supports focused activities in the fields of the analysis.

4. Increasing the quality of PDB (Produk Domestik Bruto) which is the value for goods and services produced by various production units in a country within a certain period of time, to also increase awareness of the people in Indonesia to consume local products.

1.3.3 Vision

The vision of the Institution is a portrait of its aspired future, namely:

1. To become a leading research and standardization institution that becomes an ally in the national electronics and telematics industry.

2. To become one of Indonesia's production-based institution that is able to serve national and international needs by 2025.

Through their vision, BSPJI aims to become an institution that is able to meet the needs of the industry, supported by the research and development done inline with market demands that continues to grow. As an Ls-PRO, BSPJI also aims to become more independent in conducting research to be able to effectively produce new and leading results in the fields of its expertise, in order to help similar institutions achieve the same degree of excellence.

1.3.4 Mission

In order to achieve the aforementioned vision, some steps are taken which includes:

1. Producing high-quality research and engineering design mainly for large-scale industries in the field of the institute's expertise such as physics, chemistry, and calibration.

2. Producing excellent conformity services and results for the testing, analysis, calibration, and certification of the samples submitted to BSPJI.

3. Increasing the competency of the human resources inside the institution to become leading pillars in the research and certification industry.

1.3.5 Main Values

In realizing said visions and missions, BSPJI has developed some main values to be used as reference by the members of the institution as a standard of attitude, so that in completing their responsibilities, they are able to achieve maximum excellence and optimum performance. The values in question are as follows:

1. Providing excellent service to encourage BSPJI employees to carry out their daily tasks and responsibilities while always prioritizing customer satisfaction, both internally and externally. This condition is to be achieved through intensive and developmental training which will be held continuously over a period of time in addition to the standardization work procedures carried out daily at the laboratories.

2. Preparing a conducive environment for the workers to grow into innovative and creative individuals so that in doing the responsibilities they are entitled to, all members of the institution can meet the demands of the ever-growing electronics and telematics, calibration, physics, and chemical industry in both managerial and maintenance aspects.

3. Building a compact and reliable team so that each member of the institution will be able to give mutual help and support to each other, whilst also promoting togetherness in the workplace. This will be achieved through cohesive training to empower confidence, high integrity, independence, and professionalism in each individual. Synchronization, coordination, and communication are also main aspects to be considered to build a solid working environment. The organizational structure must be upheld at all times so that every member of the institution knows their own responsibilities well.

4. Constructing and maintaining a healthy and broad working network, both internally and externally. Internally with all the members of BSPJI, and externally with other research and calibration institutions who are in the same working field as the institution. These external parties include state-owned and private-owned, international and domestic laboratory facilities, universities, industrial associations, and other relevant institutions.

1.4 Description of the Chemistry Laboratory in BSPJI

There are four main laboratories in BSPJI, namely the chemistry, physics, electronics and telematics, and calibration laboratory. All of the departments have different responsibilities and do different kinds of analysis with different kinds of samples daily. In doing said analyses, all of the laboratories refer to Peraturan Presiden No. 47 Tahun 2011 about "Jenis dan Tarif atas Jenis Penerimaan Negara Bukan Pajak yang Berlaku pada Kementerian Perindustrian" which states all of the necessary testing thresholds and policies. The internship period was spent only at the chemistry laboratory as it is the most suitable with the Biotechnology study program.

The chemistry laboratory, as its name implies, conducts mainly quantitative chemical analyses on the samples brought in for testing. The laboratory has been given a certificate of accreditation of SNI ISO 17025:2017 from KAN (National Accreditation Committee). The number of said accreditation is LP-213-IDN. As the scope of the chemical industry is considerably big, the chemistry laboratory in BSPJI is further divided into three smaller and more-focused laboratories namely the consumables (food and beverages) laboratory, the microbiology laboratory, and the environmental-chemistry laboratory. As all of these laboratories perform different analyses with different samples, each has their own lab space/room on the second floor of the institution.

1.4.1 Environmental-Chemistry Laboratory

As previously mentioned, the environmental-chemistry laboratory is one of the three laboratories under the chemistry department in BSPJI. The laboratory follows SNI with the certification number of SNI ISO/IEC 17025:2017 and mainly performs quantitative analysis on environmental-related samples such as wastewater (obtained from the customers), river and well water (obtained through sampling), and raw water (obtained from water sources). Additionally, drinking water (AMDK) is also a big part of the samples submitted to the laboratory for analysis. These said samples are primarily tested for heavy metals, organic compounds, solids, and other parameters which are dangerous if released untreated to the water bodies in the environment. There are a total of around 40 parameters of analysis done in the laboratory, focused on instrumental, titrimetry, and gravimetric analysis

1.4.2 Microbiology Laboratory

The microbiology laboratory at BSPJI is also one of the three laboratories under BSPJI's chemistry department. Similar to the environmental-chemistry laboratory, all of the materials, equipment, and standard methods used inside the laboratory follows SNI and has also been certified by the KAN, which certification was issued on February 27, 2019. The analysis performed in the microbiology lab mainly revolves around the quantification of different microorganisms grown in different agar to check and issue for standardization certification of drinking water. Other than drinking water, every once in a while, wastewater and environmentally-obtained water samples are also tested. For wastewater, some customers handed in the samples to check the purity of their filtration system. On the other hand, it is a part of the government's regulation for BSPJI to analyze and test environmentally-obtained samples such as raw water and river water. As the institution has the instruments and the means available for testing, the government has requested BSPJI to do monthly testing for these water bodies to ensure the safety of the people living close to the source of the water and for the safety of the environment as well.

INTERNSHIP ACTIVITIES

2.1 Working Conditions

The working environment in BSPJI Surabaya is overall very constructive and insightful. Over the course of the four month internship in their chemistry laboratories, 2 months were spent in the environmental-chemistry laboratory (September - October), and 2 months were spent in their microbiology laboratory (November - December). Similar to other institutions, BSPJI follows a typical working schedule starting from 7.30 AM to 4 PM every Monday to Thursday. On Fridays, the members of BSPJI are expected to arrive at 7 AM to follow the weekly morning exercise and volleyball practice session. A lunch break is also a part of the schedule, where everyone has the chance to take a break from 12 to 1 PM. On some days of the week, when there are a lot of new incoming samples to be analyzed, working overtime is common, however the internship students are not expected to stay past 4 PM and are usually asked to directly go home when the clock strikes four. Additionally, a flag ceremony is done every monday after the 17th of every month, where everyone is expected to wear their alma maters, listen to the updates from the head of the institution, and follow the ceremony earnestly.

2.2 Internship Tasks & Experiences

As previously mentioned, a four month internship was conducted in two of the three chemistry laboratories in BSPJI. In the environmental-chemistry laboratory, the laboratory work includes sample preparation, sample testing using titrimetric, gravimetric, and instrumental analysis through various available instruments, and sample destruction, including instrumental sanitation. Whilst doing said analyses, the results obtained were also given as data for this report. On the other hand, in the microbiology laboratory, the laboratory work includes sample preparation, agar making, instrument sterilization, as well as various cell culturing and counting methodologies. The results of said analysis is also given as data for this report. The analysts in the laboratories have given the interns great responsibility in doing said analysis, hence a lot of insightful experiences were gained through hands-on practice in doing most of the analysis by ourselves, with the guidance and help of the analysts.

2.3 Comparison between Theoretical and Practical

As a part of the pandemic-strucked cohort (Cohort 2019), most of the laboratory sessions during the second and third year of study (semesters 2-6) were conducted online. This was of course still very insightful and has equipped students with a lot of knowledge, however getting hands-on experience and trying to operate laboratory equipment first hand is so much different then doing it online. Over the three years of study in i3L, a lot of theoretical knowledge about the principles of the analysis conducted in BSPJI was studied and has been put into practice and good use during the internship at BSPJI. All of said knowledge gained at the university has helped build a strong foundation in understanding the principles and equipment used in the analysis done both in the environmental-chemistry and microbiology laboratory. It is safe to say that all of the knowledge gained during the study in i3L has helped the understanding of the knowledge gained during the internship period and vice versa.

2.4 Difficulties & Problems Encountered During The Internship

During the internship period, there were not many difficulties and problems personally encountered. The problems BSPJI is currently facing can be seen on section 3.4, however none was affecting on a personal level . During the first few weeks, it was difficult to get to know the analysts and the other internship students, but as time passed by the relationship was able to be well-built. As the analysts taught and informed about the processes and techniques done and used in the analysis, more responsibilities were given to the interns to the point where the entirety of the analysis was able to be solely done by ourselves. This was a little bit burdening at first, but with a lot of practice and quality control, it was handled well and was very exciting. It is rare to be getting such a privilege as an intern, hence gratitude was given to the analysts. On some weeks, especially on Mondays and Tuesdays, there were always a lot of new samples to be checked, filtered, and prepared, which made the workload a little tougher in comparison to days at the end of the week, but with the help of the other interns and the analysts, all were also bearable. All in all, the internship period was very insightful and beneficial for the future.

INTERNSHIP DESCRIPTION

3.1 Background

Water is a natural, inorganic, tasteless, odorless, and colorless substance essential and important for all known forms of life, including the human body which, in its adult stage, is 60% made out of the matter (Boyd, 2019). According to Schulkin & Sterling (2019), the body needs and uses the element to power cells, organs, as well as tissues to regulate body temperature and control other bodily functions to maintain homeostasis. Due to its importance, the human body needs to consume at least 2.7L for women, and 3.7L of water for men daily to support its healthy regulation (Rosinger, 2020). In addition to its importance to the body, water is also the main constituent of Earth's hydrosphere (Fellows, 2019). This piece of information suggests that the vitality of water does not stop with the human body but also extends to the environment.

Water for the environment helps to maintain and restore natural flow regimes to wetlands such as rivers, creeks, and oceans. Healthy water bodies will carry water to homes, farms, schools, and businesses all while nourishing the ecosystem surrounding them to provide habitat and resources for both plants and animals along the route (Arthington, et al., 2018; Winter, et al., 2020). Also affected by the flow are agriculture, industry, as well as public health which all will benefit from a robust and productive water system (Goerner, S, & Quazi., 2021). Water supports the health of its surrounding bodies, which will in turn provide for the ecosystem and human needs. It links and maintains all ecosystems on the planet with the function of propelling growth, providing permanent dwelling for species living in it, as well as serving nutrients and minerals necessary to sustain physical life (Hy, Cheng, & Tao., 2017). All reasons stated, it is of utmost importance that the quality of water is kept at its prime both for human consumption as well as for the sake of the environment.

In Indonesia, commercially-available drinking water is one of the most leading, largest and most successful business enterprises (Godar, et al., 2016). Being an archipelagic country lying between the Indian and Pacific ocean, the country is located strategically along major sea lines connecting Oceania and Asia, making it the largest islandic country with around 76.38% of its region made up of water (Pati, et al., 2016). Amongst those water bodies are 5,590 rivers,

3,404 state-funded deep-bore wells, and a lot of naturally occuring springs all over the country (Rahadianto, Fariza, & Hasim., 2015; Oehler, et al., 2019). These natural water sources are what is used by the members of said industry to be treated and processed into commercially available drinking water (Air Minum Dalam Kemasan, AMDK) (Purwanto, 2020). With that much raw water available, it is a given that a lot of businesses are interested and have since ventured into this profitable opportunity. To this day, there are as many as 541 bottled water companies in the country (Nastiti, et al., 2017). However, quantity and following the business trend isn't what is important in this industry, but rather safety and quality. As water is an essential substance for human life, it is crucial for humans to consume pure and healthy water for the body to be able to healthily regulate. That said, the quality of said drinking water is to be routinely monitored and checked for sanitation and contamination before it is able to be marketed to the public.

Wastewater problems are also one of the largest and most heavily discussed issues in the country. Being the fourth most populated country in the world with over 276 million citizens, almost 11% of the population still lacks access to an improved water source because the water bodies surrounding residential areas are heavily polluted and a large portion of the pollution comes from untreated industrial waste (effluent) (Sarin, et al., 2020). This reason, in addition to the large population growth rate of around 2% per year, has caused sustainability issues such as environmental degradation in Indonesia (Al Jufri & Pambudi, 2020). According to True, Jones, & Baumgartner (2019), over the last few years, although the change is not significant, small improvements and stabilization of certain parts of the environment have been reported in specific locations. Through the "Program for Pollution Control, Evaluation, and Rating (PROPER)" program the government has made regulations regarding wastewater disposal standards included in SNI that businesses are required to comply with to be permitted to be established in Indonesia (Wulan, et al., 2022). That said, wastewater has to be quality checked in a daily manner to further improve the stabilization of the environment as well as to ensure the health of the people residing around the industrial area.

BSPJI Surabaya, is one of the five BSPJIs in Indonesia responsible to analyze and test for the quality of, amongst other samples, water in Indonesia, specifically in Java. Drinking water, raw water (surface water, well water, natural spring water, and river water), as well as

wastewater are three of the main commodities tested and analyzed in the chemistry laboratory of the institution. As previously mentioned, the purity and safety of all these types of water are important for humans as well as for the environment as it greatly affects crucial matters such as health and sustainability. All reasons stated, the Indonesian government established the institution to perform standardization procedures and hand out certification to the businesses that are able to adhere to the standards previously set by SNI (Kusumawaty, 2017). The submission of said samples to the institution is mandatory, as the BSPJI certification of passing is not only important for the continuity of the company, but also to the environment and consumers of the products.

3.2 Scope of the Internship

The scope of this internship report mainly focuses on the different water samples analyzed in BSPJI's environmental-chemistry and microbiology laboratory. Amongst the many other samples submitted for standardization in BSPJI, water is specifically chosen because, as previously mentioned, it is one of, or not the most important substance to many living organisms. Additionally, as different sample types require different testing methodologies, focusing on one particular sample will help keep the report in a conductive and understandable manner. The report will focus on the results of said analyses and discuss how the testing helps prove the safety, consumability, and efficacy of both the sample and the testing method itself through the quality control done during each analysis.

3.3 Objectives

The main objective of this internship is to test and analyze the quality of the different kinds of water samples submitted to BSPJI, especially to the environmental-chemistry and the microbiology laboratorium. The results of said analyses and tests can be used as a benchmark for the overall water quality in Indonesia so that preventive and/or corrective measurements can be taken to produce better outcomes, both for consumable water and remediated water. In

doing so, knowledge, understanding, and skills related to instrumental usage, industrial product testing, and different parameter analysis techniques used to test for water quality samples is hoped to be achieved so that it could be used and be well- implemented in the future.

3.4 Current Problems in BSPJI

Similar to other laboratories and institutions in Indonesia, the chemistry laboratories in BSPJI also faced some challenges while conducting standardization and certification analyses. Specifically in the environmental-chemistry laboratory, most of the problems stem from the instruments and equipment used in the laboratory. Some of the equipment such as the beakers, Erlenmeyers, and pipettes, have been used for years. This prolonged usage has caused them to have some cracks, dents, as well as discoloration. Additionally, some of the instruments used in the laboratory are also in the same prolonged-usage condition. Although they are constantly calibrated over the years, some rust can be seen on the metal instruments, and some electrical instruments aren't working as powerful as they should be. All in all, most of the challenges are minor instrumental problems that can either be ignored or be fixed by exchanging them with a new one. Other than that, all the analysis runs perfectly and reliable data has been collected.

