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Research Article

Use of a convolution neural network for the classification of *E. Coli* and *V. Cholara* bacteria in wastewater

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ABSTRACT

Identifying the microbial population and type of them is a crucial measure in the water and wastewater treatment processes, reuse of wastewater, and sludge treatment system. Todays, manual methods are usually used to count and detect the type of bacteria in water and sewage laboratories which mostly suffer from human errors. This study aims at presenting an accurate method based on image analysis through the convolution neural network (CNN) to classify *Escherichia coli* (*E. coli*) and *Vibrio cholera* (*V. cholera*) bacteria, in wastewater. About 9,000 Red-Green-Blue (RGB) microscopic images of the sewage sample containing the stained bacteria were used as the input datasets. The results showed that the bacteria would be classified and counted with the accuracy of 93.01% and 97.0%, respectively. While CNN performed pretty well in counting the number of bacteria for both RGB and grayscale color models, its classification performance is only satisfactory in the RGB images. The sensitivity analysis of CNN illustrated that the Gaussian noise enhancement caused to the increment in the standard deviation (σ) that proportionally decreased the CNN accuracy.

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INTRODUCTION

Contamination identification, efficiency evaluation of the wastewater treatment system, and reusability of treated wastewater are basic principles of the microbial monitoring in the contaminated water resources with human wastewater [1–4]. In fact, according to the world health organization (WHO) guidelines, the main goal is preventing the water resources from contaminating by any type of pathogenic bacteria. One effective solution to this issue is the indicator microorganisms' contamination tests. For instance, existing the *E. coli* bacteria in the water samples has been considered as a fecal pollution indicator due to its ease and rapid detection [5, 6]. Traditionally, the manual method is used

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to count the number of cultured colonies to determine the amount of fecal pollution in water samples [7]. This method not only is time and energy consuming, but also suffers from the human errors [8]. Large number of samples as well as small size and overlapping on colonies, and diversity and large number of colonies formed in a sample are the most common reasons to make errors in this method [8–10].

Recently, employing the convolution neural network (CNN) along with the computer processing power has been playing an increasing role in the field of deep learning [11]. Some interesting applications of this method are in the environmental science, cancer detection, medical image processing. The interesting results in these applications are provided by the possibility of employing deeper layers in comparison with other artificial neural networks (ANN) [4, 12–15]. So far, CNN in measuring the concentration of cyanobacteria in water [16], pollution of the water distribution network [17], detection of water impurities [18], classification of urban wastewater microbeads [19], etc. have been studied. Akbarian Mymand et al. (2014), studied the feasibility of using an image processing method for the count of bacteria in the mixture of Quail flora, sourdough, and kefir drinks. They compared this method with colony counter method in the different dilution proportions. The results of this study indicated a significant difference between the numbers of counted bacterial in samples with low dilution proportion [20]. Huang and Wu (2018) classified the clinical bacterial colonies with different morphologies into 18 categories by a deep neural network (DNN)-based classifier. They obtained over 90% identification and classification accuracy of each bacterium category [21]. Yurtsever and Yurtsever (2018) achieved 89% accuracy to classify microbeads in the wastewater by CNN [19]. Shaily and Kala (2020) classified various shapes of bacterial particles in 20 different categories with more than 99% accuracy [22]. According to a previous study, CNN effectively improves the detection and counting accuracy of the bacterial particles [23].

In this study, by employing the capabilities of this method, we try to obtain a very high accurate classifier to identify the aforementioned bacteria in sewage. For this purpose, we design a two-part network which exploits the K-means and new data producing methods to prepare images which followed by a CNN to classify the data. First, the input images contain three components of RGB images with the resolution of 749x1000 pixels are used as the primary datasets for both bacteria. About 150 images per bacterium are considered as an initial number for the training set, which is increased to 9,000 to have more generalized dataset. One way of overfitting prevention is increasing the data which is obtained by some specific operations, such as adding Gaussian noise, rotating images at a 90o angle, upside down flip, left right flip and so on. Then, K-means clustering algorithm is used to image segmentation and masking. Finally, the prepared images are exploited as the input dataset to the CNN, and the reliability of this method is evaluated by the sensitivity analysis.

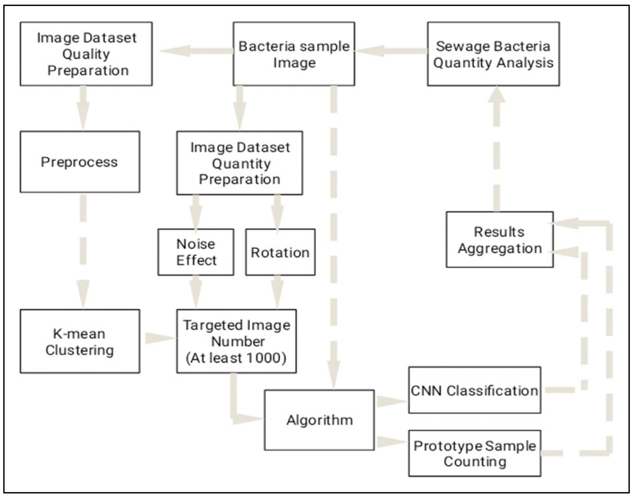

Figure 1. Main steps diageram.

