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DETECTION OF PROTOZOA IN WASTEWATER USING ANN AND ACTIVE CONTOUR IN IMAGE PROCESSING

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Abstract: *A new method is proposed to detect the location of protozoa using edge extractor and Artificial Neural Networks (ANN). In this method, pre-processing is applied to the activated sludge images. ANN is classified with seven parameters that contain general features of protozoa. The features are obtained from properties of each region after applying edge extractor. Morphological operations are applied to obtain protozoa as a whole. A system has been developed to decide the regions of protozoa using the morphological procedure after ANN process. If the determined regions are not elliptical or circular areas, the regions are expanded according to the characteristics of them. Expansion direction is found by the orientation angle of the region. Accordingly, if it is required, the starting point can be changed. Then, active contour method is applied to the regions in which the exact location can not be determined. It is important to find the correct boundaries of the object and the first mask place should be chosen correctly. To do this, the convex area of the image is used. So, the initial mask is obtained automatically. Then, the obtained regions are evaluated again at the decision stage and protozoa are detected.*

Keywords: *Activated Sludge, Protozoa Detection, Image Analysis, Active Contour, Artificial Neural Networks***.**

1. Introduction

Wastewater treatment plant includes physical, chemical and biological treatment processes. The processes in fact are considered as a parameter of an impurity. It is very important planning and management of wastewater treatment plant. Activated sludge treatment process is biological treatment process which is widely used in domestic and industrial wastewater treatment. A simple activated sludge system has aeration tank, final sedimentation tank, and loops back to the sludge. Activated sludge, wastewater containing organic and inorganic substances, is a mixture of both live and dead microorganisms. In aeration tank, microorganisms break down the organic waste and form flocs, then flocs are separated from the treated water at the final settling tank [1].

There are certain groups of organisms, plants, animals, fungi, protozoa, viruses, bacteria, and algae in domestic wastewaters. Many microorganisms (bacteria, protozoa) are useful for the treatment of biological wastewater treatment processes. Coliform bacteria are the indicators of pollution caused by human waste. Microorganisms grown in biological treatment are typically consisted of 95% bacteria, 5% higher order organisms (protozoa, rotifers, and so on.). Organic materials are broken down by microorganisms during wastewater treatment [1, 2].

Protozoa are the most important group of microorganisms after bacteria at activated sludge systems under normal operating conditions. Protozoa are fed with bacteria. Activated sludges include the amount of protozoa. The situation is very important in terms of treatment efficiency. At the same time, flocs containing the protozoa demonstrate the better settling feature. Protozoa are ten times greater than bacteria. If the deposition is fast, efficiency of treatment will be good [3]. Microbiological evaluation of activated sludge systems are directly linked to the operation of the system and provide the desired output parameters. If activated sludge has a certain number of protozoa, the process will be work well at steady state.

Active contour method minimizes the energy function with the iteration. The edge-based and region-based methods are used for active contour methods. In this study, the geodesic active contour method proposed by Caselles et al. [4] is used.

In the literature, researches were studied image processing techniques in activated sludge systems. Image processing techniques were used for separation and quantification of flocs and filamentous bacteria (diameter and track dimensions, the length of the filaments are simulated by image processing) [5, 6], sludge bulking forecast [7], protozoa recognition [8], metazoa recognition [9], and predict the activated sludge volume index [10, 11].

In [8, 9, 12] studies, the protozoa detection in activated sludge was made by identification procedures in which was selected the image of the region containing the protozoa. So, the studies were taken into account only considered the relevant region.

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This study aimed to automatically find the location of protozoa. Protozoa regions are found by ANN and edge extractor. A system has been developed to decide the regions of protozoa using the morphological procedure after ANN process. Then, active contour method is applied to the regions in which the exact location can not be found. The initial mask must be defined. Protozoa is automatically determined using the proposed method.

2. Materials and Methods

Activated sludge images were taken from the aerated tank in Konya Municipal Wastewater Treatment Plant. They were captured using Motic AE21 microscope, at 40× magnification. The images were acquired at 1288 x 966 pixels. Active contour method was used to determine the boundaries at the final stage.