On the other hand, the microbiology laboratory mainly struggles with contamination. As the laboratory work mainly revolves around culturing and counting microorganisms, it is crucial to keep the laboratory area sanitized and sterilized. However, that condition is not possible to be achieved at all times, and this will cause contamination. The contamination can root from the instruments used in the laboratory such as the oven, the autoclave, or the laminar air flow cabinet not being properly calibrated or from the samples handed in from the customers, from the container to the water samples itself. Nonetheless, a false positive is still better than a false negative result. As the microbiology laboratory is responsible for the certification of drinking water, errors while performing the analyses can be detrimental to human health. This is why all the samples are prepared in duplicates, to minimize the errors & false negative results.

LITERATURE REVIEW

4.1 Water samples in BSPJI

As previously mentioned, there are many different types of water samples submitted to be analyzed in BSPJI. Amongst these samples are bottled drinking water (AMDK), wastewater, and raw/naturally-obtained water. Their definitions are as follows:

4.1.1 Drinking water (AMDK)

According to Peraturan Menteri Perindustrian RI No. 12, (2011) about *Persyaratan Teknis Industri Air Minum Dalam Kemasan*, bottled drinking water is defined as water that has been processed in a food-grade manner, safely-packed, and has been tested for human consumption. Furthermore, the bottled water should not contain any other food additives which carry a certain color, smell, or nutritional value. As the bottled water is to be consumed immediately after purchase, it is crucial that all manufacturing and production processes are carried out aseptically whilst always prioritizing safety and purity of the water. In Indonesia, there are two types of bottled water (AMDK), which are mineral water and demineral water.

4.1.1.1 Mineral drinking water

According to *Peraturan Menteri Perindustrian RI No. 12*, (2011) about *Persyaratan Teknis Industri Air Minum dalam Kemasan*, mineral drinking water is defined as drinking water which contains minerals obtained from its source in a certain amount and concentration, not from any additives. Raw water contains specific minerals which have been approved by the SNI to be beneficial and healthy for human consumption. To reach that threshold of acceptable amount and concentration, certain processes such as sterilization and distillation are done.

4.1.1.2 Demineralized drinking water

According to *Peraturan Menteri Perindustrian RI No. 12*, (2011) about *Persyaratan Teknis Industri Air Minum dalam Kemasan*, demineralized drinking water is bottled water that does not contain any minerals. During its processing, the water is purified from any substances including

minerals and salt through distillation, deionisation, and reverse osmosis (RO). However, due to recent studies proclaiming the dangers of impure demineralized water which is able to cause bone erosion, tooth decay, rashes, digestive disorders, and cancer (Zahed, et al., 2020), its production and analysis has been closely monitored, especially in Indonesia.

4.1.1.3 Quality thresholds for AMDK in Indonesia

As previously mentioned, certain factors could affect the purity and quality of drinking water, both mineral drinking water and demineralized drinking water. Hence, there needs to be certain quality thresholds/requirements manufactures are required to follow in the production of AMDK to ensure that the final products are safe for consumption. In Indonesia, SNI arranged said thresholds in SNI-01-3553-2006, with requirements as follows:

Table 1. Quality Requirements of bottled drinking water (AMDK) according to SNI, (2006)

No.	Parameter	Unit of Measurement	Commodity	
			Mineral Water	**Demineralized Water**
1	Smell	-	No smell	No smell
	Taste	-	Normal	Normal
	Color	PtCo	≤5	≤5
2	pH	-	6.0-8.0	5.0-7.5
3	Turbidity	NT	≤1.5	≤1.5
4	Total organic compound	mg/L	0	≤0.5
5	Nitrate ($NO3$)	mg/L	≤45	0
6	Nitrite (NO_2)	mg/L	≤0.005	0
7	Ammonia (NH_3)	mg/L	≤0.5	≤0.5
8	Sulfate (SO_4)	mg/L	≤200	0

9	Chloride (Cl⁻)	mg/L	≤250	0
10	Fluoride (F⁻)	mg/L	≤1	0
11	Cyanide (CN⁻)	mg/L	≤0.05	0
12	Iron (Fe)	mg/L	≤0.1	0
13	Manganese (Mn)	mg/L	≤0.05	0
14	Chlorine (Cl)	mg/L	≤0.1	0
15	Chromium (Cr)	mg/L	≤0.05	0
16	Barium (Ba)	mg/L	≤0.7	0
17	Selenium (Se)	mg/L	≤0.01	0
18	Lead (Pb)	mg/L	≤0.005	≤0.005
19	Cooper (Cu)	mg/L	≤0.5	≤0.1
20	Cadmium (Cd)	mg/L	≤0.003	≤0.003
21	Mercury (Hg)	mg/L	≤0.001	≤0.001
22	Silver (Ag)	mg/L	0	≤0.025
23	Cobalt (Co)	mg/L	0	≤0.01
24	Arsenic (As)	mg/L	≤0.01	≤0.02
25	Total Viable Count (TVC)	colony/mL	≤100	≤100
26	Coliform Bacteria	colony/mL	negative	negative
27	*Salmonella sp.*	colony/mL	negative	negative
28	*Pseudomonas aeruginosa*	colony/mL	negative	negative

Amongst these parameters, most are able to be done at BSPJI Surabaya. From numbers 1-24, the analysis is done in the environmental-chemistry laboratory whereas parameters numbers 25-28 are done in the microbiology laboratory. If the samples are within the quality threshold required by SNI, a certification will be given from BSPJI to the company/business.

4.1.2 Raw Water

According to Guchi (2015), Raw water is water naturally found and obtained in and/or from the environment. This water has not been treated from the minerals, ions, particles, and bacteria it contains hence it is treated as "Type 1" wastewater, which quality requirements can be found on Table 2. Raw water includes, amongst other sources, rainwater, groundwater, well water, and water from bodies such as lakes, rivers, and ponds which are commonly used as raw materials for drinking water after it is treated through various filtration processes and has been certified by BSPJI for safe consumption. Once in a while, these types of water are also tested as a requirement from the government to ensure environmental stability (Permenkes, 2017).

4.1.3 Wastewater

According to Kumar, Sahoo, & GM (2019), wastewater is water that has been previously used by households and communities which also comes from industrial groundwater, surface water, and other general wastes. It can be processed in many different ways to support different sectors of the industry such as for agriculture and fishery (Smol, Adam, & Preisner, 2022). In Indonesia, wastewater is divided into two types, type 1 and type 2, based on the concentration, the sources, and the dangers of the wastewater.

4.1.3.1 Type I Wastewater

According to *Peraturan Menteri Lingkungan Hidup RI No. 5,* (2014) about *Baku Mutu Air Limbah*, type I wastewater is defined as water which Biological Oxygen Demand (BOD) concentration is under 50 mg/L and is disposed in natural water bodies such as lakes, rivers, and ponds. Additionally, water obtained from said natural sources are also deemed as Group 1 Wastewater. The quality standards of type I wastewater can be seen on Table 2.

4.1.3.2 Type II Wastewater

According to *Peraturan Menteri Lingkungan Hidup RI No. 5, (* 2014) about *Baku Mutu Air Limbah*, type II wastewater is defined as water which Biological Oxygen Demand (BOD) concentration is above 50 mg/L hence is not eligible to be disposed of in natural water bodies.

These types of waterbodies are to be treated with more filtration processes to fit SNI's quality requirements before it is eligible for discharge. The quality standards of type II wastewater can be seen on Table 2.

4.1.3.3. Quality thresholds for wastewater in Indonesia

The two different types of wastewater have different quality requirements because of the differences they uphold (dangers, concentration, source, and BOD concentration). Quality testing of these wastewaters are important as their untreated discharge will directly affect all of the living organisms they surround. Displayed below on Table 2., are the quality thresholds for these two types of wastewater based on *Peraturan Menteri Lingkungan Hidup Republik Indonesia No. 5 tahun 2014* about *Baku Mutu Air Limbah*. If the threshold for Type 1 is not sufficient for the sample, the sample will be directly considered as Type II wastewater.

Table 2. Quality Requirements of wastewater according to PerMen LH RI, (2006)

No.	Parameter	Unit of Measurement	Wastewater	
			Type I	Type II
1	Temperature	°C	38	40
2	pH	-	6.0 - 9.0	6.0 - 9.0
3	Biological Oxygen Demand (BOD)	mg/L	≤50	≤150
4	Chemical Oxygen Demand (COD)	mg/L	≤100	≤200
5	Total Dissolved Solid (TDS)	mg/L	≤1000	≤2000
6	Total Suspended Solid (TSS)	mg/L	≤20	≤40
7	Oil and Fat	mg/L	≤10	≤20
8	Iron (Fe)	mg/L	≤5	≤10
9	Manganese (Mn)	mg/L	≤2	≤5

10	Barium (Ba)	mg/L	≤2	≤3
11	Cooper (Cu)	mg/L	≤2	≤3
12	Zinc (Zn)	mg/L	≤5	≤10
13	Hexavalent Chromium (Cr6++)	mg/L	≤0.1	≤0.5
14	Total chrome (Cr)	mg/L	≤0.5	≤1
15	Cadmium (Cd)	mg/L	≤0.05	≤0.5
16	Mercury (Hg)	mg/L	≤0.002	≤0.005
17	Lead (Pb)	mg/L	≤0.1	≤1
18	Tin (Sn)	mg/L	≤2	≤3
19	Arsenic (As)	mg/L	≤0.1	≤0.5
20	Nickel (Ni)	mg/L	≤0.2	≤0.5
21	Cobalt (Co)	mg/L	≤0.4	≤0.6
22	Cyanide (CN)	mg/L	≤0.05	≤0.5
23	Hydrogen Sulfide (H2S)	mg/L	≤0.5	≤1
24	Fluoride (F)	mg/L	≤2	≤3
25	Free Chlorine (Cl2)	mg/L	≤1	≤2
26	Ammonia (NH3)	mg/L	≤1	≤1.5
27	Nitrate (NO3)	mg/L	≤20	≤30
28	Nitrite (NO2)	mg/L	≤1	≤3
29	Total Nitrogen	mg/L	≤30	≤60
30	Detergen	mg/L	≤5	≤10
31	Total coliform bacteria	MPN/100mL	≤10,000	≤10,000

Similar to the quality testing of AMDK, most of the analysis of the parameters displayed on the table above are done in BSPJI Surabaya. Parameters from numbers 1-30 are done in the environmental-chemistry lab, whereas number 31 is done in the microbiology laboratory. BSPJI will release certification if the samples are within/under the quality threshold.

4.2 Environmental Chemistry Laboratory

Displayed below are the definitions and principles of the parameters and instruments used, done, and related to the environmental-chemistry laboratory of BSPJI.

4.2.1 Chemical Oxygen Demand (COD)

According to Zahmatkesh, et al., (2022), Chemical Oxygen Demand (COD) is a measure of how much organic materials are present in a given sample. The analysis is done by measuring the amount of oxygen consumed during the reactions based on the understanding that almost every organic compound is able to fully oxidize to carbon dioxide under acidic conditions with the help of a strong oxidizing agent (Hompland, et al., 2018; Sawalha, et al., 2019). That said, COD is often expressed in mass of oxygen over the volume of the solution (mg/L) and its analysis is done using spectrophotometry (Aniyikaiye, et al., 2019).

COD analysis is often conducted to measure the amount of organic pollutants in water bodies as it has been long used as a matrix to quantify water quality (Duan, et al., 2022). Especially in Indonesia, it is used to provide an index to evaluate the impact of the release of processed wastewater into the environment, to determine its safety upon discharge (Brontowiyono, et al., 2022). High COD levels are dangerous as it can lead to the reduction of dissolved oxygen (DO) in the water, which is harmful for higher aquatic life as it can lead to anaerobic conditions (Garg, et al., 2022). Other than for aquatic life, exposure to high levels of organic materials are also harmful for the environment as it may lead to surface crusting and soil acidification. (Chang & Cockerham, 2019).

4.2.2 Biological Oxygen Demand (BOD)

Similar yet different with COD, Biological Oxygen Demand (BOD) is also the measure of the presence of organic materials in a sample, but only those which are biodegradable, which essentially means simpler organic matters. That said, BOD testing is more specific, targeting only the organic materials degradable by biological agents at a certain temperature over a period of time (Wang, et al., 2022; Prokkola, et al., 2022). It is often expressed in mg/L and is measured using titration before and after a period of incubation (Hussain, et al., 2021).

BOD is primarily used as a quantitative analysis test to measure the efficiency and effectiveness of wastewater treatment plants. Results of the measurement are used as an indicator to see the short-term impact on the oxygen levels of the wastewater. (Tchinda, et al., 2019; Jiang, et al., 2021). High levels of BOD may suggest contamination, blockage, and failure/errors in the instruments hence further testing should be performed. Impacts of high BOD levels are similar to COD, which includes dangers to aquatic life and the environment.

4.2.3 Total Suspended Solid (TSS)

According to Schumann & Brinker (2020), Total Suspended Solid (TDS) is defined as the solids present in water bodies or water samples, whose size is about or bigger than 2μm. Said solids may be waterborne particles, inorganic materials, algae, bacteria, sediments, planktons, or contaminants such as from decaying organic matter (Omer, 2019). The suspended solids are separated from the water sample through filtration using a vacuum and a 2μm filter paper. That way, it will be trapped and used for calculation using gravimetric analysis (Johnson, et al., 2022).

TSS analysis is usually done to test the quality of wastewaters (Jasim, 2020). Low levels of TSS are primarily not toxic nor dangerous as they are a group of very common matters in the water, however if the quantity is above the expected threshold from SNI, it may cause turbidity problems, which may affect aquatic life. High levels of TSS may also indicate contamination from previously mentioned decaying organic matter, which is dangerous to the environment (Jeong, et al., 2020; Victoriano, et al., 2020).

4.2.4 Total Dissolved Solid (TDS)

Comparable to TSS, Total Dissolved Solid (TDS) is an analysis to see the dissolved solids present in water samples, whose size is smaller than 2μm. Said solids may include inorganic or organic substances in a molecular, ionized, or colloidal suspended form (Becke, 2020; Schuman, 2021). Like TSS, TDS analysis is conducted with the help of a vacuum and a 2μm filter paper. For TDS, the water filtered will be used for calculation using gravimetric analysis (Anis, et al., 2019).

TDS is also one of the many parameters to test for water quality, mainly used for wastewater and drinking water, specifically done to check the instruments used for the filtration process (Ohmer, 2019; Hairom, et al., 2021). As primary sources of TDS are agricultural runoffs and non harmful chemical elements, low levels of TDS is also not toxic nor harmful, however higher levels of it may be dangerous for consumption as it may also contain heavier metals, sediments, and organic compounds (Sankhla, et al., 2019; Raimi, et al., 2022).

4.2.5 Cadmium (Cd)

Cadmium (Cd) is a chemical element, which tiny amounts can be naturally found in the air, water, and soil (Kubier, Wilkin, & Pichler, 2019). It is famous for its soft, silvery white color which can be found in steel, as it is used as an anti-corrosive agent. Additionally, cadmium is also used in the manufacture of batteries, solar cells, silver soldering, and electroplating (Hayat, et al., 2019; Goosey & Goosey, 2019). Amidst its benefits for human life, it is very toxic when consumed. Cadmium is known to be able to cause mutations and chromosomal deletions (potentially the 17th). Once consumed, it will deplete the body of reduced glutathione (GSH) as it binds sulfhydryl groups with protein, causing the production of reactive oxygen species (ROS) which can cause damage to DNA, proteins, and lipids (Khan, et al.,2022; Lee, et al., 2022). Additionally, although it is insoluble and not flammable in its liquid form, cadmium in its powder form is able to burn and will release toxic fumes dangerous when inhaled (Li, et al., 2018)

3.2.6 Ammonia (NH_3)

Ammonia is an inorganic substance made of nitrogen and hydrogen (NH_3). It is most commonly found in its gaseous form, where it is colorless but has a strong pungent odor (Jiang,

et al., 2021). The gas has a relatively low melting as well as boiling point, making it quite stable in room temperature (Wan, et al., 2021). Ammonia is a very common biological waste, found as left-overs of biological and chemical reactions particularly in the aquatic environment. It is also largely used in the industry to make fertilizers, cleaning products, and is also one of the raw materials for pharmaceutical products (Sulok, et al., 2021; Kim, et al., 2022).