METHOD AND MATERIAL

Overview

In this study, the *E. coli* and *V. cholera* bacteria image dataset is obtained from the central water and wastewater laboratory of Ardabil University of medical science. These images are 1000×749-pixel sized in the RGB color model. The main steps of this study are shown in Figure 1. K-means clustering algorithm is employed to sparse and keep only few meaningful features in the input images as an input unit for the next network (Fig. 2). This helps to have a clear separation between features which makes the classification more accurate. Furthermore, different techniques, such as image 90º rotation, upside-down flip, left-right flip, and Gaussian noise are used to generate new input images in the datasets for training and validation of CNN. Then, the input layer in the CNN will be a multidimensional array including 1000x749x3 units that will be employed to train the network for the bacteria classification and counting. It is worth noting that the applied images are in both RGB and Grayscale models that generalizes the proposed method for different color models.

Bacteria Specifications

E. coli is an anaerobic Gram-negative bacterium considered as an indicator to identify fecal pollution in the water resources. It is also applied to evaluate the performance of the disinfection system in the wastewater treatment plants and reusing effluent for irrigation. According to the WHO guideline, the allowable limit of this pollutant varies depending on the type of usage. For example the maximum acceptable level of *E. coli* for recreation water use and general irrigation, recommended less than 385 and 1000 MPN/100ml, respectively [24]. The morphology of *E. coli* similar to most of the gram-negative bacteria bacillus is in rod shape (Fig. 3).

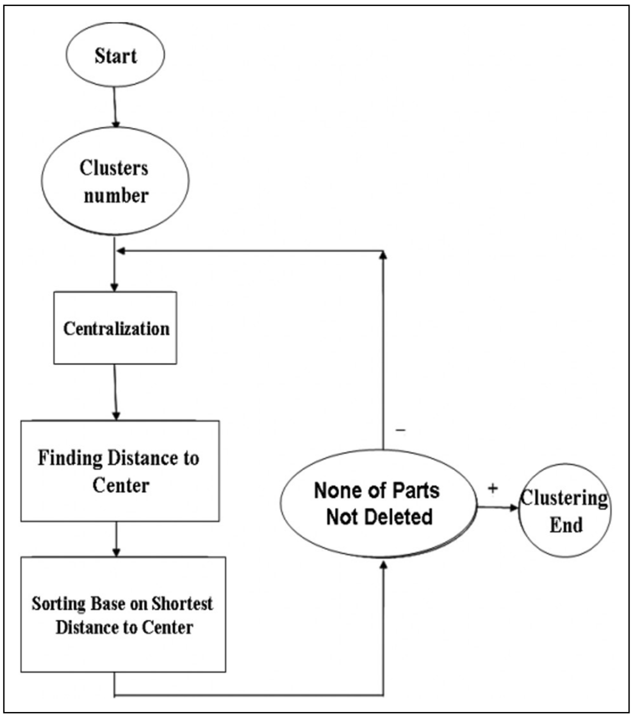

Figure 2. K-means clustering algorithm.

V. cholera is a Gram-negative comma shape bacterium which has been widely distributed in the water environment [25, 26]. Figure 3 shows a *V. cholera* bacterium with a length and width of 2.7-3.5 µm and 0.36-0.4 µm, respectively. This bacterium can be transmitted to the human intestine through fruits and vegetables irrigated with water polluted with this bacterium.

Image Preprocessing

Generally, image is a matrix that pixels are its entries that the values of these entries show the intensity of each pixel in the image. Image processing includes methods to enhance the quality of an image, and search to extract the relevant information in order to the following analysis in an algorithm. Firstly, the quality of image is improved by filtering unwanted effects, such as noises, lights, etc. Noise capturing filters are mostly categorized from the simple average and median filters to the more complicated Gaussian and adaptive filters [27]. Next step is image segmentation to keep the most meaningful information in it. There are also several approaches to image segmentation and masking, such as K-means clustering, threshold algorithm, watershed algorithm, neural network, and deep learning methods [23]. Since this study is aimed on two categories of bacteria, preprocessed images of each category imported as the input data to the CNN. In this study, the gram-negative bacteria considered as red/violet-color cells. All images used in RGB (Red, Green, and Blue) and grayscale modes and operations repeated for each mode. MATLAB 2015 Image Processing Toolbox used for CNN operation while supported with the Visual Studio 2013.