2.1. Artificial Neural Network (ANN)

In this study, a three-layer feed-forward network model is used. ANN architecture is composed as 7:10:1. Here, input nodes, hidden nodes, and output node are 7, 10, and 1 respectively. Activation function of the hidden nodes is sigmoid function. The number of hidden nodes is found experimentally. Learning rate is chosen as 1 in the training phase. The Back Propagation algorithm is used for training of ANN. The mean squares of the errors are used as a performance (error) function with its goal set to zero. ANN input parameters are the standard deviation, mean, entropy, field, solidity (Area/Convex Area), extent (Computed as the Area divided by the area of the bounding box), and perimeter/area. These parameters are converted into a vector for using the input of ANN. The vector is normalized between 0 and 1. The output of the ANN is classified to protozoa or not. Desired value of the output node of the output layer is logic-1 or logic-0. The detection procedure is performed on data saved before as off-line.

We introduce the procedure based on ANNs for the automatic detection of protozoa in activated sludge images. The ANN classifier separates possible protozoa and possible non-protozoa.

2.2. Active Contour Models

Snakes or “active contour models” represent a special case of the general multidimensional deformable model theory. Active Contour Models were firstly proposed by Kass at al. [13] in 1988 as an Interactive Segmentation method for 2D images. Snakes are planar deformable contours that are useful in several image analysis tasks.

The active contour model snakes are used to obtain object boundaries as curves using internal forces coming from the curves itself and image forces computed from the image data. Geometrically, a snake is a parametric contour embedded in the image plane (x, y) € ℜ2’de. The contour is represented as:

$$v(s) = [\, x(s), y(s)\,]^T \tag{1}$$

where x and y are the coordinate functions and s € [0, 1] is the parametric domain. The shape of the contour subject to an image I(x, y) is dictated by the following equation [14, 15].

$$E\,(v) = S\,(v) + P\,(v) \tag{2}$$

Eqn (2) can be viewed as a representation of the energy of the contour and the final shape of the contour corresponds to the minimum of this energy. The first term of the functional,

$$S(v) = \int_0^1 w_1(s)\left|\frac{\partial v}{\partial s}\right|^2 + w_2(s)\left|\frac{\partial^2 v}{\partial^2 s}\right|^2 ds \tag{3}$$

S is the internal deformation energy. It characterizes the deformation of a stretchy, flexible contour. Two physical parameter functions dictate the simulated physical characteristics of the contour: w1(s) controls the “tension” of the contour while w2(s) controls its “rigidity”. The second term in Eqn (2) couples the snake to the image. Generally,

$$P(v) = \int_0^1 P(v(s))ds \tag{4}$$

where P(x, y) denotes a scalar potential function defined on the image plane. To apply snakes to images, external potentials are designed whose local minima coincide with intensity extreme, edges, and other image features of interest. For example, the contour will be attracted to the intensity extreme in an image I(x, y) by choosing a potential;

$$P(x,y) = -c\left|\nabla\left[G_\sigma * I(x,y)\right]\right| \tag{5}$$

where c controls the magnitude of the potential, ∇ is the gradient operator, and G_σ* I denotes the image convolved with a (Gaussian) smoothing filter whose characteristic width σ, controls the spatial extent of the local minima of P. In accordance with the calculation of the variations, the contour v(s) which minimizes the energy E (v) must satisfy the Euler-Lagrange equation as:

$$-\frac{\partial}{\partial s}(w_1 \frac{\partial v}{\partial s}) + \frac{\partial^2}{\partial s^2}(w_2 \frac{\partial^2 v}{\partial s^2}) + \nabla P(v(s,t)) = 0 \tag{6}$$

This equation expresses the balance of internal and external forces when the contour rests at equilibrium. The first two terms is the internal stretching and bending forces, respectively. The third term is the external forces that couple the snake to the image data. The general approach for solving Eqn (6) is application of numerical algorithms [15].

The "active contours" start with an initial contour and deform actively themselves to the desired border. For the each iteration, the defined energy is reduced until convergence. When the balance is occurred between the external powers and the internal powers, the convergence is achieved. The external powers attracts the contour to its desired location using image features, the internal powers keeps the contour regular by maintaining some function of its curvature [16].

2.3. Proposed Method

The main steps of the image analysis procedure are summarized in Figure 1.