Despite the fact that it is very commonly found in nature and daily life, ammonia in its raw and untreated gaseous form is very hazardous and corrosive (Rafiee, et al., 2021). High levels of exposure may lead to severe irritation and burns in the skin, eyes, mouth, throat, and lungs which may lead to death (Slaughter, et al., 2019; Ismail, Abidin, & Rasdi, 2020). According to Padappayil & Borger, (2020), concentrations of 2500 to 4500 ppm can be fatal for humans, only taking 30 minutes to completely burn the lungs. According to Badan Standardisasi Nasional (2005), for drinking water and wastewater standardization purposes, ammonia is analyzed so that its concentration can be monitored. The analysis is done using UV-Vis spectrophotometry and is read at 615 nm wavelength, using hypochlorite and phenol as reactants as well as sodium nitroprusside as a catalyst.

3.2.8 Titrimetry

According to Gill & Zheng (2020), titrimetry is a quantitative analysis method in which an analyte's concentration is determined based on its stoichiometric reaction with a reagent of known concentration. This analysis is usually done to determine the concentration of said analyte, known as the sample (Goeltz & Cuevas, 2021). The reagent will be added gradually to the analyte until stoichiometry is achieved. In most cases, the end of the reaction can be visibly detected with a change in color (Kozak & Townshend, 2018; Mtewa, et al., 2022). In the analyses included in this report, biological oxygen demand (BOD) testing uses this methodology. .

4.2.8 Gravimetry

In chemistry, gravimetric analysis is a quantitative methodology used to determine the quantity of a certain analyte/sample based on the mass of the solid (Sargent, 2020). This analysis, similar to titrimetry, is usually performed to determine the mass or concentration of a substance through measuring the change of its mass. Additional reagents are sometimes added as a catalyst to speed up the process or the reaction (Wu & Piszczek, 2020; Olivo, et al., 2021). Amongst the many parameters included in this report, TDS and TSS uses this methodology.

4.2.9 Spectrophotometry

According to Hodgkinson & Tatam (2012), spectrophotometry is a standard laboratory and analysis technique used in many different industries, particularly those involving science, technology, and engineering, specifically to measure the light absorption ability of a certain solution (sample). The instrument used in said analysis is called a spectrophotometer and it uses a light beam to measure the light intensity of said sample through the measurement and monitoring of color (Anagnostaki, et al., 2020). The principle of the spectrophotometer is based on Beer-Lambert's law who states that the amount of light absorbed by a (colored) solution, is directly proportional to the concentration of the solution (Cole & Levine, 2020).

As its name suggests, a spectrophotometer is made of two instruments, a spectrometer and a photometer. The spectrometer is responsible for generating light of a specific wavelength, set by the analyst (Masson, et al., 2018). On the other hand, the photometer is responsible for measuring the amount of light which passed through the sample through its absorption spectrum (Valentini, et al., 2021). There are many different types of spectrophotometer, two of which are used in the analyses included in this report is the UV-Vis spectrophotometer and the Atomic Absorption Spectrophotometer (AAS)

4.2.13.1 UV-Vis Spectrophotometer

The UV-Vis spectrophotometer is a commonly used instrument in laboratories specifying for biology and chemistry especially in bacteria identification, nucleic acid quantitation, as well as quality control for wastewater and drinking water samples (Anderson, et al., 2019; Jiang, et

al., 2021). The equipment is specifically designed to measure the ability of the samples to catch and absorb light in the UV-Vis scale, which is at 190 to 900 nm (Ren, et al., 2022). Like all spectrophotometers, the light source will produce a light beam that will be caught by the particles on the sample, which will then produce an absorbance value signifying the ability of the sample to catch a certain value of light intensity (Shao, et al., 2022). In BSPJI, the UV-Vis Spectrophotometer used is from Shimadzu, able to measure wavelengths 190 to 900 nm.

4.2.13.2 VIS Spectrophotometer

The Vis spectrophotometer, very similar to the UV-Vis spectrophotometer, is also a very common laboratory instrument used to measure the amount of light absorbed and transmitted by the sample. As its name suggests however, the Vis spectrophotometer is only able to transmit visible light, which is at 390 to 700 nm (Tang, et al., 2019). Other than the difference in wavelength, all other working principles are the same. The light will beam at a certain wavelength which will be captured by the particles in the sample, producing an absorbance measurement (Ma, et al., 2020). In BSPJI, the Vis spectrophotometer used is from "Hach", able to measure wavelengths 390 to 700 nm.

4.2.13.4 Atomic Absorption Spectrophotometer (AAS)

Different from the other spectrophotometers and their principles, Atomic Absorption Spectrophotometer (AAS) is an instrument mainly used in elemental analysis, specifically for heavy metals. The equipment is used to determine the concentration of certain elements in the sample with the understanding that these elements are able to absorb light in a specific and unique wavelength (Jin, et al., 2020). When this specific wavelength is beamed through the spectrophotometer, it will be absorbed by the element's atom, which is present in the sample. The electrons inside the atom will then move from its ground state into an excited state. Through this, the absorption and concentration of the element can be calculated (Varma, 2019).

As its name implies, the elements inside the sample which is to be analyzed have to be in its atom form. That is why, in each AAS there is an atomizer present. There are many different types of atomizer, divided into two different groups based on how it atomizes the samples. The

first technique is through vaporizing using a chemical reaction, which is present in MVU (mercury vapor unit) AAS as well as HVG (hydride vapor generator) AAS. These two types of AAS are not as popular as the others as it is specific and can only be used for the identification of one metal, as well as are not as safe as the others (Perdana Harahap, et al., 2020; Huong, 2020). The more popular atomizing method is through heating. There are two main AAS methods which uses high temperature to atomize samples, namely flame AAS which makes use of either acetylene (C_2H_2) or nitrous (N_2O) (Dias, et al., 2019) as well as Graphite Furnace AAS, which is used for the analysis of Cadmium, included in this report.

Graphite Furnace AAS makes use of a graphite-coated furnace to atomize the sample. Inside the atomizer is a closed graphite tube with transparent end windows, clamped by two electrodes on its side. Electricity will flow from each of the electrodes, therefore heating up the contents inside the graphite tube and atomizing the sample. A lamp unique for the element will then be put inside the furnace and it will beam at a specific wavelength from the sides and through the tubes, exciting the electrons, hence measuring the absorption and concentration of the analyzed element (Schneider, et al., 2018; Vinhal & Cassella, 2019). The instrument is almost 100% self-run, with only a few steps of inputs needed to be done in-person. In BSPJI, the graphite furnace used is from Thermo Scientific iCE 3000 Series.

4.3 Microbiology Laboratory

4.2.1 Microorganisms

According to Zhou, et al., (2020), microorganisms are defined as living organisms that are microscopic, or simply can not be seen with the naked eye. They may exist in a single cell, or form a group called a colony. Although unseen, microorganisms are everywhere, some are beneficial while some others are harmful (Baron, Eilers, & Haverkate, 2022). It is important that microorganisms which are harmful and bring damage to both the human body and the environment be noticed, so that they don't bring further contamination.

Water is as essential to microorganisms as it is to humans and the environment, hence they grow rapidly inside water bodies (Chowdhary, et al., 2020). This is why microbial quality requirements are some of the threshold when testing for drinking water and wastewater. It is

important that the presence of these microorganisms are noticed and tested before it is consumed by humans and is given back to the environment. As previously mentioned, there are a lot of different microorganisms which bring various detrimental effects to the human body (Peng, et al., 2020), some of which are tested in BSPJI to ensure that the bottled drinking water available for commercial consumption and the wastewater disposed back to water bodies are within SNI's threshold and are therefore safe.

4.2.2 Coliform Bacteria

Coliforms are very common animal digestive tract dwellers (Herrmann, 2021). They are gram-negative non-spore forming Bacilli which are also commonly found in the soil, vegetation, and unsanitary water (Mian, et al., 2020). This is why coliform has become one of the most analyzed parameters for water quality measurement. As they are found in these unhygienic places, it is crucial that drinking water is safe and free of coliform bacteria, as it signifies contamination from feces as well as unclean filtration process. Consumption of coliform can lead to diarrhea, vomiting, fever, and severe intestinal infection (Humes, et al., 2020; Butler, McLoughlin, & Flaherty, 2022). Not only is it dangerous for humans, too much of it in the environment can be dangerous as well for the soil and the organisms that dwell in it, as it can lead to unbalanced food chains and diseases in bigger animals (Schmithausen, et al., 2018). There are a few coliform bacterias which are more dangerous than others, hence further confirmation testing is usually needed for drinking water and wastewater analysis. Said coliform bacterias are as follows:

4.2.1 *Escherichia coli*

Escherichia coli (E. coli) is a very commonly found coliform bacteria, usually dwelling in the lower intestines of warm-blooded organisms such as humans and certain animals. Inside the body, they are responsible for the production of K2 vitamins, which is beneficial for the hosts (Poirel, et al., 2020). The bacterias are then brought back into the environment through fecal matters, which when exposed to drinking water and wastewater, can cause serious harm both to the human body as well as to the environment. Most of *E. coli*'s strains are not dangerous or

harmful, but some of them, such as strain O157:H7, is dangerous as it produces toxins which may lead to hemorrhagic diarrhea and kidney failure (Davis, et al., 2022; Good, 2022)

4.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative rod-shaped bacterium commonly found in the soil and water. The bacterium thrives in water, specifically amongst liquid waste (Weimer, et al., 2020; Kristanti, et al., 2022). It is famous for causing numerous diseases in plants, animals, as well as humans, with pneumonia and cystic fibrosis being two of the most notable ones (Qin, et al., 2022). As the microorganism lives freely amongst humans and in the environment, the spreading and contamination of which is very hard to restrain (Rahman, et al., 2022), which is why it is important to test for the signs of the bacteria while testing for drinking water and wastewater as a positive result may indicate contamination from liquid waste.

Another thing *P. aeruginosa* is famous for is its neon-green/blue glow under the UV light (Momtaz, 2022). This is possible because of the color pigments they have namely pyoverdin and pyocyanin. Pyoverdine is the yellow-green pigment, whereas pyocyanin is the bluish-gray pigment. These two pigments combined will result in a neon blue/green glow under the UV light (Poppe, Reichelt, & Blankenfeldt, 2018; Mishra, et al., 2022).

4.2.4 Total Viable Count (TVC)

Total viable count (TVC) is a microbiological methodology which aims to analyze and estimate the total number of microorganisms present in a given water sample. This test is widely used as it gives a quick quantification of how pure and clean the sample is (Perrin, et al., 2019). Different from total bacteria count (TBC), TVC focuses only on living and growing colonies, hence during the testing, an incubation period is needed (Garland, 2020). The cultures are incubated at two different temperatures, namely at 22°C and 36°C. The 22°C incubation is to see the bacterias growing at an ambient temperature, this is usually used to test for yeast, mold, and fungi (Acharya & Hare, 2022). On the other hand, bacteria predominantly grows best

at 36°C, hence the incubation period is done to see the growth of bacteria which are usually harmful for humans (Savitskaya, et al., 2019).

As previously mentioned, TVC is one of the many water quality test requirements in Indonesia. A high result in TVC indicates poor water quality, which signifies contamination and/or filtration problems and might cause serious as well as detrimental health impacts to the human body, especially when found in drinking water which is to be consumed on a daily basis (Kochi, et al., 2020). According to SNI (2015), to make sure that all the testing is done maximally, several dilution steps are also taken to ensure that the samples don't give either a false positive or a false negative as well as to facilitate the enumeration part of the analysis.

4.2.5 Pour-plate Method

The pour-plate method is the most commonly used plating technique for microorganism enumeration (Lenhart & Gorsuch, 2021). It was developed by Robert Koch and is still widely used to this day to isolate and trap the microorganisms present in the sample inside the agar to promote its growth (Wallace, et al., 2021). This method is based on the understanding that once they are well-mixed with the agar, each viable microorganism will grow and create a colony of its own, hence one colony can be counted as a singular microorganism (Diko, 2021).

4.2.6 Membrane Filtration

The membrane filtration technique is an effective and quantitative laboratory method used to test certain water purification parameters (Xiang, et al., 2022). The technique revolves around filtering said water sample through a membrane with a 0.45mm size pore, in hopes of hooking all the microorganisms from the sample in the membrane. The membrane will then be incubated in a selective medium to be enumerated after it has grown (Sheppard, 2021). This method is widely acceptable as it is done in a micro-scale, causing minimal contamination and producing approvable results (Hube, et al., 2020). In BSPJI, this method is used for *P. aeruginosa* and Coliform bacteria testing in bottled water samples.

MATERIALS AND METHODS

5.1 Parameters from the Environmental-Chemistry Laboratory

As previously mentioned, amongst the many parameters done in the environmental-chemistry laboratory of BSPJI, 6 were taken into consideration for the purpose of this internship report. The materials and methods used for the parameters are as follows:

5.1.1 Chemical Oxygen Demand (COD) analysis using spectrophotometry

The materials, equipment, as well as methods used and followed in COD analysis in BSPJI Surabaya is based on SNI 6989.2:2009 about *"Cara Uji Kebutuhan Oksigen Kimiawi (Chemical Oxygen Demand/COD) secara Spektrofotometri"*. Based on the guidelines, this method is used to determine the concentration of COD in water and wastewater through the reduction of $Cr_2O_7^{2-}$ and is read by a spectrophotometer at 420 nm (low COD) and 600 nm (high COD). The difference between low and high COD analysis occurs on the concentration of the sample. As the samples have an unknown concentration when they are first received, they will usually be analyzed for both high and low COD, with some exceptions such as when the sample seems very turbid, it will directly be tested for high COD and when the sample is almost clear, it will directly be tested for low COD. If the results are outside the range of the analysis of choice, a follow up test will be performed to determine the correct COD concentration of the samples.

5.1.1.1 Materials & Equipment

The materials needed for the analysis includes the samples, which are wastewater, an organic-free water, a digestion solution which is made of 1.022 g (for low COD) or 10.216 g (for high COD) $K_2Cr_2O_7$ and 33.3g $HgSO_4$ diluted in 167 mL of H_2SO_4 and 500 mL of water, sulfuric acid solution which is made of 10.12 grams of Ag_2SO_4 is diluted in 1000 mL of H_2SO_4, and KHP (potassium hydrogen phthalate) used to make the standard curve. The equipment used for the analysis are test tubes, measuring and volumetric pipettes, volumetric flasks, a VIS spectrophotometer with a wavelength of 400 nm to 700 nm, cuvettes, and a heating block to heat up the samples.

5.1.1.2 Standard Curve Preparation & Measurement

Before analyzing and measuring the samples, a standard curve is firstly prepared to establish the relationship between absorbance and concentration. 8.5 grams of KHP is diluted in 1 L to make a KHP solution with a concentration of 10,000 mg/L. From this stock solution, serial dilution is done to make a standard curve for low COD and high COD. For low COD, the stock solution is diluted to concentrations 10, 20, 30, 40, 50, 60, 70, 80, and 90 mg/L, whereas for high COD, the stock solution is diluted to concentrations 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mg/L. Each of the solutions were put in test tubes, where 1.5 mL of the digestion solution and 3.5 mL of the sulfuric acid solution is added and homogenized together with 2.5 mL of the standard solutions. The tubes were then heated at 150°C for two hours in a heating block and then read with a spectrophotometer at 420 nm and 600 nm for low COD and high COD respectively.