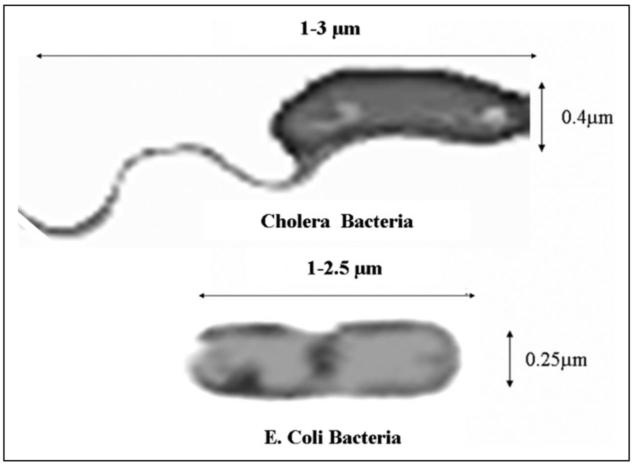

Figure 3. *E. coli* and *V. cholera* bacteria morphology.

K-means Clustering

The approach of clustering is partitioning a set of data to some clusters. K-means clustering labels each pixel of the image to a cluster with the nearest mean or shortest distance to the center of that cluster. In fact, it finds the optimum point of data by minimizing the following error function.

$$J = \sum_{i} D^2.(X, C_i), \quad (1)$$

where D is the distance of C_i as the center of i^{th} cluster in the data group X. Different distance measures, such as Euclidean and Manhattan distances can be exploited in the K-means clustering [28]. Generally, K-means clustering algorithm includes four following steps:

1- Inserting random means within the data.

2- Calculating distance of each data to the means and assigning the data to the nearest group.

3- Calculating the new mean of each group.

4- Repeating the steps 2 and 3 to converge to the target.

K-means here is applied for segmenting and masking bacteria shapes within the images. All images are segmented and masked with K-means clustering algorithm accurately. Particularly, each image is divided into two separated background and foreground parts which the former is bacteria-shaped area, and the latter is black-colored area. The bacteria-shaped area contains intensity and position of each pixel which will be used as the input of CNN for both categories of bacteria. Figure 4 shows image masking in the RGB and grayscale modes.

Convolution Neural Network (CNN)

Convolution neural network as a kind of feed forward neural network and deep learning method uses a Perceptron network with some changes on classic operation. This network is based on four characteristics which have

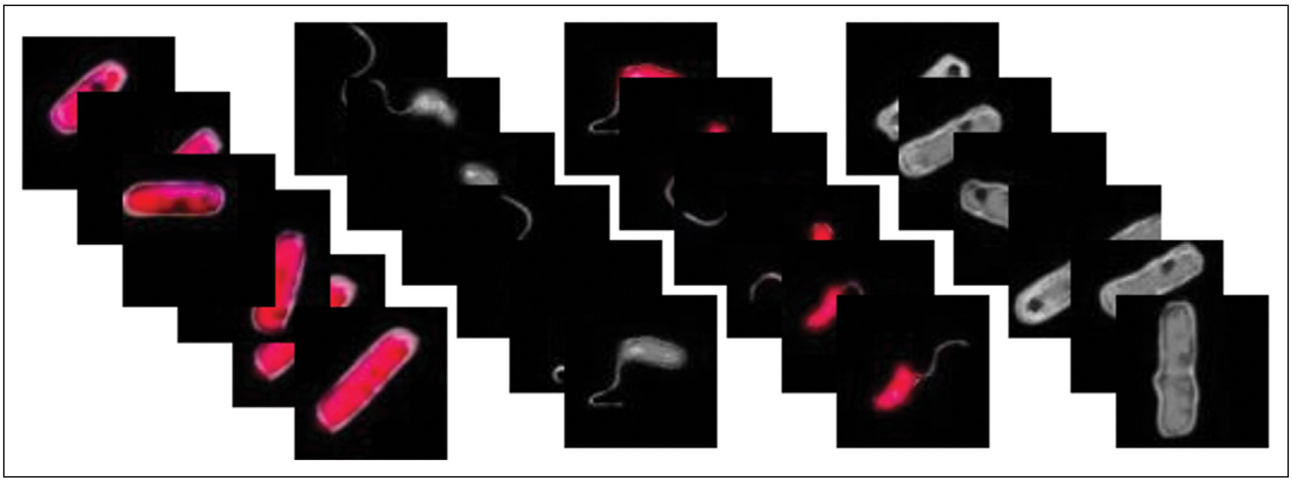

Figure 4. Masked and segmented bacteria images (RGB, grayscale).

been implied from natural signals. Therefore, CNN can be applied to the process of natural signals and multi-dimensional matrices. This characteristic includes neurons near communications, layer common weights, using numbers of layer and polling ability [29]. CNN formed in layers includes convolution layers, polling layer and fully connected layer and sub layers. Each layer connects to prier layers base on the weight. Non-linear functions and activation functions include sigmoid function or ReLU functions applied to import sum of neurons' weight in each layer. These layers extract near specification of images or in other situation continuity of common specifications. Maximum values of vicinity choice to reduce the dimension of data and summarize extracted image specifications. Each CNN contains layers for the convolution and polling operation. Layers exploit ReLU non-linear functions. Each weight of layers calculates by back propagation method [30]. Dense layer with the fully connected layer used for this study to extract specifications and assorting the data. Finally, the classified results of the dense layer are considered as the outputs. The CNN with seven major layers and sub-layers used for this study CNN structure, see Figure 5.

