Using Customized Computer Vision and Charge-Coupled Device (CCD) Sensor for the Recognition of Colony Formation and Counting of Live Bacteria in the Agricultural Industry

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***Abstract*—This paper presents an arrangement based on a customized computer and Charge-Coupled Device (CCD) sensor system to allow the counting and recognition of the colony formation, in an intelligent manner, of live bacteria. Microbes in agricultural environments are important catalysts of global carbon and nitrogen cycles, including the production and consumption of greenhouse gases in soil. The magnitude of this process is influenced by human activities and impacts the warming potential of Earth's atmosphere. The method implemented uses techniques of digital image processing, and among them, Hough Transform for circular objects. For calibration and validation of the method, a RGB (Red-Green-Blue) camera based on the CCD was used, as well as a prepared illuminated chamber, to allow the analysis of the bacteria *Escherichia Coli* and *Acidithiobacillus ferrooxidans*. The visual environment, Borland Builder C++, was used for the development, and a modeling for decision making was incorporated to aggregate intelligence. Moreover, a set of comparisons was established, taking into account the smart methods and analyses carried out by experts. The results have shown the potentiality of the method, which is applied for laboratory applications that involve the quantification and the pattern recognition of bacterial colonies in solid culture environments.**

Keywords-intelligent sensing; bacterial colonies counting; Hough Transform; computer vision.

I. INTRODUCTION

Some bacteria have properties that are beneficial to plants. They can be found in the soil, and they have the potentiality to affect, in a good way, the plants and cultures by fighting against the harmful bacteria. Also, they can be the source of providing nutrition to the crops. For instance, bacteria increase the fertility of the soil and provide nutrients, which are useful for plant growth. However, it is still open for research to better understand the role bacteria play when the plant grows. Although the cultivation of microorganisms in solid culture is a traditional technique, it continues being the compulsory step when it is necessary for the isolation and purification bacterium lines. Nowadays, the microbiology technique has been employed associated with the traditional techniques, including counting and cultivation in solid ways, when intending to quantify or isolate different groups of microorganisms.

Many institutes, laboratories and entities are concerned with establishing procedures, criteria and standards of microbiology analysis involving the counting of colonies in solid culture. The Ministry of Agriculture, Livestock and Food Supply, through the Normative Instruction nº 62, of August, 26 of 2003, established a procedure to standardize the counting of microorganism with application of samples of raw material, water and meals [1]. The National Agency of Health Surveillance (ANVISA) and the National Advice of Environment [2] are examples of worried entities with the criteria and standards of microbiology analyses.

There are different laboratory methods for counting bacteria. Among them, one may consider the Counting Chambers, which can fill in an automatic way a certain volume and uses a special microscope slide with a cover glass to calculate the number of bacteria per milliliter of the original sample from the known volume:the Most Probable Number, which estimates the number of bacteria; the Membrane Filters, in which the water is filtered on a filter which has holes smaller than bacteria and then is placed on a dish of agar, and bacteria grow on the top of the filter to be count; Photometers and Spectrometers, in which a meter reads the amount of light passing through the culture, and by consulting a standard curve which you have prepared by reading the meter and plating the bacteria, you can estimate the number of bacteria. These methods have the limitations of counting live and dead bacteria [3]-[11]. On the other hand, as a process of structural colony formation, which excludes dead bacteria and debris an opportunity is observed to have a method that uses a sensor for imaging and a computer aided system for pattern recognition and counting in an intelligent manner of the colony formation of live bacteria.

However, it has been observed that the manual counting of colonies is limited, because is a slow process and the number of analyses performed depends of visual exposure activity by the technician. Besides, in this context, the development of a method based on the use of a sensor which allows imaging for intelligent analysis of the colonies formation of the bacteria and automatic counting of the colonies formation units can speed up the number of laboratorial analyses.

This paper presents a system for the recognition of colonies formation of live bacteria and its counting. The method uses the Hough Transform, adapted for the detection of colonies with circular shapes, in order to aggregate the

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intelligence for decision making in the agricultural industry.

After this introduction, Section 2 presents the theoretical and technological background; Section 3 presents the method of aggregation of computer intelligence. Finally, the results and discussions are presented in Section 4, followed by the conclusion in Section 5.

II. THEORETICAL AND TECHNOLOGICAL BACKGROUND

A. Microbial growth phases

Most bacteria under optimal growing conditions grow and divide every half hour [12]. Thus, the increase in the population numbers from a single bacterium can be expressed as a geometric progression, as follows:

$$1 - 2^1 - 2^2 - 2^3 - \cdots - 2^n \quad (1)$$

where the exponent $(0, 1, 2, 3, \cdots n)$ refers to the number of generations. The time interval required for each microorganism to divide, or for the population of a culture to double in number, is known as generation time [13]. It should be noted that not every species of microorganisms have the same generation time. Fig. 1 illustrates the bacterial growth curve and its phases.