5.1.1.3 Sample Preparation & Analysis

For wastewater COD measurement, the first step would be to determine whether the sample is to be tested for low COD, high COD, or both. Once that is established, 2.5 mL of the sample is first pipetted into test tubes. As the testing methodology follows the requirements of SNI, quality control is also done during the sample preparation process by pipetting aquadest as blanco, duplo for one sample, as well as a standard point with a known concentration. For this specific analysis, KHP with the concentration of 60 mg/L for low COD and 300 mg/L for high COD is used and was measured. After the samples are all pipetted into the test tubes, 1.5 mL of the digestion solution and 3.5 mL of the sulfuric acid solution is added into the tube and homogenized with the sample by shaking. They were then heated in the heating block for two hours at 150°C and were read with a Vis spectrophotometer at 420 nm for low COD and 600 nm for high COD.

5.1.2 Biological Oxygen Demand (BOD) analysis using Titrimetry

The materials, equipment, and methods used for the BOD analysis in BSPJI Surabaya follows SNI 6989.72:2009 about *"Cara uji Kebutuhan Oksigen Biokimia (Biological Oxygen*

Demand/BOD). Based on said guidelines, this methodology is used to measure the amount of dissolved oxygen needed by aerobic microbes to oxidize biodegradable organic materials in wastewater effluents which do not contain toxins and other contaminants. The analysis is done over the course of a 5-day dark incubation period at 20°C using titration with thiosulfate as the titrant. As thiosulfate is not able to directly react and reduce dissolved oxygen, other reactants are added which serves as catalysts in the reaction. The BOD concentration will be measured based on the difference between the dissolved oxygen value during the first and fifth day of the incubation.

5.1.2.1 Materials & Equipment

The materials used for this analysis includes mineral-free water, a nutrient solution which consists of phosphate buffer, magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), and Iron(III) chloride ($FeCl_3$), an aerobic bacteria suspension solution, glucose-glutamic acid solution for standard which is made by mixing 150 grams of glucose and glutamic acid each to 1 L of mineral free water and is then homogenized using a magnetic stirrer, manganese (II) sulfate ($MnSO_4$) solution, alkaline iodide azide solution, sulfuric acid solution (H_2SO_4), and amylum which acts as an indicator. On the other hand, the materials used in BOD testing are a buret, a retort stand, clamps, Erlenmeyers, beaker glasses, volumetric and measuring pipettes, winkler bottles, and volumetric flasks.

5.1.2.2 Nutrient Solution Preparation

1 mL of phosphate buffer, magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), and Iron(III) chloride ($FeCl_3$), and bacteria suspension solution is added per 1000 mL of aquades. The solution is then homogenized and oxidized using an oxidizing equipment inside the incubator to keep the bacteria dormant until it is added to the sample.

5.1.2.3 Standard Solution Preparation

The standard solution used for BOD analysis is a mixture of glucose and glutamic acid. As mentioned in the materials and equipment section, the glucose and glutamic acid powder are heated at 103°C for an hour to remove any unwanted contaminants. 150 grams of each are then

weighed and diluted together in 1 L of aquadest. This will serve as one of the samples during the analysis for quality control purposes.

5.1.2.4 Sample Preparation & Analysis

For wastewater BOD measurement, different volumes of each sample are first pipetted into 250 mL volumetric flasks. The volumes of the samples are set based on their turbidity, if the sample is very turbid, only 5-10 mL of it is taken to be diluted, whereas if the sample is clear, 100 mL of it will be pipetted for dilution. The dilution is done using the nutrient solution previously made. Along with the samples, blanks, duploids, as well as a standard solution is also pipetted for quality control purposes. After the samples are pipetted to their respective volumetric flasks they are poured into two different winkler bottles per sample, one to be read on the day of the analysis (DO_0) and the other to be read after 5 days of incubation (DO_5). The samples that are prepared for incubation are then moved inside the incubator.

The samples that are to be measured on the day of the analysis are then added with 1 mL of manganese (II) sulfate ($MnSO_4$) solution and 1 mL of alkaline iodide azide solution before being homogenized by shaking. The bottles are set for around 10 minutes so that the manganese oxide can precipitate to the bottom of the flask. Titration then begins by first adding 1 mL of concentrated sulfuric acid into the bottle. This will turn the color of the solution into a brownish yellow color. Thiosulfate is then carefully added from the burette into the bottle until it turns pale yellow. A few drops of amylum is then added as an indicator, turning the solution into black, before it is titrated back with thiosulfate until it turns colorless. The volume of the bottle and thiosulfate is written down for calculation. The same steps are repeated after 5 days for the bottles that have been incubated (DO_5), with their bottle and thiosulfate volumes written as well.

5.1.2.5 BOD Concentration Calculation

The titration and winkler bottle volumes that have previously been written are then used to calculate first the dissolved oxygen concentration (mg/L) of the samples. This is done using the following simplified formula:

$$Dissolved\ Oxygen\ (mg/L)\ =\ \frac{N\ (thiosulfate)\ x\ V\ (thiosulfate)\ x\ Be\ O2\ x\ 1000}{Sample\ volume\ (bottle\ volume) - 2}$$

After the dissolved oxygen concentrations of the samples are calculated, their BOD concentration can be measured using the following formula:

$$BOD\ (mg/L)\ =\ [(DO0\ -\ DO5)\ sample] - (DO0\ -\ DO5)blank]\ x\ fp$$

The blank used in this analysis is the nutrient solution in which the samples are diluted in, hence the BOD concentration of the samples are to be subtracted by it to ensure that the concentration at the end of the calculation is solely the concentration of the samples.

5.1.3 Total Suspended Solid (TSS) analysis using Gravimetry

The materials, equipment, and methods used in the analysis of TSS in BSPJI is adapted from SNI 06-6989.3-2004 about *"Cara Uji Padatan Tersuspensi Total (Total Suspended Solid, TSS) secara Gravimetri".* Based on the national standard, this method is used to determine the total suspended solids concentration in wastewater samples, with the exception of evaporated and floating suspended solids as well as decomposed mineral salt. The process is done through gravimetric analysis which makes use of the difference in the weight of the porcelain cups, used to hold the sample during the analysis.

5.1.3.1 Materials & Equipment

The materials needed for the analysis includes a Whatman 45 filter paper, whose pore size is 0,45 μm, aquadest, as well as the wastewater samples to be analyzed. On the other hand, the equipment used for the analysis are an evaporating dish, vacuum filter/pomp with all of its parts, an oven that can heat up to 101˚C - 105˚C, a desiccator with silica gel ,an analytical weigh, beaker glasses, volumetric pipettes, and porcelain/gooch cups.

5.1.3.2 Filter Paper Preparation

As gravimetric analysis is the preferred method for this analysis, the filter papers later used to filter the samples are first weighed to know its original weight. The weighing was done three times using an analytical balance after the filters are named according to the samples so

that they don't get mixed up during the filtering process. After all of the weights of the filter papers are written down, they are put in a porcelain cup for after the filtration process.

5.1.3.3 Sample Preparation & Analysis

Before the samples are filtered through the vacuum pump, it is first homogenized so that all of the suspended particles are evenly distributed. The vacuum filtration system was then turned on and the whatman filter, which had previously been weighed and named was put in. 100 - 500 mL of the wastewater samples were then filtered through the system. After all of the samples were filtered, all the filter papers were heated up in the oven at 105°C for two hours and were cooled to room temperature inside the desiccator for an hour. Weighing was then done to see how much TSS is present in the sample. This process was repeated until the weights of the filter paper reached equilibrium, with only changes of ±0.0005 grams.

5.1.3.4 TSS Calculation & Measurement

The concentration of the total suspended solids present in the samples are calculated with the following formula:

$$TSS = \frac{Wf - Wi\,(mg)}{V(L)}$$

From the formula, W_f is the final weight of the filter paper with the total suspended solid, W_i is the initial weight of the filter paper which was taken during the preparation step mentioned above, and V is the volume of the sample, which differs in accordance with its color and turbidity. TSS concentration is expressed in mg/L.

5.1.4 Total Dissolved Solid (TDS) analysis using Gravimetry

The materials, equipment, and procedure used for TDS analysis in BSPJI follows SNI 06-6989.27-2005 about "*Cara Uji Kadar Padatan Terlarut Total (Total Dissolved Solids, TDS) secara Gravimetri".* According to the guideline, this methodology is specifically used to measure the amount of total suspended solid in wastewater samples, which includes evaporated and dissolved solids which are tied in the water. Similar to TSS, the procedure makes use of the

principles of gravimetric analysis, which focuses on the difference in weight between the final and initial measurement.

5.1.4.1 Materials & Equipment

The materials used in the sample are aquadest, a Whatman 45 filter paper with 0.45 μm sized pores, as well as the wastewater samples for analysis. The equipment used, on the other hand are an analytical weight, porcelain cups, an oven that can heat up to 180˚C, a water bath, a measuring cylinder, vacuum filter/pump with all of its components, as well as a desiccator.

5.1.4.2 Porcelain Cup Preparation & Weighing

As the samples to be analyzed are in liquid form, a medium to hold them is needed. Porcelain cups are mainly used because of their durability and heat resistance, hence the first step of the analysis was to prepare the cups. Porcelain cups as many as the samples are first heated in the oven at 180˚C for an hour and were then cooled down to room temperature in a desiccator. These steps are necessarily taken to ensure that the cup is free of any contaminants. This step was repeated until the weights of the cups were stable

5.1.4.3 Sample Preparation & Analysis

Similar to TDS analysis, the samples to be analyzed were first homogenized so that all the dissolved solids are evenly distributed. The vacuum filtration system was then put in place with the filter paper as well as the measuring cylinder inside the vacuum glass. 100 mL of the samples were then filtered and moved into the pre-weighed and named porcelain cups. The cups were then heated at 180˚C in the water bath until all of the water evaporated. Then, the cups are put inside a 180˚C oven for an hour, cooled down to room temperature for an hour inside a desiccator, and weighed again. These steps were also repeated until the weight of the porcelain cups reached an equilibrium with changes less than ±0.0005 grams

5.1.4.4 TDS Calculation & Measurement

The concentration of the total dissolved solids present in the samples are calculated with the following formula:

$$TDS = \frac{Wf - Wi\ (mg)}{V(L)}$$

Similar to TSS, W_f signifies the final weight of the porcelain cup already with dissolved solids attached to its walls, whereas W_i is the initial weight of the porcelain cup. V signifies the volume of the samples taken for filtration, which is 100 mL or 0.1 L as TDS is expressed in mg/L.

5.1.5 Cadmium (Cd) analysis using Graphite Furnace Analyzer (GFA) Atomic Absorption Spectrophotometry (AAS)

The materials, equipment, and procedure used to test for Cadmium concentration in BSPJI is adapted from SNI 3554:2015 about *"Cara uji Air Minum Dalam Kemasan"*. According to the standard method, this technique is performed to determine the concentration of Cadmium in drinking water and wastewater samples with a concentration range of 0 - 0.003 mg/L or 3 µg/L, with a wavelength of 228.8 nm which is the specific length in which Cadmium atoms are able to absorb light. As mentioned, the procedure is done in a graphite furnace atomic absorption spectrophotometer.

5.1.5.1 Materials & Equipment

The materials needed for the analysis are aquadest, Cadmium standard solution with a concentration of 10 mg/L to be used to generate a standard curve, nitric acid for destruction, the drinking water samples to be analyzed, and argon gas to power up the graphite furnace atomizer. The equipments used for the analysis includes a graphite furnace atomic absorption spectrophotometer with a Cadmium hollow cathode lamp (wavelength of 228.8 nm), small plastic vials to put the samples and standard solution in the autosampler, measuring and volumetric pipettes, whatman 42 millipore, a hot plate, and volumetric flasks

5.1.5.2 Sample preparation and destruction

Before the samples are analyzed for their cadmium concentration, they must first be filtered and "destructed" through the addition of nitric acid. The drinking water samples were filtered through a Whatman millipore filter, with a pore size of 0.42 µm until around 50 mL. After the filtration process, 3mL of HNO_3 was added into 50 mL of the samples for destruction.

Nitric acid was added because an acidic condition will help metals, including Cadmium, to be able to stand on its own without needing to react and bind to another element. The mixture was then homogenized by slight mixing and was kept inside the acid room on the laboratory until all of the samples are readily prepared for testing

5.1.5.3 Sample analysis & Cadmium concentration measurement

After all of the samples were prepared, the graphite furnace AAS equipment was first turned on, including all of its components namely the computer and the program to read the results of the analysis, the UPS battery box, the graphite furnace, the autosampler, the main unit, as well as the blower, cooling water, and the argon gas. The samples and the standard solution were then poured into the plastic vials in the autosampler as the program was set to instruct the machine on how many samples are present and how many sampling it needed to take of the sample. After everything was set in place, as the machine is almost fully able to run on auto, it was left until the samples were produced.

Before the autosampler started sampling the samples, a standard curve was first made through serial dilution. As previously stated, a cadmium standard solution with a concentration of 10 µg/L was poured into one of the plastic vials, for it to be used to make the standard curve. Through the dilution process, 7 concentration points namely 0, 0.25, 0.5, 1, 1.5, 2, and 3 µg/L were measured for their absorbance. The equation generated from the graph was then used to calculate the concentration of the samples once their absorbance values were measured by the atomic absorption spectrophotometer.

5.1.6 Ammonia (NH3) analysis using Spectrophotometry

The materials, methods, and equipment used for this analysis were obtained from SNI 06-6989.30-2005 about *"Cara uji Kadar Amonia dengan Spektrofotometer secara Fenat"*. The module specifies that this testing is done to measure the concentration of Ammonia (NH_3) in drinking water (AMDK) and wastewater samples with a range of 0.1 to 2 mg/L. The analysis follows the reaction of phenol and sodium prusside with the help of an oxidizing agent to the

sample, changing the solution's color to blue in the presence of ammonia. This change of color is then read using the UV-Vis spectrophotometer and at the wavelength of 640 nm.

5.1.6.1 Materials & Equipments

The materials used in the analysis includes the drinking water and wastewater samples, ammonium chloride (NH_4Cl) standard solution, phenol solution (C_6H_5OH) which is made by mixing 11.1 mL of 89% phenol to 100 mL of 95% ethyl alcohol, 0.5% sodium nitroprusside ($C_5FeN_6Na_2O$), and an oxidizing solution made by diluting 25 mL sodium hypochlorite (NaOCl) in 100 mL of alkaline citrate. On the other hand, the equipment used for this analysis includes a UV-Vis spectrophotometer, an analytical scale, Erlenmeyers, beakers, and volumetric flasks, as well as measuring and volumetric pipettes.

5.1.6.2 Standard Curve Preparation & Measurement

For the calibration curve, a standard ammonium chloride solution with a concentration of 100 mg/L was used. From this stock, the solution was dissolved with distilled water into concentrations of 0, 0.2, 0.4, 0.6, 1, and 2 mg/L. These solutions with different concentrations were then read using the UV-Vis spectrophotometer at 640 nm, and their absorbance values were measured. From these data sets, a standard curve was generated, which can be seen on Figure 8. The data sets used are also included in Table 9. Although the analysis was done to measure ammonia (NH_3) concentration, an ammonium (NH_4) standard curve was used as it is easier and more affordable to make.