Equation 2 defines CNN output layer operation function (f)

$$Y_{w,h,m} = f(Y_{w,h,m}) = f\left(\sum_{i=(w-1)s+1}^{(w-1)s+k} \sum_{j=(h-1)s+1}^{(h-1)s+k} \sum_{K=1}^{N} W_{k,m} x_{i,j,k} + b_m\right), \quad (2)$$

where $Y_{w,h,m}$ regards as convolution output layer with the dimension of h, w and m. Parameters bm and $W_{k,m}$ are neurons bios and weights, respectively. The input of the network is $x_{i,j,k}$ as $(i,j)^{th}$ pixel of the k^{th} component of an RGB image.

A pooling layer as a sub-sampling layer is exploited after every convolution layer to summarize specifications and bold characteristics of the previous layer. In fact, accuracy in characteristics improves the training operation accuracy. In this regard, two possible sub-sampling strategies in this layer are maximum pooling and average pooling.

RESULTS AND FINDING

Images and CNN Layers

The images after noise reduction and removing unwanted lights rectified by the average filter. Then, the preprocessed images masked and segmented by the K-means clustering algorithm. In this step, 150 extracted bacteria units extracted to categorize each bacterium. Furthermore, the number of images in the primary dataset increased to train and validate the CNN. Rotation process employing 90° rotation, upside-down flip, and left-right flip, in collaboration with Gaussian noise addition used to increase the number of images in the dataset, see Fig 6 and 7. The resulted 9,000 units of data in the size of 85×85-pixel considered as the input layer in the CNN.

Input image dataset included both RGB and grayscale modes. Size of images regarded 85×85 pixels while other image sizes like 64×64-pixel and 125×125-pixel had relatively poor results. In fact, the dimension of the images in the input layer for RGB and grayscale modes are subsequently 85×85×3 and 85×85×1.

Counting algorithm is employed to count the number of bacteria in each microscopic image. In addition to the input layer, the network has three convolutional, pooling, fully connected layers. The trend of CNN operation includes epochs of training, frequency of validation and rate of learning. The algorithm chose 70.0% of the dataset for the training procedure including 6300 images per each labeled category of *E. coli* and *V. cholera*. It also selected 30.0% or 2700 images of the dataset as the validation set. Furthermore, for counting the number of bacteria, image ingredient counting algorithm used in the CNN.

Classification

Primary results of the CNN revealed that although the grayscale images due to the smaller size had a faster training/validation, the accuracy of that was poor. However, the

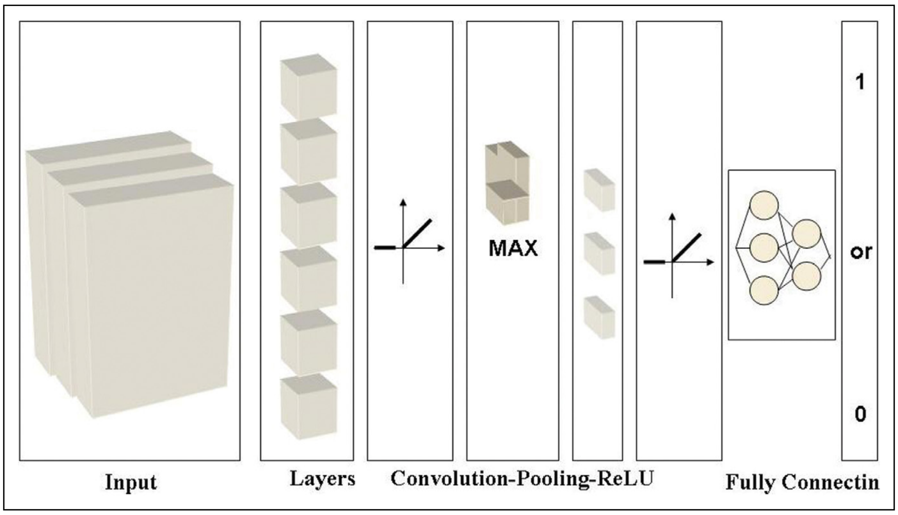

Figure 5. CNN structure.