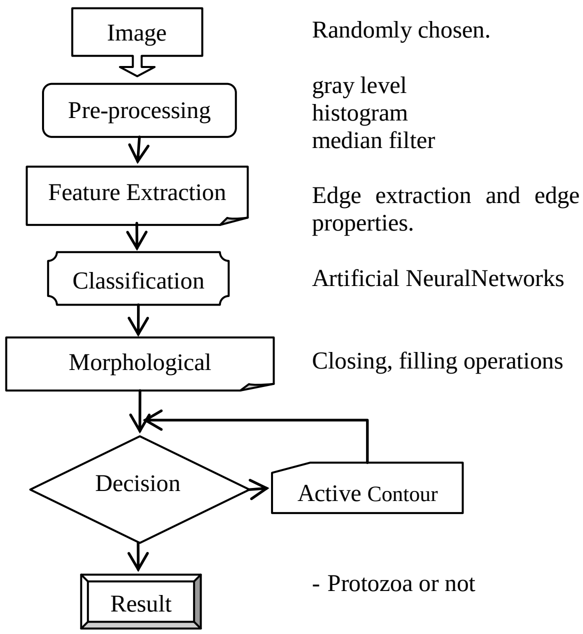


Figure 1. The proposed method.

The received activated sludge images may be seen in different shades of color. Figure 2 shows the images of some protozoa detected in wastewater treatment plant.

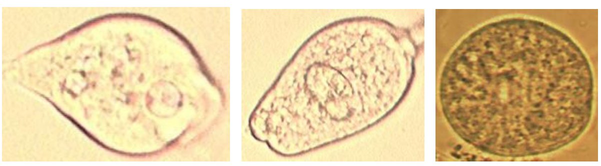
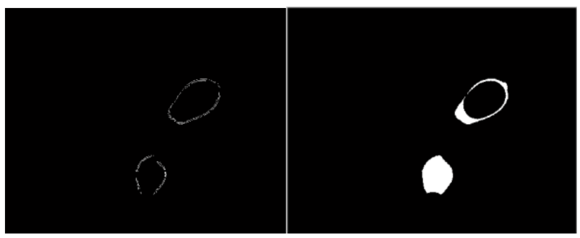


Figure 2. The images of some protozoa detected in wastewater treatment plant.

Some of the protozoa have a filamentary structure. However the filamentary structure of received images from the wastewater was not too explicit, and was lost in flocs. Therefore, it was ignored. The images which are converted to gray level are enhanced by histogram equalization. Mean filter is used to eliminate the problems that make it difficult to identify on the image (noise, contrast, impairment, etc.) Then, prewitt edge extractor is applied to the images. Small areas are removed from image after applying the prewitt edge extractor. Characteristics of each region are determined by connected component analysis which connected regions in binary digital images are detected. ANN input parameters are obtained from these characteristics.

Seven image parameters which can be considered as general characteristics of protozoa are determined. These characteristics are standard deviation, mean, entropy, area, solidity, extent, and perimeter/area. Entropy is calculated to measure the amount of the average information content of the image. Solidity is a scalar quantity specifying the proportion of the pixels in the convex hull and also in the region. It is computed as Area/Convex Area. Extent is a scalar quantity. It is specified by the ratio of pixels between on the region and on the bounding box. It is computed as the area divided by the area of the bounding box. Convex area is the smallest convex polygon that can be considered into the area of the object. Each of these attributes is calculated for all regions and is classified using artificial neural networks. The ANN classifier is used to separate possible protozoa and possible non-protozoa.

Regions which may be protozoa are obtained using neural networks. This regions are collected into a single image (Figure 3-a). Protozoa must be obtained as a whole to detect the number of protozoa automatically. To do this, morphological operations are performed such as close and filling (Figure 3-b).



(a) (b)

Figure 3. (a) The regions may be protozoa, (b) morphological closing and filling on binary image

All edges of protozoa are usually not clear in the images of the wastewater. Medium and short edges of protozoa are unclear. Other long edges are usually very obvious. Therefore, whole protozoa can not be obtained automatically by using only the edge information. In general, these areas which are combination of small dots and spaces can be applied to obtain completely the closing process. In that case, it will result in the combination of flocs with protozoa. Moreover, protozoa are intertwined with the flocs at some of the images (Figure 4). Thus, protozoa segmentation is not possible directly with many morphological processes. Therefore, obtained edges are not applied the merge (close) process. In this case, one or two edges of a protozoan are obtained. To solve this problem, the regions of protozoa are determined and merge process is performed.