5.1.6.3 Sample Preparation & Analysis

To measure the concentration of ammonia, 25 mL of each sample was first pipetted into 50 mL Erlenmeyers. 1 mL of phenol solution, 1 mL of sodium nitroprusside, and 2.5 mL of oxidizing solution is then individually added into the Erlenmeyer and was homogenized after each addition. The Erlenmeyers were then closed using parafilm and were left to react for around an hour. During this time, the samples containing ammonia would have changed color

into blue. This change of color and the intensity of the blue color itself is what was then measured using the UV-Vis spectrophotometer at 640 nm.

5.1.6.4 Ammonia Calculation & Measurement

As previously stated, the standard curve generated was for ammonium (NH_4), hence further calculations were done to calculate the concentration in ammonia (NH_3), the formula to calculate ammonia from ammonium is as follows:

$$Ammonia\ concentration\ = \frac{Mr\ of\ Ammonia\ (NH3)}{Mr\ of\ Ammonium\ (NH4)}\ x\ Concentration\ of\ Ammonium$$

As the relative atomic mass of Ammonia is 17.0306 and the relative atomic mass of ammonium is 18.0306, the concentration of ammonia can be calculated by simply multiplying the ammonium concentration with 0.945. This will result in its ammonia concentration in mg/L.

5.2 Parameters from the Microbiology Laboratory

From BSPJI's microbiology laboratory, 4 parameters were taken into account for the writing of this report. The samples analyzed were drinking water as well as wastewaters. The parameters previously mentioned were as follows:

5.2.1 Total Viable Cell (TVC) analysis on drinking water samples using Pour-Plate Method

The materials, methods, as well as equipment used in this analysis is adapted from SNI 3554:2015 about *"Cara Uji Air Minum dalam Kemasan".* As stated in the standard method, this analysis focuses on inoculating drinking water samples at specific volumes in a petri dish filled with agar and incubating them to later enumerate the viable organisms grown on the plate.

5.2.1.1 Materials & Equipment

The materials used for this analysis are the drinking water itself, peptone water to dilute the samples, as well as the agar used to grow the organisms, in this case BSPJI uses the Yeast Extract Agar (YEA). On the other hand, the equipment used are an autoclave, a 36°C incubator

and a 22°C incubator, Petri dishes, a micropipette and its tips, an analytical weight, as well as a colony counter to count the microorganisms grown on the Petri dishes.

5.2.1.2 Agar Making & Preparation

As previously mentioned, the agar used for this analysis in BSPJI is YEA (Yeast Extract Agar). The agar is usually prepared 500 mL at a time, as it is the best volume to fit the number of samples analyzed daily in the microbiology laboratory. To make the agar, 17.5 grams of its powder was weighed using the analytical weight and was diluted in aquadest. The solution was then homogenized by mixing and was heated in boiling water while being agitated frequently until the powder had completely dissolved. It was then autoclaved at 121°C for 15 minutes.

5.2.1.3 Sample Preparation & Dilution

All of the samples must be prepared aseptically as it is to be tested for its microbiological contents. Before the samples were submitted to the laboratory, BSPJI has given a note to the customers to use sterilized glassware to put the samples. Once the sample has arrived, it was first put in the refrigerator to keep the bacteria in its dormant state. After the agar has been prepared, the samples were then let out of the refrigerator until it reaches room temperature.

For the analysis, pour-plating method was used as it is deemed to be the most efficient amongst the other methods. To ensure the quality of the analysis, the samples were prepared in 3 different volumes, namely 10^0, 10^{-1}, and 10^{-2} through serial dilution using peptone water. 10^0 represents the original sample, and was diluted twice, taking 1 mL and mixing it into 9 mL of peptone water. After all of the samples and their dilutions were prepared, 1 mL of each was moved into the Petri dishes. The 10^0 analysis was done twice as a quality standard from SNI.

After the media has cooled down to room temperature, it was then poured into the Petri dishes containing the samples. The mixture was then homogenized by shaking to ensure that the samples were well-dispersed into the agar. For each sample, 2 of each set of 3 were prepared to be incubated at 36°C and 22°C. The 36°C incubation was done to grow all of the microbes present in the sample as it is the most optimal temperature for their growth, whereas the 22°C incubation was done to monitor the growth of fungi and mushrooms. The Petri dishes

inside the 36°C incubator were grown for 44 ± 4 hours, whereas the Petri dishes inside the 22°C incubator were kept for 68 ± 4 hours.

5.2.1.4 Sample Enumeration & Analysis

After the incubation period, the Petri dishes were taken out for enumeration, which was originally done using a cell counter. The equipment, however, was broken and was not able to automatically count the bacteria colonies, hence it was counted manually under the light of the cell counter. The results of the enumeration were then noted down to be reported and the samples were washed and discarded.

5.2.2 Identification and Enumeration of Total Coliform Bacteria in bottled drinking water samples using Membrane Filtration

The materials, equipment, and methods used for the total coliform bacteria analysis in drinking water samples were sourced from SNI 3554:2015 about *"Cara Uji Air Minum dalam Kemasan".* According to SNI, the procedure was done to specifically grow Coliform bacteria present in the samples, for them to be later enumerated after incubation.

5.2.2.1 Materials & Equipment

The materials used for the analysis were the drinking water (AMDK) samples, Coliform Agar (CCA), as well as the 0.45 μm 47 mm membrane filter. On the other hand, the equipment used is a 36°C incubator, as well as the membrane filter set which includes a vacuum filter, a clamp, a set of open beakers, as well as a piping system to discard the water.

5.2.2.2 Agar Making & Preparation

As the analysis was done to specifically enumerate the coliform bacteria present in the samples, a specific coliform-growing agar was used namely the Coliform Agar (CCA). Similar to YEA, this media was usually prepared in 500 mL volume to match the numbers of samples analyzed on a daily basis. Firstly, 13.25 grams of the powder was measured using an analytical scale and was diluted in 500 mL of sterilized distilled water as coliform agar should not be autoclaved (its nutrient content may be reduced). After the powder has been homogenized, it is

heated in boiling water while frequently agitated to ensure that all of the powder has been diluted. It was then poured into petri dishes once it reached room temperature.

5.2.2.3 Sample Preparation & Filtration

Similar to TVC analysis, all of the samples were aseptically prepared in a glass bottle and has been refrigerated beforehand. As the samples were left out to reach room temperature, the membrane filtration system was prepared. The vacuum filter, clamps, open beakers, and pipes were put in place and were left for 15 minutes with the UV light on inside the laminar air flow to minimize contamination. After the samples were ready, the filtration began with putting a 0.45μm filter paper in-between the open beakers and pouring 250 mL of the sample into them. The vacuum pomp was then turned on to absorb the water as the filter paper filtered it. The filter paper was then moved into the already hardened coliform agar and put faced down to prevent water droplets from dropping and contaminating the paper. The petri dish was then put inside a 36°C incubator and was left for 21 ± 3 hours.

5.2.2.4 Sample Enumeration & Analysis

After around 21-24 hours, the petri dishes were taken out for enumeration. As the cell counter was still unable to automatically count the colonies, enumeration was done manually. Amongst the microbes which grew on the filter paper, the purple/bluish colonies were the ones counted as coliform bacteria whereas the others are ignored. After the enumeration process was done and noted down, the petri dishes were washed and sterilized.

5.2.3 Identification and Enumeration of *Pseudomonas aeruginosa* analysis in bottled drinking water samples using Membrane Filtration

The materials, methods, as well as equipment used in the enumeration analysis of *P. aeruginosa* is based from SNI 3554:2015 about "*Cara Uji Air Minum dalam Kemasan".* According to the standard method, this analysis is specifically done by growing *P. aeruginosa* in a specific agar and enumerating them after the incubation period. As the microbe is considered dangerous to human health, the drinking water samples tested should not contain any microbe for it to receive certification, hence the threshold from SNI is 0 colonies.

5.2.3.1 Materials & Equipment

The materials used in this experiment includes the drinking water samples to be tested, 5% glycerol, 1 vial of Pseudomonas CN selective supplement, as well as Pseudomonas CFC/CN agar. On the other hand, the equipment used are Petri dishes, a 36°C incubator, an autoclave, a micropipette and its tips, a UV light, and the membrane filter set which includes a vacuum filter, a clamp, a set of open beakers, as well as a piping system to discard the water.

5.2.3.2 Agar Making & Preparation

As mentioned before, the agar used for this analysis is the Pseudomonas CFC/CN agar. This media is used because it is able to specifically grow *P. aeruginosa* and highlight the color pigments it has, enabling it to glow under the UV light hence easing the enumeration process. To prepare the agar, firstly 24.2 grams of its powder was weighed with an analytical scale and homogenized with aquadest. 5 mL of glycerol was then added into the mixture and was once again homogenized by vigorous shaking. The mixture was then heated in boiling water until all of the powder had dissolved and then autoclaved at 121°C for 15 minutes. Once the agar has reached around room temperature, 1 vial of CN selective supplement was added into the agar and was homogenized before it was poured into petri dishes to harden.

5.2.3.3 Sample Preparation & Filtration

Similar to other microbiology sample preparation, the drinking water to be tested has been sterilized, put inside a glass bottle, and refrigerated overnight before it is filtered. On the day of the filtration, the samples were let out of the refrigerator to reach room temperature while the membrane filtration system was set in place. The open beakers and clamps as well as the piping system were left under the UV light for 15 minutes to minimize contamination risks. After the samples have reached around room temperature, they were filtered with a 0.45 μm filter paper, and just like the coliform analysis, the filter papers were then put inside the agar face down and incubated at 36°C incubator for 44 ± 4 hours.

5.2.3.4 Sample Enumeration & Analysis

After the petri dishes were incubated for around 2 days, they were moved out for the enumeration process. As mentioned, this was done under the UV light as *P. aeruginosa* contains 2 specific color pigments, namely pyoverdin and pyocyanin, enabling the bacteria to emit a vibrant blue/green color under the UV light. These very colonies were the ones searched and counted during the enumeration process, whereas the other colonies which didn't glow were ignored. The results of the enumeration process were then noted and reported.

RESULTS AND DISCUSSIONS

6.1 Parameters from the Environmental-Chemistry Laboratory

6.1.1 Chemical Oxygen Demand (COD) analysis using spectrophotometry

The standard curves made for the measurement are displayed below on Figure 1 for low COD analysis and Figure 2 for high COD analysis, whereas the concentration points used to make the curves are listed on Tables 3 and 5 for low and high COD concentrations respectively.

Table 3. Concentration and absorbance values for low COD standard curve

Concentration (mg/L)	Absorbance		
	Reading 1	**Reading 2**	**Average Absorbance**
0	0.0020	0.0010	**0.0015**
10	0.0010	0.0020	**-0.0015**
20	-0.0430	-0.0450	**-0.0440**
30	-0.0700	-0.0700	**-0.0700**
40	-0.0970	-0.0990	**-0.0980**
50	-0.1200	-0.1220	**-0.1210**
60	-0.1480	-0.1490	**-0.1485**
70	-0.1730	-0.1730	**-0.1730**
80	-0.1930	-0.1940	**-0.1935**
90	-0.2130	-0.2170	**-0.2150**

10 concentration points were used to generate the standard curve for low COD. According to SNI, (2009) a minimum of 5 concentration points are enough for the standard curve, hence the omitting the 100 mg/L point is allowed.

Low COD Standard Curve

$y = -0.0025x + 0.004$
$R^2 = 0.9981$
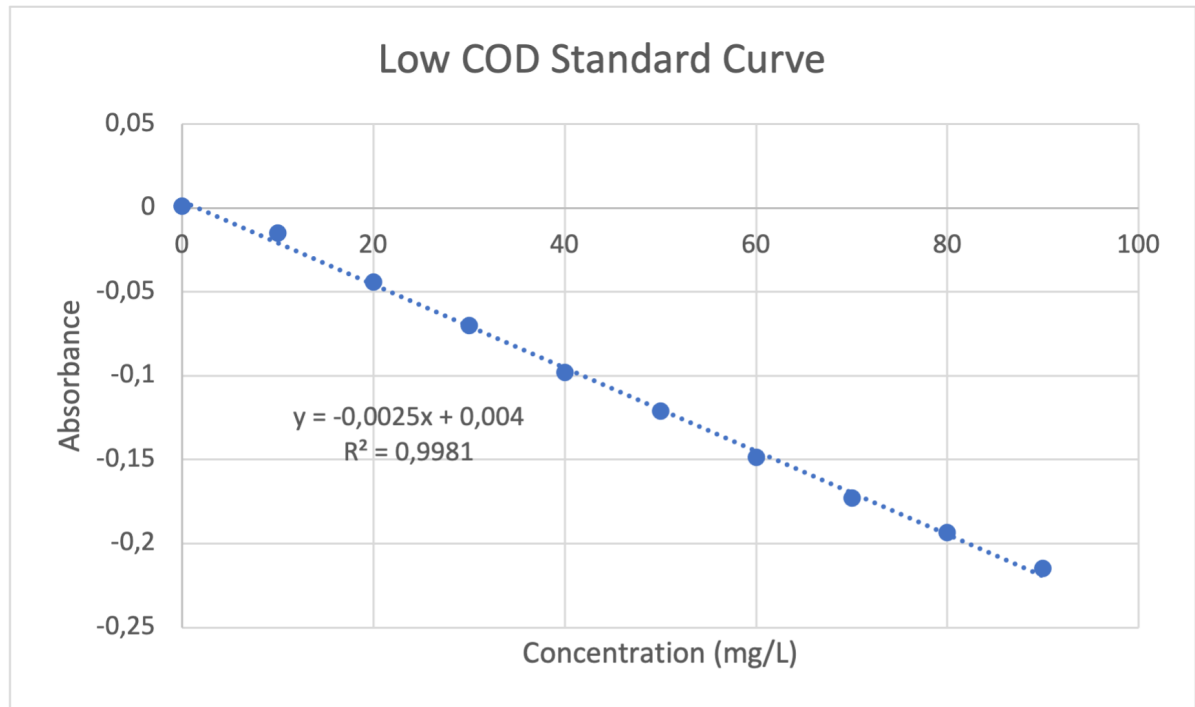
Figure 1. Low COD Standard Curve

Displayed above is the standard curve for low COD analysis & measurement. As mentioned, 9 concentration points are included in the graph from 0 mg/L to 90 mg/L. The R square value generated from the graph is 0.9981, which is above SNI's threshold of ≥0.995 hence the graph is deemed acceptable as a standard curve. The equation generated from the graph was then used to calculate the concentration of the samples, whose absorbance value has been measured by the Vis spectrophotometer at 420 nm. The results of the concentration calculation of low COD can be seen on Table 4. As can be observed, the graph does not follow the Lambert-Beer law. Especially for low COD analysis, the concentration and absorbance values are not proportional to each other, but rather the opposite. This is because of the reagent used for the reaction, namely Potassium dichromate ($K_2Cr_2O_7$) which has a bright yellow color. When

homogenized with a solution with high concentrations of organic matter, the color will fade, leaving a colorless liquid, on the other hand, when mixed with a solution with low concentration of organic matter, the color will stay vibrant yellow. This is why the curve is sloped downwards, because the higher the concentration, the more colorless the substance will be, which will result in a lower absorbance reading in the spectrophotometer.

