Abstract—Wastewater is a combination of the liquid wastes conducted away from residences, hospitals and industrial establishments and treatment of wastewater is crucial for environmental and public health as treated wastewater is reused in many ways including drinking water. In a wastewater treatment plant, microorganisms and toxic chemical compounds are removed from wastewater by physical, chemical and biological methods to generate effluent wastewater. Microorganisms play a critical role in activated sludge systems.

A culture media is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms which must contain all the nutrients required for the growth of the microorganism. The function of the media depends on its composition as well as the nutrients needed for the growth of all bacteria. It has been suggested that, no definitive standard exists for the assessment of culture media.

Herein, the aim of our study was to evaluate the effects of influent and effluent wastewater samples used in Mac Conkey agar on the growth of E. coli. Our preliminary results showed that influent wastewater was more effective on bacterial growth compared to effluent wastewater. The results of our preliminary findings need to be strengthened with additional studies conducted using wastewater samples.

Index Terms—Mac Conkey agar, E.coli, toxic chemicals, wastewater, nutrients.

I. INTRODUCTION

The production of waste from human activities is unavoidable. This waste will end up as wastewater. In literature, wastewater has been described as “a combination of the liquid wastes conducted away from residences, offices, business premises and institutions as well as from industrial establishments, where such ground, surface and storm water may also be admitted to or find its way into the sewers”, by the Committee on Sewerage and Sewage Disposal of the American Public Health Association. Therefore, wastewater is constructed from residential sources that are used, going down the drain from baths, sinks, dishwashers and toilets and also from commercial and industrial sources [1], [2].

Treatment of wastewater is crucial for environment and public health, as treated wastewater is reused in a many ways, including drinking water, golf course irrigation, cooling water for power plants and oil refineries, processing water for mills, plants, toilet flushing, construction activities, concrete mixing, artificial lakes, etc [2].

On the other hand if wastewater is not adequately treated spreading of diseases, fish kills and destruction of other forms of aquatic life may occur. The pollution of water has deleterious effects on all living creatures. The mission of a wastewater treatment plant is to treat the wastewater before it is returned to the environment. Therefore in every developing and developed country, wastewater treatment plants operate at a critical point of the water cycle, preventing the water from excessive pollution [2]-[4].

In wastewater treatment, most of the pathogenic microorganisms and toxic chemical compounds are removed from wastewater by physical, chemical and biological methods. Measuring the quality of influents and effluents has critical importance as it reflects the performance of the plant. Besides, microorganisms play a critical role in the activated sludge systems [4], [5].

In wastewater treatment plants an aerobic activated sludge system decomposes organic compounds via microorganisms. Microorganisms grow on the aerated organic matter and the newly formed microbial biomass is eventually consumed to meet maintenance energy needs [2]-[4]. Finally, wastewater effluent is produced as the final product of all earlier treatment processes, which can be discharged to a stream, river, bay, lagoon or wetland and can be used to irrigate parks, corps or to recharge groundwater and additionally it can also be used in industrial applications as well [5].

In literature, a culture media is defined as a solid or liquid preparation used for the growth, transport, and storage of microorganisms which must contain all the nutrients required for the growth of a microorganism. In culture media, usually tap water or distilled water is preferably used [6].

Escherichia coli (E. coli) was first isolated from the feces of a child in 1885 by the Austrian pediatrician Theodor Escherich [7]. E. coli is the common and most prevalent inhabitant of terminal small intestine and large intestine of human and many warm blooded animals belonging to Enterobacteriaceae family [8]-[10]. They are the most abundant facultative anaerobes and nonsporeforming bacilli in the environment [10], [11]. The optimum pH for E. coli growth is 6 to 8, however, growth can occur as low as pH 4 and as high as pH 9 as well. The optimal conditions for growth are a temperature of 37°C. It can survive between 4-45°C. On the other hand vegetative cells killed by pasteurization and excess heat [7], [10], [12]. E. Coli is almost 0.5 μm in diameter and 1.0–2.0 μm in length. Within the periplasm, single (thickness of 2.5 nm) to three layered (7nm thick) of peptidoglycan can be found. E. coli are commonly motile in liquid by means of peritrichous flagella. It can be found secondarily in soil and water as a consequence of fecal contamination. In general, its determination has been used as an indicator of poor water
quality [13]-[15]. Most strains of E. coli are harmless and healthy intestinal tract can synthesize vitamin K2 by the help of these bacterial strains. Moreover, it suppresses the growth of several pathogenic bacteria [16].