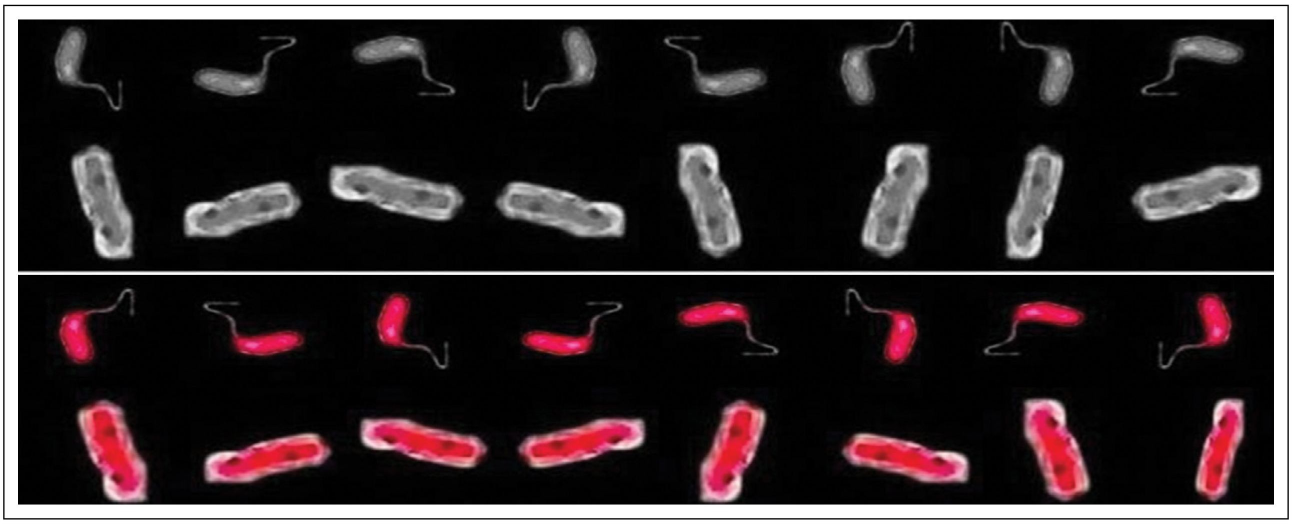

Figure 6. Masked and segmented images rotation.

RGB mode provided more data in the different color channels which resulted in higher accuracy in the cost of higher training/validation time. Color model of the images as the basic parts of this procedure are determinative factor for the bacteria classification. Since the RGB images contain more data, it provides better performance in the classification. Thus, training/validation in the grayscale space achieved 59.0% accuracy while RGB mode provides higher performance in the training/validation with accuracy of 93.01%. These results show the outstanding performance of the CNN in the classification of wastewater bacteria for the RGB images. Table 1 presents the training/validation results.

CNN training set exploited 6300 images for each bacterium and totally 12,600 images in the training procedure. CNN reached to the best point of the training in the 246th repetition. Results show that the CNN operation accuracy achieves 93.01% with least squares error (LSE) of 0.087 in the RGB mode. Figure 8 shows how the proposed method meets the highest accuracy in the RGB mode classification. Last optimizing point achieved in 246th training and validation repetitions. Figure 9 illustrates the LSE convergence of the method which is saturated at the 246th iteration with the error of 0.087.

Table 1. Training and validation results

Parameter	RGB	Grayscale
Accuracy (training - validation)	93.01%	59%
Error (training - validation)	0.087	1.9