Table 4. Concentration and absorbance values for low COD samples

Sample	Commodity	Absorbance			COD (mg/L)
		Reading 1	Reading 2	Average	
Blanko	Aquadest	0.0000	0.0020	**0.0010**	**1.2000**
Standard	KHP 60 mg/L	-0.1540	-0.1540	**-0.1540**	**63.2000**
4418.1	Domestic Wastewater	-0.0540	-0.0580	**-0.0560**	**24.0000**
4418.2		-0.0550	-0.0550	**-0.0550**	**23.6000**
4418 (Xr)					**23.8000**
4233		-0.0540	-0.0550	**-0.0545**	**23.4000**
4234		-0.0230	-0.0190	**-0.0210**	**2.4400**
4430		-0.0910	-0.0930	**-0.0920**	**38.4000**
Quality Control Requirements					
4418	% RPD	$= \lvert x1 - x2 \rvert / \bar{x} \text{ x } 100\%$ $= \frac{\lvert 24 - 23.6 \rvert}{23.8} x\ 100\%$			**1.6807%** (<10%) approved
Standard	% R	$= \frac{Measured\ Standard\ Concentration}{Known\ Standard\ Concentration} x\ 100\%$ $= \frac{63.2}{60} x\ 100\%$			**105.3000%** (85% - 115%) approved

Based on Table 4, the concentrations of all the samples tested for low COD are within the threshold written on Peraturan Gubernur Jawa Timur Nomor 72 Tahun 2013 about *"Baku Mutu Air Limbah Bagi Industri dan/atau Kegiatan Usaha Lainnya"*, which is under 100 mg/L as all of said samples are classified as Type I wastewater. The highest concentration amongst the samples are from sample 4430, which turns out to be wastewater from a chemical-processing company at 38.4 mg/L which is still considered as safe. Apart from the low COD concentrations, the quality control done during the analysis also produced good results as the %RPD and %R numbers are below/within the desired threshold. %RPD or relative percent difference is calculated through diploid testing from sample 4418 to measure the precision between the tests, measured by dividing the change in value to the average of the value. %R on the other hand, is measured to ensure the accuracy of the analysis being done.

On the other hand, as previously mentioned, high COD analysis requires a separate standard curve with different standard points. As the concentration range of COD analysis falls between 100 - 1000 mg/L, the calibration curve made is also in between those values. 9 concentration points were used to generate the curve, and as the minimum concentration point is 5 (SNI, 2009), the remaining 8 points are enough for the curve to be accepted. The table and the curve can be seen on Table 5 and Figure 2 below.

Table 5. Concentration and absorbance values for high COD standard curve

Concentration (mg/L)	Absorbance		
	Reading 1	Reading 2	Average Absorbance
0	0.0000	0.0010	**0.0005**
100	0.0300	0.0340	**0.0320**
200	0.0680	0.0680	**0.0680**
300	0.1065	0.1065	**0.1065**
400	0.1340	0.1360	**0.1350**

500	0.1780	0.1780	**0.1780**
600	0.2000	0.2000	**0.2000**
700	0.2300	0.2330	**0.2315**
1000	0.3420	0.3420	**0.3420**
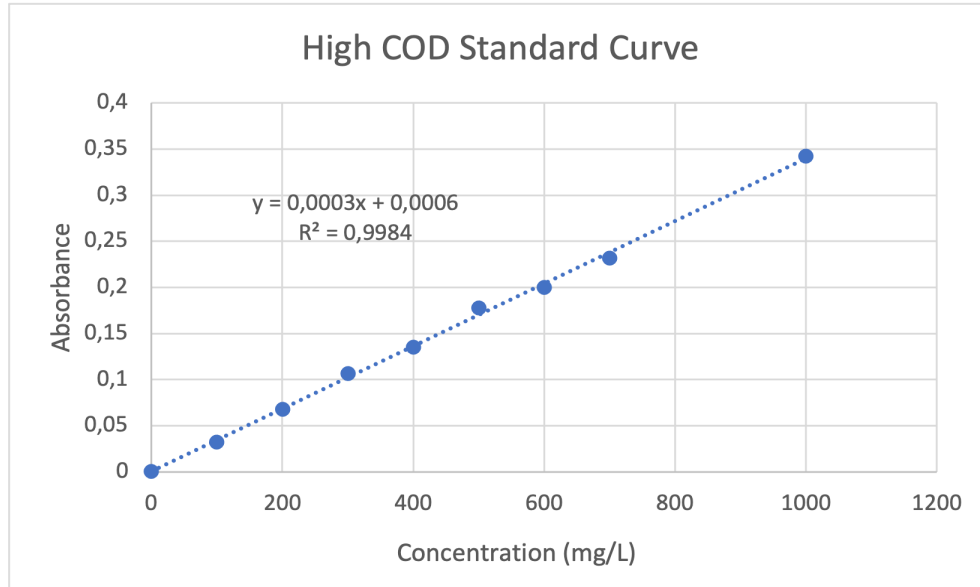
Figure 2. High COD Standard Curve

As can be seen above, the relationship between absorbance and concentration for high COD measurement has been established through the curve. The absorbance value is displayed on the y-axis and the concentration is displayed on the x-axis in mg/L. The R squared value generated from the graph is 0,9984, which is above 0.995 and is therefore deemed acceptable by SNI (2009)'s standards. The equation is then used to calculate the sample's concentration, which can be seen on Table 6.

Table 6. Concentration and absorbance values for high COD samples

Sample	Commodity	Absorbance			COD (mg/L)
		Reading 1	Reading 2	Average	
Blanko	Aquadest	0.0010	0.0010	**0.0010**	**1.3333**
Standard	KHP 300 mg/L	0.0970	0.0990	**0.0980**	**324.6667**
4384.1		0.0710	0.0710	**0.0710**	**234.6667**
4384.2		0.0690	0.0670	**0.0680**	**224.6667**
4384 (Xr)					**229.6667**
4409	Industrial Wastewater	0.0740	0.0740	**0.0740**	**244.6667**
4410		0.0590	0.0630	**0.0610**	**201.3333**
4411		0.0660	0.0680	**0.0670**	**221.3333**
4437		0.0610	0.0610	**0.0610**	**201.3333**
4438	Slaughterhouse wastewater	0.1400	0.1400	**0.1400**	**464.6667**
Quality Control Requirements					
4384	% RPD	$= \lvert x1 - x2 \rvert / \bar{x} \times 100\%$ $= \dfrac{\lvert 234.6667 - 224.6667 \rvert}{229.6667} \times 100\%$			**4.3541%** (<10%) approved
Standard	% R	$= \dfrac{Measured\ Standard\ Concentration}{Known\ Standard\ Concentration} \times 100\%$ $= \dfrac{324.6667}{300} \times 100\%$			**108.2222%** (85% - 115%) approved

Results of the high COD analysis shows that all of the samples tested are within Peraturan Gubernur Jawa Timur Nomor 72 Tahun 2013's threshold of under 250 mg/L except for sample 4438, which turned out to be wastewater from a local slaughterhouse. Slaughterhouses are known to have high concentrations of COD as the waste consists of mainly animal by-products, hence further treatments should be carried out to ensure the safety of its surrounding environment. Other than the COD analysis, the quality control requirements are all

within the range of SNI's threshold which is below 10% for RPD and 85%-115% for %R. This ensures that the standard used for the making of the curve is calibrated and that the analytical method/procedures are correctly followed.

6.1.2 Biological Oxygen Demand (BOD) analysis using Titrimetry

The results of the BOD analysis are displayed below on Table 7. As previously stated, the dissolved oxygen concentration is the first step to measuring the sample's BOD concentration, hence it is also displayed in the table below along with the volumes of the sample (inside the winkler bottle), the titration volume, and the commodity of the sample.

Table 7. BOD Concentrations of wastewater samples

Code	Commodity	Sample Vol (mL)	Dilution Factor	DO_0			DO_5			BOD (mg/L)
				Sample Vol (mL)	Titration Vol (ml)	DO0 (mg/L)	Sample Vol (mL)	Titration Vol (mL)	DO05 (mg/L)	
ABM	Aquadest	-	-	100.45	4.20	8.5520	98.32	3.98	8.2542	**0.2678**
BL	Nutrient solution	-	-	98.75	4.12	8.5066	99.36	3.76	7.7146	**0.7919**
STD	Standard Solution	5	50	100.07	4.22	8.5958	99.98	2.16	4.4038	**170.005**
4384.1	Waste-water	5	50	98.25	4.04	8.3847	98.40	2.66	5.5120	**104.04**
4384.2		5	50	98.81	4.20	8.6664	99.50	2.78	5.6957	**108.94**
4384 (Xr)		-					-			**106.49**
Inlet		20	12.5	99.35	4.06	8.3310	100.97	2.32	4.6827	**35.7063**
Outlet		100	2.5	100.93	4.22	8.5210	100.98	3.14	6.3371	**3.48**
4301	River Water	150	100	99.86	4.16	8.4917	100.54	2.32	4.7031	**7.4918**
4302		150	100	100.01	4.30	8.7641	99.66	2.98	6.0955	**4.6918**
4303		150	100	99.01	4.10	8.4426	101.44	2.90	5.8257	**4.5625**
4304		150	100	101.88	4.20	8.4000	103.99	3.18	6.2284	**3.4493**

Quality Control Requirements				
ABM	Aquadest		= 0.2678 mg/L	(<0.4 mg/L) Approved
BL	Nutrient Solution		= 0.7919 mg/L	(0.6-1.0 mg/L) Approved
4384	%RPD	$= \lvert x1 - x2 \rvert\ /\ \bar{x}\ \text{x } 100\%$ $= \dfrac{\lvert 104.04 - 108.94 \rvert}{106.49}\ x\ 100\%$	= 4.6016%	(<30%) Approved
STD	%R	$= \dfrac{Measured\ Standard\ Concentration}{Known\ Standard\ Concentration}\ x\ 100\%$ $= \dfrac{170.005}{193.0} x\ 100\%$	= 88.0855%	(85-115%) Approved

Table 7 shows the calculation and overall BOD concentration for the samples tested. There are two types of water analyzed for this dataset namely wastewater and river water. For the wastewater samples, samples "inlet" and "outlet" are wastewater samples from BSPJI. Inlet is wastewater which has not been treated, whereas outlet is for wastewater which has been treated locally by BSPJI, which can be seen by its low BOD concentration in comparison to Inlet. The sample 4384 on the other hand, has a relatively high BOD concentration with an average of 106.49 mg/L. This concentration is above Peraturan Gubernur Jawa Timur No. 72 Tahun 2013's quality threshold of 100 mg/L for industrial wastewater, hence the wastewater should be taken back for analysis & to be filtered out more heavily before it is able to be brought back to the environment. The other set of samples are the river water samples, whose analysis results are all within Peraturan Pemerintah No. 22 Tahun 2021's safe-range of below 12 mg/L as it is classified as Type 4 river water. The quality control done is also approved by the standards of SNI which proves that the analysis was done in an acceptable manner. The BOD values of ABM (mineral-free water) and BL (nutrient solution) are within SNI's safe range hence are eligible for calculation. The %RPD and %R values are also approved as their values are 4.6016% and

88.0855% respectively, which is below 30% (for RPD) and inside the range of 85% t0 115% (for %R).

6.1.3 Total Suspended Solid (TSS) analysis using Gravimetry

The results of the TSS concentration of the wastewater samples analyzed are displayed below on Table 8. As previously mentioned, gravimetric analysis was performed to measure the concentration of TSS in these water samples, hence multiple weighing were done to ensure that all the samples had reached stability. That said, although the measurement was done three times, only the last measurement was used to calculate the TSS concentration of the samples.

Table 8. TSS Concentration of wastewater samples

Code	Commodity	Sample Vol (mL)	W_i (mg)			W_f (mg)			W_f-W_i (g)	TSS (mg/L)
			1	2	3	1	2	3		
4795.1	Irrigation Wastewater	500	0.1166	0.1165	0.1165	0.1216	0.1198	0.1196	3.1	**6.200**
4795.2		500	0.1180	0.1179	0.1179	0.1222	0.1210	0.1209	3	**6.00**
4795 (Xr)		-								**6.10**
4792	Chicken Slaughterhouse Wastewater	100	0.1165	0.1164	0.1164	0.1228	0.1203	0.1201	3.7	**37.00**
4793		250	0.1170	0.1169	0.1169	0.1198	0.1184	0.1183	1.4	**5.60**
4766	Slaughterhouse Wastewater	200	0.1178	0.1177	0.1177	0.1225	0.1205	0.1203	2.6	**13.00**
4665	Domestic Wastewater	400	0.1170	0.1169	0.1169	0.1207	0.1198	0.1196	2.7	**6.75**
4717		400	0.1172	0.1171	0.1171	0.1210	0.1195	0.1192	2.1	**5.25**
4828		500	0.1178	0.1177	0.1177	0.1225	0.1205	0.1203	2.2	**4.40**
4883		400	0.1171	0.1171	0.1170	0.1219	0.1199	0.1198	2.8	**7.00**
4884	Industrial Wastewater	250	0.1175	0.1173	0.1172	0.1202	0.1194	0.1193	2.1	**8.40**
4885		100	0.1193	0.1191	0.1191	0.1218	0.1211	0.1212	2.1	**21.00**
Quality Control Requirements										
4795	%RPD	$= \lvert x1 - x2 \rvert / \bar{x} \times 100\%$						**= 3.2787%**		<5%

		$= \dfrac{|6.2-6|}{6.1} \; x \; 100\%$		Approved

From the samples present, irrigation and domestic wastewaters are considered type I wastewater, whereas industrial and slaughterhouse wastewaters are considered type II wastewater. Based on the results of the analysis, all seems to be within the safe threshold from Peraturan Gubernur No. 72 Tahun 2013 which is below 20 mg/L for type I wastewater and below 40 mg/L for type II wastewater. The quality control done for this analysis also produced great results, with an RPD value of 3.2787%, which is below 5% and is therefore approved.

6.1.4 Total Dissolved Solid (TDS) analysis using Gravimetry

The result of the TDS analysis of various wastewater samples using gravimetric analysis is displayed below on Table 9. In the table the initial weight of the porcelain cup is written as W_i, whereas the final weight of the cup with the dissolved solid is written as W_f. Similar to the TSS analysis results, although the measurement was done three times for both W_f and W_i, only the last of the three measurements were used for calculation as that is when the cups are stable.

Table 9. TDS Concentration of wastewater samples

Code	Commodity	Sample Vol (mL)	W_i (mg)			W_f (mg)			W_f-W_i (g)	TDS (mg/L)
			1	2	3	1	2	3		
4471.1	Cooling Wastewater	100	54.6501	54.6492	54.6433	54.6709	54.6712	54.6713	28.0	**280**
4471.2		100	50.6843	50.6841	50.6841	50.7137	50.7139	50.7138	29.7	**297**
4471 (Xr)		-								**288.5**
4468	Domestic Wastewater	100	56.8803	56.881	56.881	56.9596	56.9594	56.9594	78.4	**784**
4469		100	54.6344	54.6343	54.6344	54.7089	54.7088	54.7088	74.4	**744**
Outlet	Industrial Wastewater	100	52.0499	52.0494	52.0494	52.1646	52.1596	52.1595	110.1	**1101**

Inlet		100	45.5974	45.5983	45.5983	45.7267	45.7248	45.7250	126.7	**1267**
Quality Control Requirements										
4795	%RPD	$= \lvert x1 - x2 \rvert / \bar{x} \text{ x } 100\%$ $= \dfrac{\lvert 280 - 297 \rvert}{288.5} \; x \; 100\%$							**= 5.8925%**	<10% Approved

Based on the results obtained from the calculation, all of the samples tested are still within the threshold set by Peraturan Gubernur Jawa Timur Nomor 72 Tahun 2013. Amongst the samples, cooling and domestic wastewaters are considered type I wastewater, with a maximum TDS concentration of 1000 mg/L, whereas the industrial wastewater samples, inlet and outlet which is BSPJI's wastewater, are considered type II wastewater with a maximum TDS concentration of 2000 mg/L. Additionally, according to the quality control assessment, the analysis is accurate enough to be accepted with a %RPD value of 3.2787%, which is below 10%.