Consequently, the aim of our study was to evaluate the effects of influent and effluent wastewater samples used in Mac Conkey agar on the growth of E. coli.

II. MATERIALS AND METHODS

A. Preparing Media with Wastewater

Influent and effluent wastewater samples collected between May and August from wastewater treatment plant in Istanbul in 2015 were used as study material. Samples were transported in sterilized glass bottles with screw cap in opaque special transport containers to microbiology laboratory. After collection of samples, they were immediately transported to laboratory on ice and studied on the same day.

B. Growth Media Prepared Using Wastewater

Growth media was prepared using wastewater according to the standard procedures to produce the Mac Conkey agar media. MacConkey Agar is generally used for the selective isolation and identification of Enterobacteriaceae family from feces, urine, water, wastewater and foods. It’s selective and differential culture media containing peptons, bile salts, lactose, crystal violet, NaCl, neutral red dye, agar and water. It isolates Gram-negative and enteric bacteria and differentiate them based on the lactose fermentation [16]-[19]. Then, media was sterilized for 15 minutes at 121°C in an autoclave and was cooled down to 40-50°C and was poured into petri dishes. Plates were stored in the refrigerator after they were solidified.

C. Growth Media Prepared Using Distilled Water

The same procedure was applied for standard media [17]-[19].

D. E. coli Identification of Wastewater Samples

Wastewater samples were brought to laboratory in 300 ml opaque sterile glass bottles with screw cap in special transport containers and diluted in specific proportions for microbiological examination. The prepared samples were inoculated on Mac Conkey agar, plates were incubated at 36.5°C for 24-48 hours and the colonies were observed. Agar plates with bacterial growth were examined macroscopically and microscopically.

After macroscopic examinations, colonies were transferred onto slides using a needle and were stained with various painting methods. Then they were microscopically examined and photographed under a light microscope. At the end of microscopic examinations classical biochemical tests, Indole, Methyl red, Voges-Prokauer, Citrate (IMVIC) tests, triple sugar iron agar (TSI) tests and API kits were performed for accurate species identification.

The identified E. coli species were inoculated on Mac Conkey agar prepared by using wastewater and distilled water at 36.5°C for 24-48 hours to interpret the growth densities.

III. RESULTS

Biochemical diagnostic test result for E. coli (IMVIC tests +, +, -, -) was evaluated. H2S formation was not observed in TSI media, yellow acid reaction was observed both in the bottom and on the surface of the media. E. coli was found to give an opaque image as brick red color colonies on Mac Conkey agar. E. coli strains were confirmed with rapid identification of the samples with high standard API test.

In our preliminary study, 10 wastewater samples (as 5 influent and 5 effluent samples) obtained from wastewater treatment plant at different time points were used to prepare the Mac Conkey agar. The results of E. coli growth of each sample is given in Table I and the averages of the results are given in Table II.

E. coli growing areas were evaluated on the standard prepared media plates and on the media plates with wastewater samples. An average of 90 colonies were examined on Mac Conkey agar media prepared with influent wastewater obtained from treatment plant (10^-2 dilution) whereas 66 colonies were observed on the media prepared with effluent wastewater obtained from treatment plant (10^-3 dilution). As controls, Mac Conkey agar prepared with distilled water was observed to have 95 colonies of E. coli (Table II).

E. coli growing areas were evaluated on the standard prepared media plates and on the media plates with wastewater samples after 10^-3 dilution as well. An average of 18 colonies were examined on Mac Conkey agar media prepared with influent wastewater (10^-3 dilution) whereas 13 colonies were observed on the media prepared with effluent wastewater (10^-3 dilution). Mac Conkey media prepared with distilled water was observed to have 20 colonies of E. coli (Table II).