Table 2. CNN counting results

Parameter	RGB	Grayscale
Accuracy	97%	97%
Error	0.03%	0.03%

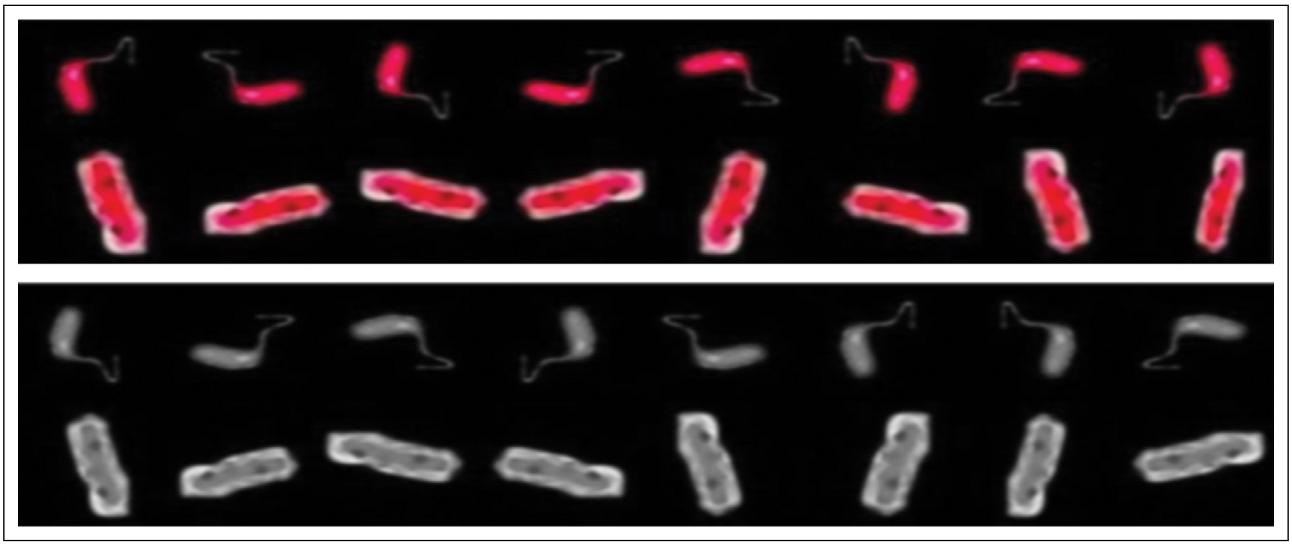

Figure 7. Adding Gaussian noise (σ=30) to images after rotation.

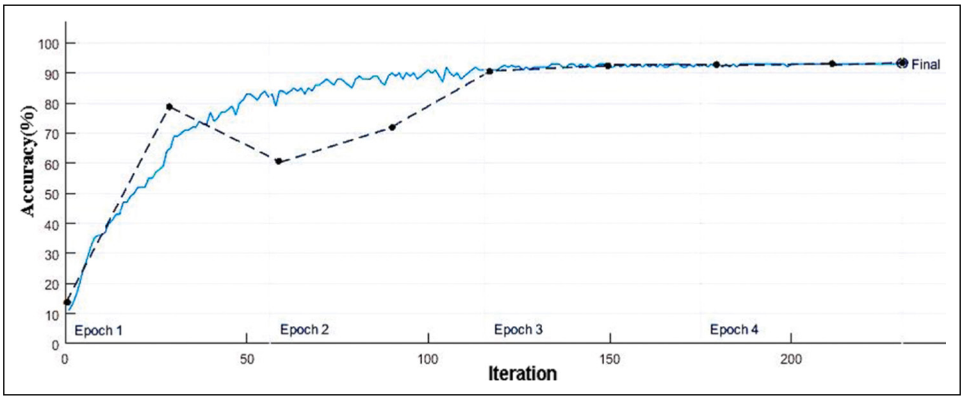

Figure 8. Procedure of accuracy optimizing (RGB), training, validation.

Counting Sample Bacteria

Images ingredient counting algorithm used to count the number of bacteria in the CNN. This algorithm employs "Morphological opening" operations to keep each bacteria shape skeleton. Per each defined morph of bacteria up counter counted to add number to counter clipboard. Procedure is repeating for all morphs to the end. Total values of counter regarded as number of the bacteria for each microscopic image of bacteria. Figure 10 and 11 show the counting procedure of the microscopic sample image. This section of CNN operation's result achieved 97.0% of accuracy. Related results are presented in the Table 2.

Sensitivity Analysis

Sensitivity analysis used to evaluate the effects of the images' quality on the accuracy. Different situation regarded as changes in primary image to increase numbers to 9,000 images for each category. These situations including normal images, image 90° rotation, upside-down flip images, and left-right flip images while Gaussian's noise (σ) added to them. Accuracy of results evaluated for each situation. Enhancing the Gaussian noise caused to higher standard deviation (σ) per pixel. Standard deviation relates directly to the histogram width or variance of the dataset. Figure 12 shows noise enhancement and histogram changes and the results of the sensitivity analysis are in the Table 3.

Figure 13 illustrates the effect of the Gaussian noise on four states of the images. The result reveals the inverse relation between the Gaussian noise and system accuracy. Generally, enhancing the gaussian noise totally decreases the CNN accuracy.

DISCUSSION

According to this study, microbial monitoring is used to analysis water, wastewater, and other water resources with high efficiency. Furthermore, evaluating wastewater treatment analysis efficiency is important. CNN model helps to analysis wastewater ingredients through the image processing methods. Monitoring the ingredients helps to analysis the qualities and effects of waste-

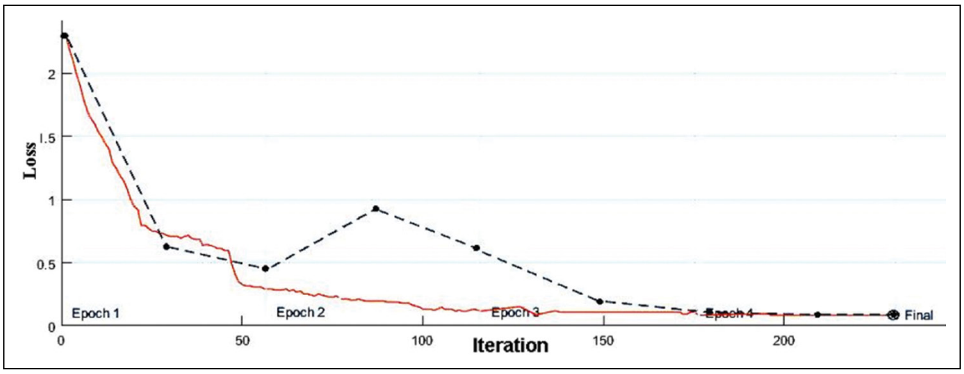

Figure 9. Procedure of error optimizing (RGB), training, validation.