6.1.5 Cadmium(Cd) analysis using Graphite Furnace Analyzer (GFA) Atomic Absorption Spectrophotometry (AAS)

As previously mentioned, GFA AAS is used to measure the concentration of Cadmium in water samples. Specifically for this analysis, all the samples analyzed are bottled drinking water (AMDK). The threshold of Cadmium concentration in drinking water is ≤ 0.003 mg/L or 3μg/L, because of that the points used in making the standard curve displayed below on Table 10 ranges from 0 - 3 μg/L. All of the serial dilution is done through the AAS equipment and was read 3 times.

Table 10. Concentration and absorbance values for Cadmium concentration analysis standard curve

Concentration (μg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Average Absorbance

0.0000	0.0021	0.0025	0.0036	**0.0023**
0.2500	0.0176	0.0205	0.0222	**0.0201**
0.5000	0.0471	0.0480	0.0497	**0.0483**
1.0000	0.1074	0.0975	0.1046	**0.1060**
1.5000	0.1717	0.1817	0.1543	**0.1692**
2.0000	0.2522	0.2538	0.2164	**0.2408**
3.0000	0.3639	0.3811	0.3892	**0.3725**

From the absorbance and concentration points calculated above, a standard curve was generated as can be seen on Figure 3. The graph shows a good regression line with an R value of 0.9965, which is approved by SNI's standard of above 0.995.

Figure 3. Cadmium Concentration Analysis Standard Curve

Cadmium (Cd) Standard Curve

$y = 0.1254x - 0.0108$
$R^2 = 0.9965$
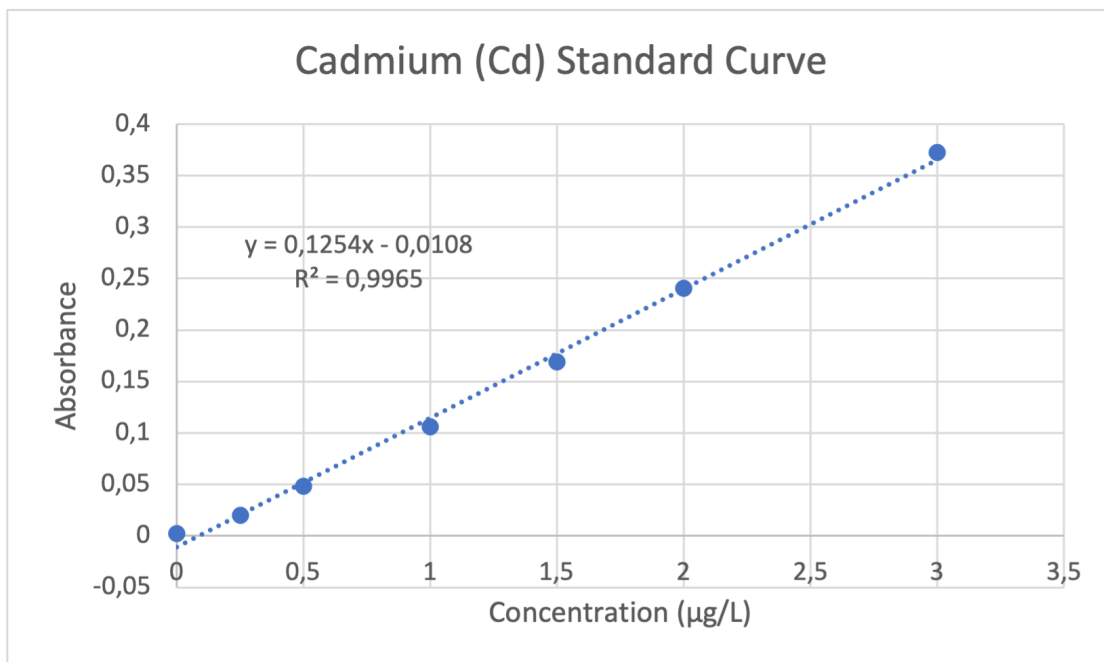
The equation projected from the graph, $y = 0.1254x - 0.0108$ is then used to calculate the samples' Cadmium concentration, which can be observed on Table 11.

Table 11. Concentration and absorbance values of Cadmium analysis samples

Sample	Commodity	Absorbance				Cd Concentration (µg/L/)
		Reading 1	Reading 2	Reading 3	Average	
Blanko	Aquadest	0.0023	0.0011	0.0027	**0.0017**	**0.0994**
4703.1	Bottled Drinking Bottle (AMDK)	0.0023	0.0028	0.0025	**0.0025**	**0.1060**
4703.2		0.0036	0.0019	0.0026	**0.0027**	**0.1073**
4703 (Xr)						**0.10665**
4703+sp		0.0330	0.0323	0.0310	**0.0321**	**0.3418**
4477		0.0037	0.0029	0.0017	**0.0028**	**0.1079**
4752		0.0027	0.0018	0.0037	**0.0027**	**0.1076**
4753		0.0034	0.0029	0.0028	**0.0030**	**0.1100**
4676		0.0016	0.0044	0.044	**0.0035**	**0.1135**
4678		0.0017	0.0023	0.0029	**0.0023**	**0.1042**
4468		0.0057	0.0053	0.0055	**0.0055**	**0.1297**
Quality Control Requirements						
4703	% RPD	$= \lvert x1 - x2 \rvert / \bar{x} \times 100\%$ $= \dfrac{\lvert 0.1060 - 0.1073 \rvert}{0.10665} \times 100\%$				**1.2189%** (<10%) approved
4703+sp	%R	$= \dfrac{spike\ concentration - sample\ concentration}{known\ standard\ concentration} \times 100\%$ $= \dfrac{0.3418 - 0.10665}{0.2500} \times 100\%$				**94.06%** (85-115%) approved

Based on the results portrayed in Table 11., all of the drinking water that was tested has a Cadmium concentration lower than 3 µg/L with the highest concentration being only at 0.1135 µg/L, far from the maximum threshold set by SNI. As for the quality control requirement done for the analysis, the RPD value, similar to the other testings, has a percentage of 1.2189%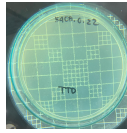 which is lower than 10% and is therefore approved. For the %R value, it was calculated to be at 94.06%, which is still inside the range set by SNI, and with that it can be concluded that the analysis was done well and produced accurate results.

6.1.6 Ammonia (NH3) analysis using UV-VIS Spectrophotometry

Following the methods written in SNI 06-6989.30-2005, spectrophotometry was the preferred method to analyze ammonia concentrations in drinking water and wastewater. The threshold for ammonia contents in drinking water and wastewater are 0.5 mg/L and 1-1.5 mg/L respectively. Following that, the standard curve was made with concentration points from 0 to 2 mg/L using the ammonium stock solution previously prepared. The standard curve can be seen on Figure 2 with the concentration points used visible in Table 12.

Table 12. Concentration and Absorbance values for Ammonium (NH4) Standard Curve

Concentration (mg/L)	Absorbance		
	Reading 1	Reading 2	Average Absorbance
0	0.0000	0.0020	**0.0010**
0.2	0.0910	0.0910	**0.0910**
0.4	0.1550	0.1590	**0.1570**
0.6	0.2350	0.2350	**0.2350**
1	0.4100	0.4120	**0.4110**
2	0.7750	0.7750	**0.7750**

From the points written above, a standard curve was generated. The curve which can be observed on Figure 4 has an equation of $y = 0.387x + 0.0074$ and a regression value of 0.9989, which is considered to be near perfect as the highest R square value is 1. The equation previously mentioned is then used to calculate the ammonium concentration of each sample before they are then calculated for their ammonia concentration. Said concentrations can be seen on Table 13., complete with the quality requirements from SNI included.

Figure 4. Ammonium (NH$_4$) Concentration Analysis Standard Curve
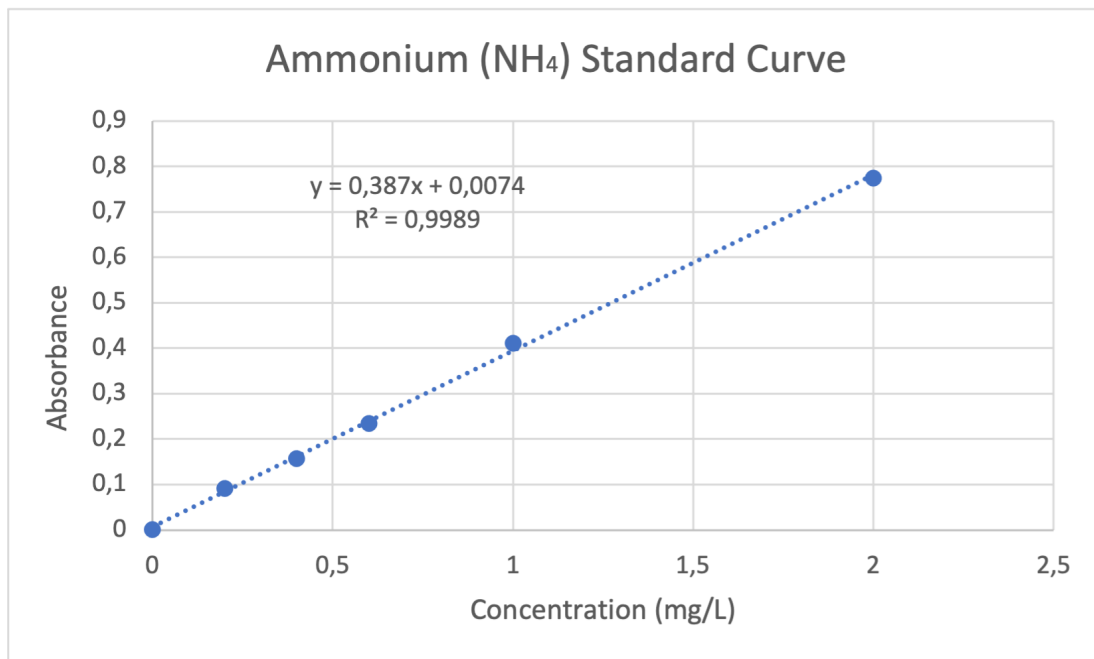
Table 13. Concentration and absorbance values of Ammonia analysis samples

Sample	Commodity	Dilution Factor (fp)	Absorbance	Concentration	
				Ammonium (NH4)	Ammonia (NH3)
Blanko	Aquadest	1	-0.0320	**-0.0050**	**<0.0196**
4154.1	Bottled drinking	1	0.0060	**-0.0030**	**<0.0196**
4154.2		1	0.0040	**-0.0090**	**<0.0196**

4154 (Xr)	water (AMDK)			**-0.0060**	**<0.0196**
4154+s		1	0.5670	**0.5670**	**0.5360**
3975	Wastewater samples	1	0.0340	**0.0700**	**0.0660**
3811		10	0.0650	**1.4988**	**1.4156**
Inlet		100	0.0370	**7.7519**	**7.3220**
Outlet		1	0.1820	**0.4522**	**0.4271**
Quality Control Requirements					
4384	% RPD	$= \lvert x1 - x2 \rvert / \bar{x}$ x 100% $= \frac{\lvert 271.3333 - 258 \rvert}{264.6667} \, x \, 100\%$			**5.0378%** (<10%) approved
Standard	% R	$= \frac{Measured\ Standard\ Concentration}{Known\ Standard\ Concentration} \, x \, 100\%$ $= \frac{324.6667}{300} \, x \, 100\%$			**108.2222%** (85% - 115%) approved
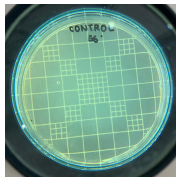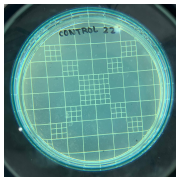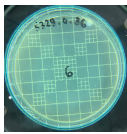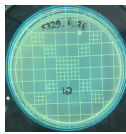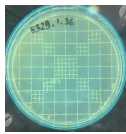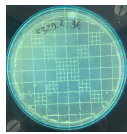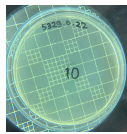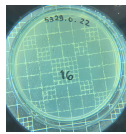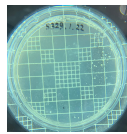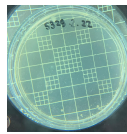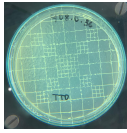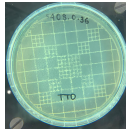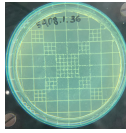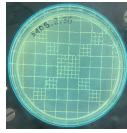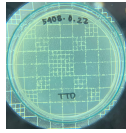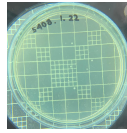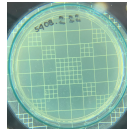
Based on the results portrayed in Table 13., the drinking water sample's ammonia concentration is less than 0.0196 which is below SNI's standard of 0.5 mg/L and is deemed safe for consumption. As for the wastewater, all are classified as type II wastewater because they were obtained from industrial plants as well as BSPJI's own wastewater. After the analysis, all except for the inlet samples have an ammonia concentration of under 1.5 mg/L, which is considered safe by Peraturan Gubernur Jawa Timur No. 72 Tahun 2013's standard. As for the inlet sample, as have been explained before, it is BSPJI's pure waste before it is processed, so it will not be released to the environment, hence BSPJI's wastewater is also considered safe. For the quality control, both the RPD and %R value showed great results, hence the analysis is deemed safe and accurate.
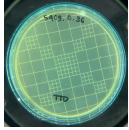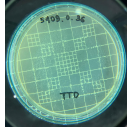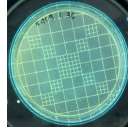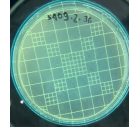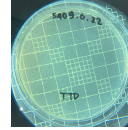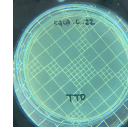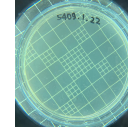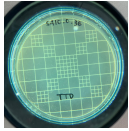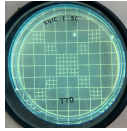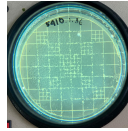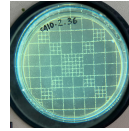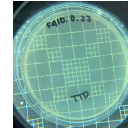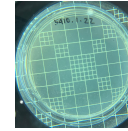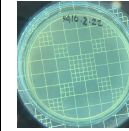
6.2 Parameters from the Microbiology Laboratory

6.2.1 Total Viable Cell (TVC) analysis on drinking water samples using Pour-Plate Method

The results of the total viable cell analysis on drinking water samples can be observed on Table 14 below. On the table, the sample number, commodity, as well as the results of its TVC analysis both at 36°C and 22°C can be observed. Additionally, as all of the enumeration was done manually, the pictures displayed on the table serve as proof of the amount of colonies present in the samples. For cell counting, if the colonies are already readable at 10^0, the 10^1 and 10^2 samples can be overlooked.