A decision-making mechanism is developed by taking into account the convex areas of the obtained regions. Active contour method is applied to the regions in which exact location may not be determined (Figure 4). If the obtained regions are not elliptical or circular areas (Figure 3), the regions are expanded according to the characteristics of them. These are length, width, maximum and minimum axial length of region.

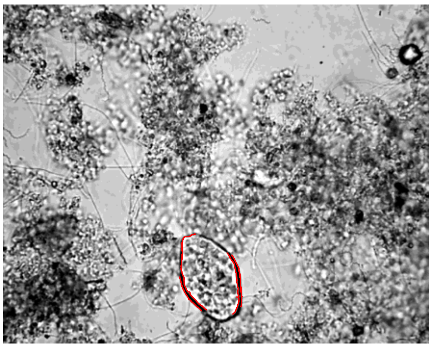
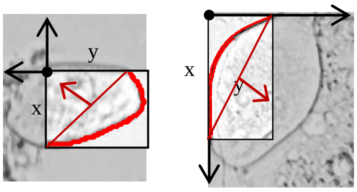


Figure 4. A protozoa (exact edges may not be determined)

Expansion direction is selected according to the orientation of the region. It is determined whether replaced starting point's locations of the expanded regions. Figure-5a and Figure-5b show the images in which the location of starting point is replaced or not replaced, respectively. The regions are automatically expanded according to the length, the width, maximum and minimum axial length of the regions (Figure 5).



(a) (b)

Figure 5. Illustration of the expanded regions. (a) expanded regions that replaced locations of starting point (b) not replaced starting point's locations.

For protozoa detection, the initial position of the snake is important, because the snake’s position is optimized locally close to its initial position. The initial mask is automatically detected and not defined by user. The initial position of the snake is defined by using the convex areas of regions automatically (Figure 6).

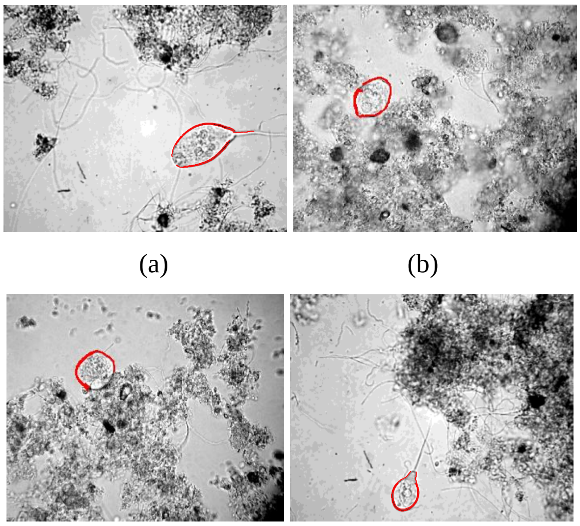
Figure 6. Convex areas of regions (initial snakes)

Gaussian smoothing filter is applied before applying the active contour method. The operator is a 2-D convolution operator that is used `blur' images and removes detail and noise.

The active contour method starts with initial mask and iteratively deforms it to get the final, best describing objects contour. The methods iteratively minimize a pre-defined energy functional which is usually a balance of two forces: external forces, that attract the contour to its place and internal forces that keep the contour smooth. Energy minimization is a main logic of snake for the feature extraction. Minimum energy of snake means that edge of the feature is detected and final shape of snake is created in optimization procedure.

5. Results

If the activated sludge has a certain number of protozoa, the process will work well at steady state. Some of the protozoa have a filamentary structure. However, the filamentary structure of the received images from the wastewater is not too obvious, and was lost in flocs. Therefore, the filamentary structure of the protozoa was ignored. All edges of protozoa are usually not clear in wastewater images. Medium and short sides of protozoa are unclear. Other long edges are usually very obvious. Small areas are removed from the image after applying the prewitt edge extractor. Features of each region are determined by component analysis. The features are applied to ANN input. ANN is trained with the seven images parameters. Figure 7 shows the obtained protozoa after ANN process and decision stage.