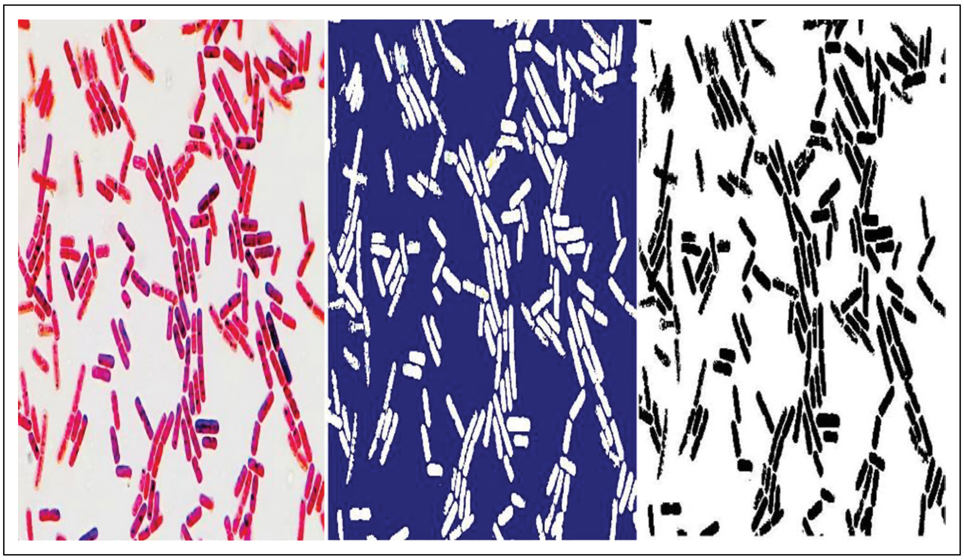

Figure 10. *E. coli* counting processing procedure.

water secondary uses on the environment and human. In this study, CNN analysis used as the major approach to classify and count the bacteria in the wastewater. All operations in the CNN have been used in a fast and inexpensive manner while results achieved to high accuracy. Along with the computer processing improvement and in combination with the artificial intelligent development, new methods are competing as well with classic approaches and even are advance rather than classic approaches. This study used classification and counting bacteria simultaneously as a new method for the RGB and grayscale modes dataset. All operations are applicable to other types of the bacteria as well.

Using two categories of bacteria improves the CNN promptitude in comparison to high number of the bacteria categories. This point regarded to evaluate the CNN operation fast and remake it for better operation. Grayscale mode achieved poor accuracy in comparison to the RGB mode while counting results were equal. Increasing the number of images will improve the performance of the CNN. Clear images in the large datasets of bacteria are ideal cases but in common situations different sort of images in variety range of qualities are available. Hence, removing Gaussian noise will be such as overfit results with high accuracy of classification only in the high-quality image datasets. Therefore, providing a variety of different images in the dataset will be logical solution to elate the deep learning procedure.

The CNN used in this study made in seven layers and sub-layers, and the operation showed flexible performance in comparison to the pre-trained CNN, such as AlexNet, VGGNet, ResNet, etc. Pre-trained CNN uses more layer of convolution to achieve high accuracy of classification. These kinds of the convolution neural networks are not easily adaptable to the different cases of datasets and require more time and high-performance hardware to train and validate the datasets.

Result of sensitivity analysis verified the Gaussian noise has an inverse relation with the accuracy. Upside-down flip images took most effect of the Gaussian noise with poor accuracy of 58.0% while left-right flip images had most similar results to the normal images.