TABLE I: E. coli GROWTH REPRESENTED AS COLONY COUNT ON MAC CONKEY AGAR

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Colonies)</td>
<td>113</td>
<td>27</td>
<td>92</td>
<td>11</td>
<td>107</td>
</tr>
<tr>
<td>Influent (Colonies)</td>
<td>95</td>
<td>22</td>
<td>79</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>Effluent (Colonies)</td>
<td>63</td>
<td>17</td>
<td>48</td>
<td>8</td>
<td>74</td>
</tr>
</tbody>
</table>

TABLE II: AVERAGE E. coli GROWTH REPRESENTED AS COLONY COUNT ON MAC CONKEY AGAR

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Control</th>
<th>Influent</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-2 dilution</td>
<td>95 Colonies</td>
<td>90 Colonies</td>
<td>66 Colonies</td>
</tr>
<tr>
<td>10^-3 dilution</td>
<td>20 Colonies</td>
<td>18 Colonies</td>
<td>13 Colonies</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

The appropriate culture media is very important for the growth of microorganisms in laboratory conditions. For the analysis of water and food, microorganism identification as well as analyzing antibiotic sensitivities, some specialized media has been used by microbiologists since the nineteenth century. Although the use of rapid methods increase, most of the techniques applied in the pharmaceutical quality control laboratories still need growth media [17].
Therefore culture media is of fundamental importance for most microbiological tests in order to obtain pure cultures to grow and count microbial cells and to cultivate and select microorganisms. Without the appropriate and high-quality media, achieving accurate, reproducible and repeatable microbiological test results would be very difficult [17, 18]. A microbiological culture media can be defined as a substance that encourages the growth, support, and survival of microorganisms. Accordingly, a culture media contains nutrients, growth promoting factors, energy sources, salt, minerals, metals, and gelling agents [19].

E. coli ATCC 25922 is a nonpathogenic strain of E. coli. It is also easily cultured, well characterized as lactose fermenting Gram negative bacteria and additionally studied as a model organism in the field of microbiology and biotechnology [16], [20]-[22]. Lactose fermenting bacteria likeE. coli develop as red or pink color due to the acid production from lactose. Precipitated bile salts are observed around the proliferated colonies. Thered color is due to the production of acid from lactose. When the pH of media drops below 6.8, absorption of neutral red and a subsequent color change of the dye occurs. Bacteria that can’t have the ability to ferment lactose are colorless and transparent. The selective action of Mac Conkey agar is attributed to bile salts and crystal violet dye, as they have inhibitory effects on most of the Gram positive bacteria and conversely, Mac Conkey agar allow gram-negative organisms to grow. For these reasons, Mac Conkey agar is generally referred as “selective-differential” media [16]-[19].

It has been suggested that no one definitive standart exists for the assessment of culture media [19]. Under the light of these data, the aim of our study was to evaluate the effects of influent and effluent wastewater samples used in Mac Conkey agar on the growth of E. coli. Our preliminary results showed that influent wastewater was more effective on bacterial growth compared to effluent wastewater. The number of colonies observed on culture media prepared using the effluent wastewater were less than that of the culture media prepared using the influent wastewater and the control Mac Conkey media.

Although all microorganisms need sources of energy, nitrogen, carbon, phosphorus, sulfur, and various minerals, the exact composition of a satisfactory media depends on the species to be identified as nutritional requirements differ among the microorganisms. Therefore, it is important to know microorganism’s normal habitat to select a suitable culture media since its nutrient requirements reflect its natural surroundings. The function of the media also depends on its composition as well as the nutrients needed for the growth of all bacteria. Moreover, some special-purpose media contain one or more chemical compounds that are essential for their functional specificity [1]-[4].

Wastewater constituted from residential sources contain the necessary nutrients for the growth of bacteria. In our case, wastewater was constructed mainly from domestic sources and mostly greywater and blackwater. Chemically, wastewater is made up of organic and inorganic compounds. Organic compounds are mainly carbohydrates, proteins and fats which reflects the diet of the people. Carbohydrates and proteins are perfect diet for bacteria. On the other hand, in organisms are consist of heavy metals, nitrogen, nitrate, nitrite, ammonium ions, phosphorus, sulphur, chlorides and other toxic compounds. During the treatment processes, most of the essential organic materials such as nitrogen and phosphorus are removed during the secondary treatment. This may be the reason for the reduced bacterial growth in culture medium prepared with the effluent wastewater.

To the best of our knowledge, this is the first study in literature that investigates the effects of wastewater samples in culture media on the growth of bacteria. The results of our preliminary findings need to be strengthened with additional studies.

REFERENCES


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