(a) (b)

(c) (d)

Figure 7. The obtained protozoa after ANN process and decision stage

In some cases, only one edge of protozoa may be obtained. A second process is applied to these images at the decision process. If the obtained regions are not elliptical or circular areas, the regions are expanded according to the characteristics of them. Expansion direction is determined by the orientation of the region. Accordingly, the starting point is replaced. Noise is reduced by a Gaussian filter. Then, the active contour algorithm is applied. To determine the correct boundaries of the object is important, and the first mask place should be chosen correctly. For this aim, the convex area of the image is used. The initial mask is determined automatically. Number of iterations is taken as 600. Figure 8 shows the initial state, the image with 300 iterations and the image with 600 iterations.

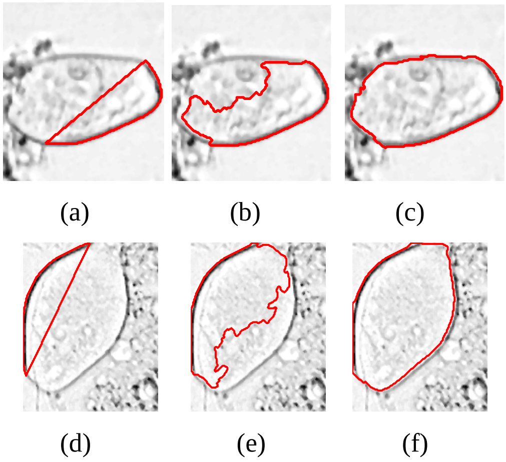


Figure 8. (a) and (d) The initial state, (b) and (e) the images with 300 iterations, (c) and (f) the images with 600 iterations.

Figure 9 shows the segmented images with 600 iterations. Protozoa are obtained as a whole instead of partially. Then, the segmented images are evaluated again at the decision stage and protozoa are detected.

Figure 9. The segmented images

The success of the proposed system is shown in Table 1. It has been discussed about 30 images in Table. The system is evaluated in stages. It has achieved 200 regions. 148 of them are eliminated with ANNs. Other 12 regions are sent to active contour in the decision stage. The boundaries of the protozoa are successfully determined with the active contour except for a region. This region is reevaluated in the decision stage after the active contour. The final stage of the protozoa detection system combines the outcomes of the active contour to make a decision about the presence of protozoa. It is eliminated the regions which may not be protozoa. The method determined 29 pcs protozoa, although there are 27 pcs protozoa. The reason for these protozoa is very similar to flocs. Protozoa segmentation is very difficult to be distinguished combined with flocs. Protozoa may be determined by the proposed method. It is observed that more protozoa are detected by the proposed method. The more protozoa may be eliminated in recognition stage.

Table 1. Evaluation of results in stages

Number of Regions	ANN		Decision		Active Contour	
	Non Protozoa	Candidate Regions	Protozoa	Active Contour	Protozoa	Non Protozoa
200	148	52	19	12	10	1

The previous studies have been made with identification procedures, or by selecting of the regions containing the protozoa. Only the relevant region was investigated in [8, 9, 15]. A polygonal region of interest is user defined manually around the selected organism using the mouse. In this study, an image processing application is developed for the detection of protozoa automatically. Disadvantage of the proposed method is ignoring the filamentary structure. Because the filamentary structure is not clear in the images or it is nested flocs structures and it is vague. The regions which may be protozoa are obtained using neural networks. These regions are collected into a single image. Morphological operations are applied in order to obtain as a whole. A decision-making mechanism is developed to take into account the convex areas of the obtained regions. If the obtained regions are not elliptical or circular areas, the regions are expanded according to the characteristics of them. To find the correct boundaries of the object, the first mask place should be chosen correctly. To do this, the convex area of the image is used. Then, active contour method is applied to the region that exact location can not be determined. The initial mask is defined automatically.

The final stage of the protozoa detection system combines the outcomes of the active contour to make a decision about the presence of protozoa. Protozoa are determined automatically using the proposed method without any choice to user. Information about the amount of microorganisms is obtained. Microbiological evaluation of activated sludge systems is directly related to operation of the system and provides the desired output parameters.

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