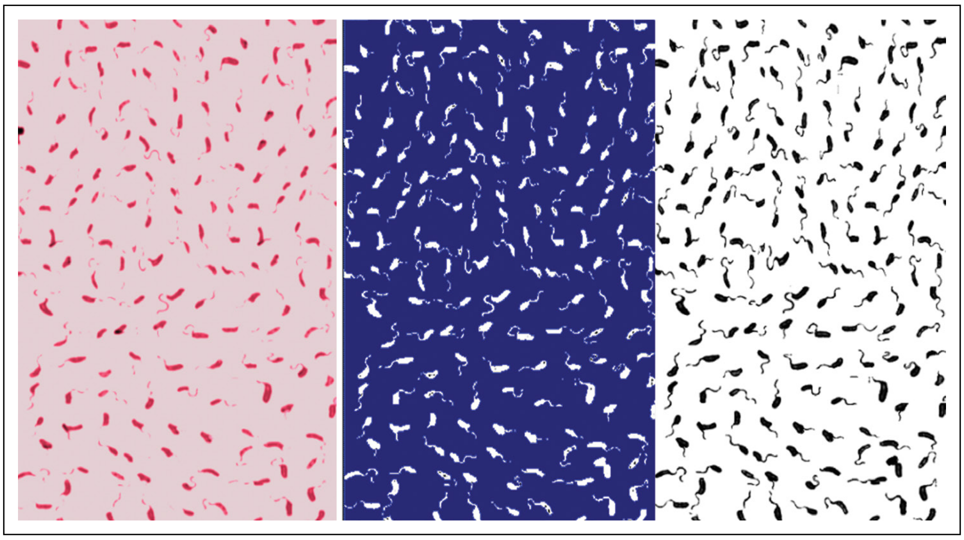

Figure 11. *V. cholera* counting processing procedure.

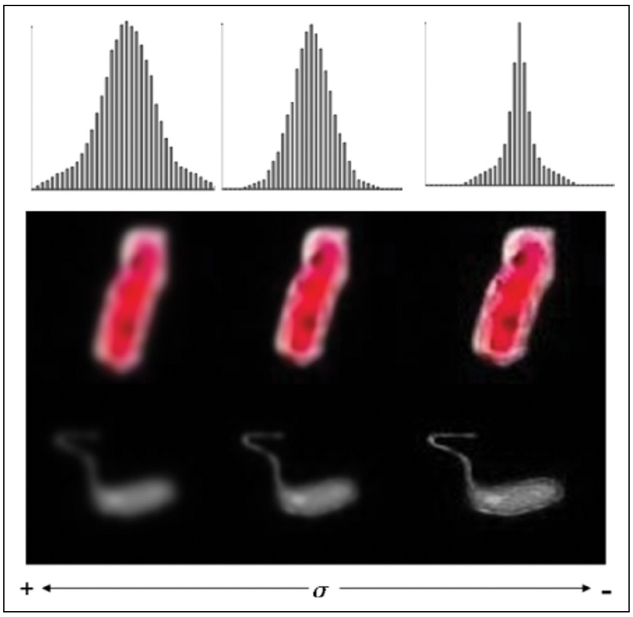

Figure 12. Gaussian noise histogram.

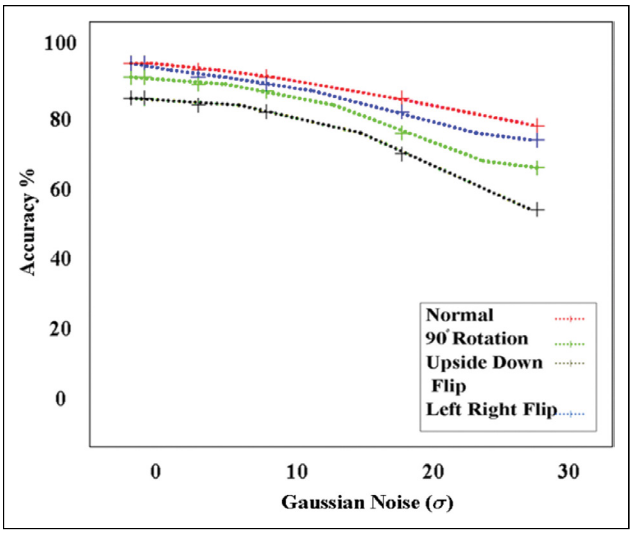

Figure 13. Plot of the sensitivity analysis.

CONCLUSION

This study used an image processing-based method through a CNN model in seven layers and sub-layers to classify and count bacteria in the RGB and grayscale images. *E. coli* and *V. cholera* bacteria considered as the most common bacteria and wastewater indicators.

From a computational viewpoint, this study verified the CNN performance for combining duties of classification and counting bacteria. The results revealed that the CNN model in the RGB mode achieved high efficiency with the accuracy of 93.01% and LSE of 0.087 to classify the bacteria. Also, counting results verified the CNN counting efficiency with accuracy of 97.0% for both RGB and grayscale images.

The used method in this study can classify and count other bacteria which confirms the high flexibility of the model. Accuracy of 93.01% verifies high efficiency of classification in comparison with the pre-trained CNN with high number of layers and greater dataset. Moreover, all operations are faster and simple in use with low GPU system requirements.

Results of the sensitivity analysis indicated that Gaussian noise affects the performance of the CNN model. In the presence of the additive Gaussian noise (σ=30), upside-down flip images took most effect of that which resulted in decreasing the accuracy to 58.0%. Left-right flip and 90°-rotated images had most similar results to the normal images with the accuracy of 78.0% and 75.0%, respectively.

In principle, our study presents an inexpensive, fast, rather simple image processing-based method by employing the CNN. This method can be used to classify and count the number of bacteria in the wastewater, and in the similar approach sort the microbial. Adapting the used CNN for the classification and counting samples can be beneficial in the wastewater treatment, education, and microbial researchers.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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