Table 14. Results of the Total Viable Cell (TVC) analysis on drinking water samples using Pour-Plating Method

Sample	Commodity	Samples incubated at 36°C				Samples incubated at 22°C			
		10^0	10^0	10^1	10^2	10^0	10^0	10^1	10^2
C	Control								
		Not Detected (ND)				Not Detected (ND)			
5329	Drinking Water (AMDK)								
		6 colonies	12 colonies	overlooked	overlooked	10 colonies	16 colonies	overlooked	overlooked
5408									
		ND	ND	overlooked	overlooked	ND	ND	overlooked	overlooked

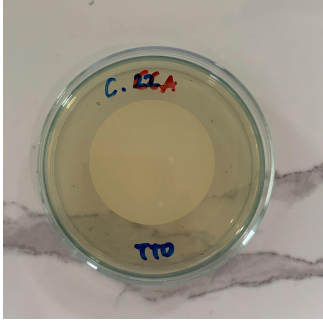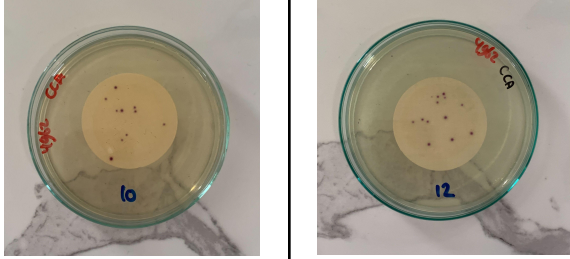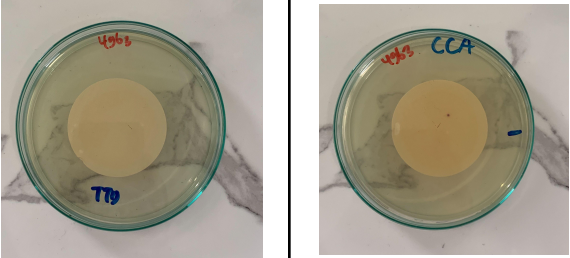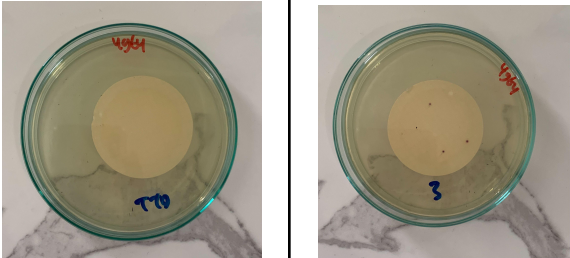| 5409									
		ND	ND	overlooked	overlooked	ND	ND	overlooked	overlooked
5410									
		ND	ND	overlooked	overlooked	ND	ND	overlooked	overlooked

Based on the results of the TVC analysis displayed above on Table 14, most of the drinking water samples are free from any microorganisms and contaminants, as most of the results of the testing are ND, which means that there are no microorganism colonies detected. For sample 5329, however, several bacterial colonies were seen present. As the colonies were visible enough to be enumerated at 10^0, enumeration was only done at 10^0, overlooking samples 10^1 and 10^2. After the enumeration, 6 & 12 colonies were found at the sample which was incubated at 36°C whereas 10 and 16 colonies were found at the sample which was incubated at 22°C. The threshold value stated from SNI is 250 colonies, since the number of the colonies present are still below 100 colonies, sample 5329 is still deemed safe for consumption.

6.2.2 Identification and Enumeration of Total Coliform Bacterias in bottled drinking water samples using Membrane Filtration
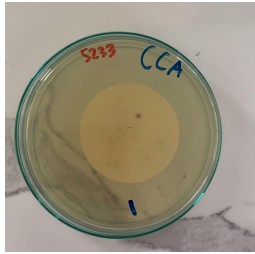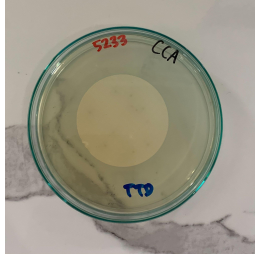
The results of the total Coliform Bacteria analysis on bottled drinking water samples using the membrane filtration process can be observed on Table 15 below. Similar to the TVC analysis, as the colonies were counted manually, the pictures presented below serve as proof on how many bacterial colonies were present in the samples.

Table 15. Results of total coliform analysis on drinking water samples using membrane filtration system

Sample	Commodity	Results		Average

		1	**2**	
C	Control			ND (not detected)
		Not detected (ND)		Approved
4962	Bottled drinking water sample (AMDK)			11
		10	12	Not Approved
4963				ND (not detected)
	Bottled drinking water samples (AMDK)	ND (not detected)	1	Approved
4964				1.5 colonies
		ND (not detected)	3	Not Approved

5233				ND (not detected)
		1	ND (not detected)	Approved
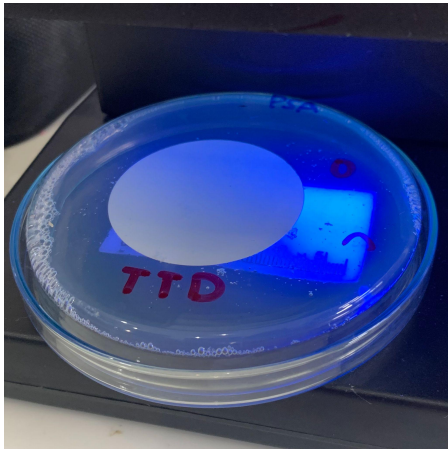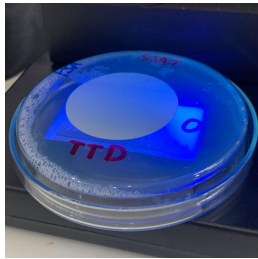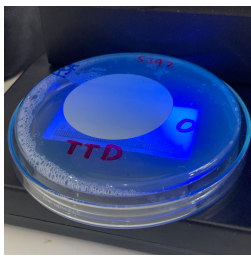
The results of the total coliform analysis on drinking water samples showed diverse results. Starting from sample 4962, the average number of colonies formed were 11 colonies. As the threshold for coliform bacteria from SNI is <1 as it is dangerous for human health, this water is not safe from consumption, hence is not eligible for commercialization. For sample 4964, although one of the results of the analysis came out as ND, the other result is 3 colonies, hence averaging to 1.5 colonies and is therefore also not eligible for consumption. For sample 5233 whose results are all ND as well as 4963 which results in 1 ND and 1 colony, both passed the analysis and is issued standardization from BSPJI. Specifically for sample 4963, although 1 colony was present, when averaged with 0, it amounts only to 0.5 colonies which is less than one, which is why it is still safe for consumption.
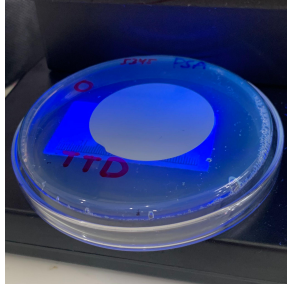
6.2.3 Identification and Enumeration of *Pseudomonas aeruginosa* analysis in bottled drinking water samples using Membrane Filtration

The results of the *P. aeruginosa* analysis on bottled drinking water samples using the membrane filtration system can be observed below on Table 16. On the table, the sample, commodity, as well as the picture for the analysis which is done in duploids can be seen. Again, as the samples were manually enumerated, the pictures presented below shows the total number of colonies present in each drinking water sample.

Table 16. Results of *Pseudomonas aureus* analysis on drinking water samples using membrane filtration system

Sample	Commodity	Results		Average

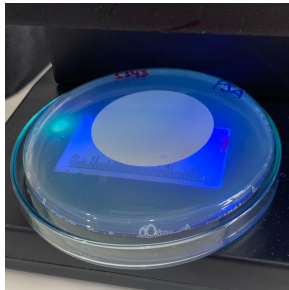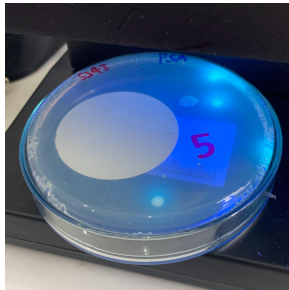|		**1**	**2**	
C	Control			ND (not detected)
		Not detected (ND)		Approved
5342	Bottled drinking water sample (AMDK)			ND (not detected)
		ND (not detected)	ND (not detected)	Approved
5343	Bottled drinking water samples (AMDK)			3
		1	5	Not Approved
5344				ND (not detected)

		ND (not detected)	ND (not detected	Approved
5345				ND (not detected)
		ND (not detected)	ND (not detected)	Approved

Based on the results displayed on Table 16 above, all of the samples except for sample 5343 has no *P. aeruginosa* colony detected, hence all are approved for consumption and general commercialization. As there are 1 and 5 *P. aeruginosa* colonies on sample 5343, and SNI has a threshold of 0 for these microorganisms because of its dangers to human health,it is deemed not safe for consumption, hence certification could not be given to the company. The company was instructed to do a follow-up testing once the problem has been identified & fixed.

SELF REFLECTION

This internship opportunity at BSPJI has given the writer a lot of opportunities to learn, grow, as well as develop both as a student and as a member of the biotechnology industry. Over the course of the four month internship, a lot of skills and knowledge has been gained through the countless analysis methodologies to test for many different parameters present in BSPJI's environmental-chemistry and microbiology laboratory. Most of the knowledge gained stems from the principles of the equipment used during the analysis and the chemical reactions that serve as a foundation for the testing. Similarly, most of the skills achieved and accumulated are also from continuously and repetitively using the equipment and machineries during the testing process. As all of the analysis conducted in BSPJI follows Indonesia's national standard (SNI), the writer firmly believes that all of the skills and knowledge acquired during the internship will be greatly beneficial to employment interest after the completion of the degree.

As previously mentioned, i3L has also taken a big part in the success and the completion of this internship process. Through the academic and non-academic activities conducted and learned from i3L, the writer was able to know some of the principles of the analysis done in BSPJI ahead of time, making the learning process so much easier as the foundation was already there. The laboratorium in i3L has also facilitated the writer with adequate knowledge as well as skills for the internship process, also giving the writer the foundation she needs to work in a more professional laboratory environment. Non-academically speaking, the BRIGHT sessions held throughout the years has also positively contributed to how the writer interacted with the members of BSPJI as well as her internship peers, acting as a guideline on how to communicate in a formal and non-formal basis and how to professionally commit to the work done in the lab.

All in all, the internship period was able to be finished well without any major problems. The writer was given enough responsibility to carry out different analysis independently, which indicates the level of trust the analysts have given as well as impact to the workplace. One of the weaknesses faced throughout the internship only lies on some poor instrumentation which needs to be changed because of time constraints, but apart from that, the internship period was completed well and the writer is content with the relationships built and results obtained from the four-month internship at BSPJI.

CONCLUSION & RECOMMENDATION

In conclusion, the aims and objectives of this internship have been fulfilled. Different quantitative methodologies have been applied to analyze many different water samples, which includes drinking water, wastewater, and raw water in BSPJI's environmental-chemistry and microbiology laboratory. Most of the results of the analyses turned out to be satisfactory hence certification was issued to said samples, however some samples such as the wastewater from the slaughterhouse and some new bottled drinking water brands have yet to fulfill the threshold set by SNI, hence certification could not be issued to said brands/companies and they are obligated to do a follow-up testing once the issues have been resolved. All in all, the writer has learnt a lot of valuable lessons during the 4-month internship period, which will certainly be beneficial for the future, especially for working in a professional industrial environment.

As previously stated before, some of the instruments and machineries used for analyses in BSPJI has been used for a long time, causing them to not be as calibrated/accurate as it should be, hence a suitable recommendation would be to calibrate said instruments as well as purchase new ones to ensure that all of the analyses are done accurately. For the report itself, as this report was only able to discuss 9 out of the many parameters tested in BSPJI, hopefully more people in the future are open to the idea of spending their internship period in this very institution, where they will be able to write and discuss about other sample types tested here, visit different laboratories, as well as learn about the many parameters being done in BSPJI. In short, the institution has all the necessary equipment and skills to carry out many important water quality tests, however some improvements are essential to be made, to ensure the quality of said analysis.

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APPENDICES

Appendix 1. The writer in front of BSPJI's laboratory at Jl. Jagir, Surabaya

Appendix 2. Example of water samples in BSPJI's environmental-chemistry laboratory

Appendix 3. Graphite furnace Atomic Absorption Spectrophotometer (AAS) used in BSPJI
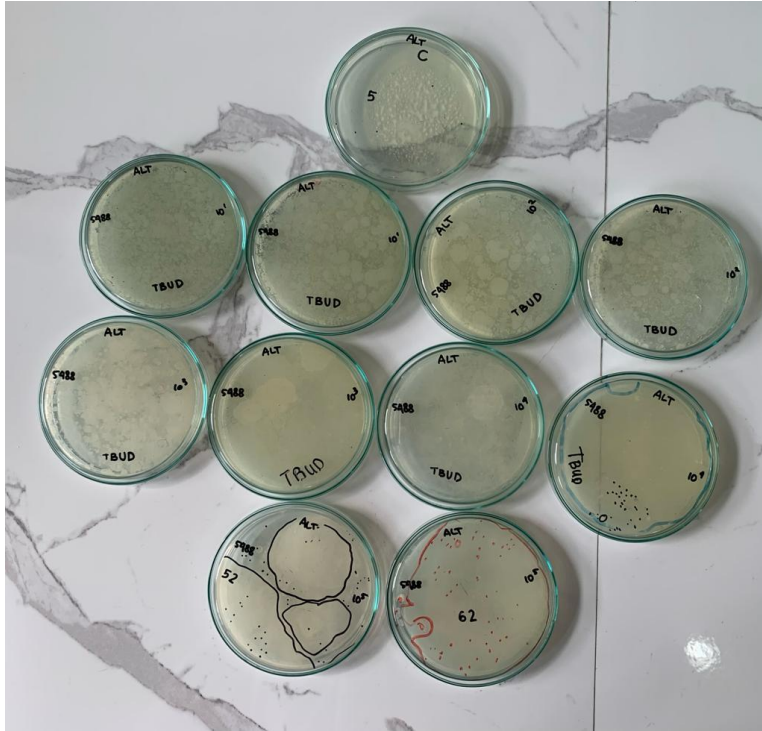

Appendix 4. Chemical Oxygen Demand (COD) analysis samples after heating

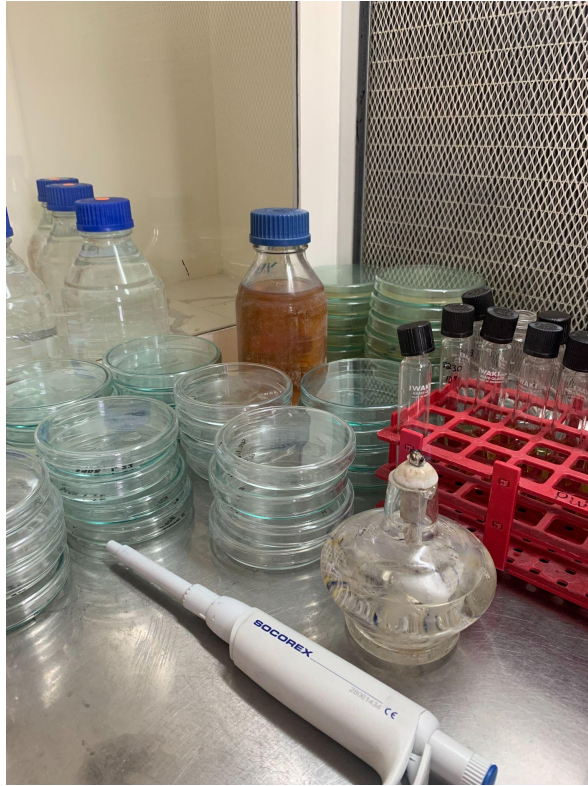**Appendix 5.** Total Suspended Solid (TSS) analysis in BSPJI's environmental-chemistry laboratory

Appendix 6. Biological Oxygen Demand (COD) analysis before titration

Appendix 7. Poor Total Viable Count (TVC) analysis results

Appendix 8. Membrane filtration apparatus in BSPJI's microbiology laboratory

Appendix 9. Workbench inside the laminar air flow in BSPJI's microbiology laboratory

Appendix 10. Monthly (every 17th of the month) flag ceremony in BSPJI

Turnitin (Plagiarism) Report

Flavia Nadine (19010056) - Biotechnology 2019