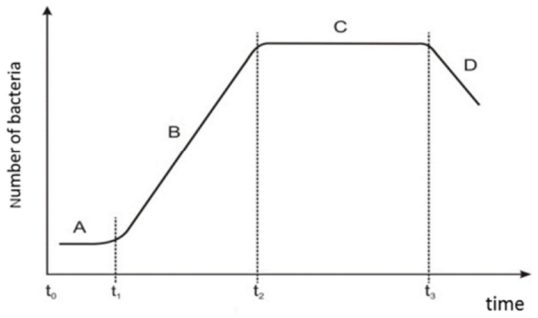

Figure 1. The phases of the bacteria growth: A - lag phase;B - exponential phase; C - stationary phase;D - declining phase (adapted from [12]).

There are four stages of growth that characterize the bacterial growth curve: lag phase, exponential phase, stationary phase and decline phase or cell death. Table I helps the understanding of the phases of bacteria growth, i.e., Figure 1, where each phase represents the notion in relation to its growing status.

TABLE I. THE MICROBIAL GROWTH PHASES.

Lag phase	$t_0 \leq t < t_1$
Exponential phase	$t_1 \leq t < t_2$
Stationary phase	$t_2 \leq t < t_3$
Declining phase	$t \geq t_3$

The lag phase occurs after the inoculation medium. The cells begin to adjust to the physical condition and available nutrients. During this time, the cells are in a latency period where there is an intense metabolic activity that is not reflected in the increase in cell number. This phase can continue for one hour to several days.

In the exponential phase, all cells are divided at regular intervals of time, resulting in an exponential increase in the number of individuals in the population. This phase is the period of high metabolic activity of the cell; however, the organisms are particularly sensitive to environmental changes.

The stationary phase occurs when the growth rate slows down and strikes a balance between the rate of death and the rate of divisions in the population.

Finally, the decline phase, or cell death, occurs when the rate of death exceeds the rate of divisions. This phase continues until the population disappears completely.

B. Manual Counting Method by Experts

The method based on manual analysis, employed in this work for comparison purposes with the automated method, uses a device which has a reticulated and illuminated acrylic surface wherein the Petri dish is placed, and above the surface, there is as increased magnifying 1.5 times with a flexible rod that allows the experts to visually count the existing bacteria colonies on the plate. For this research, the experts used an apparatus named Colony Counter Phoenix Luferco, model CP-608.

C. Sensor and Illumination System

A CCD camera is a semi-conductor device that acts as a transducer between incoming light and electrical charge. For the CCD sensor operation, it is important to consider the level of the environmental illumination in relation to the object one is going to work with. The lighting is an important factor to be considered in forming the image, as it can influence the final result of analysis, since the level of the pixel intensity will be a function of the illumination and the angle of its incidence over the object during the image acquisition process.

In systems for the automatic counting of colonies, generally, the main difficulty is the lighting system that requires high power lamps to be dimmer; in some cases, the use of special lenses or a combination of all these components is necessary. There are at least two methods of lighting that can be employed in an imaging system for bacteria counting. One is the method that uses backlight, and the second one is the method that uses front lighting. In the first method, the Petri dish is placed on a light source under the CCD camera. Generally, this method uses a white acrylic plate between the light source and the plate to produce a more uniform lighting. In the second, front lighting method, the Petri dish is placed below both the light source and the CCD camera. The camera captures the light of the environment and performs the registration of the frames for processing.

In order to get a better arrangement, for this research, a front lighting system was developed for the acquisition of the information from the bacterial colonies, which were located into Petri dishes. For the lighting system four fluorescent lamps of 20 watts connected by two electrical ballasts were used. The use of electrical ballasts avoided the effect of the flicker of the fluorescent lamps, which can be captured by the CCD cameras, i.e., generated undesirable noise. The box surrounding the set of bulbs was produced in wood. The box that packages the lamps and the Petri dish has the following

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internal measurements: 500 mm long by 250 mm wide and 500 mm in height. It has a front cover that allows the manipulation of samples and a top cover that closes the box. Internally there is a support for the lights and the camera (Fig. 3). The CCD involves that photons striking a silicon surface create free electrons through the photoelectric effect [14][15]. Nature abhors a vacuum and, thus, a concomitant positive charge (called as a hole) is generated, as well. If nothing else is done, the hole and the electron will recombine and release energy in the form of heat. This is accomplished by positively biasing discrete areas to attract electrons generated while the photons come onto the surface. The substrate of a CCD is made of silicon, but this is not where most of the action occurs. Photons coming from above the gate strike the epitaxial layer and generate photoelectrons. The gate is held at a positive charge in relation to the rest of the device, which attracts the electrons to it. Because of the insulating layer the electrons can't make it through to the gate and are held in place by the positive charge above them.

Fig. 2 shows a basic diagram of a CCD and the arrangement used for the serial image frame readout mode. The image acquisition process starts when the incoming photons reach the sensitive sensor array. To readout, the sensor of the accumulated charge must then be shifted vertically row by row into the serial output register. Besides, for each row the readout register must be shifted horizontally to allow for readout of each individual pixel.

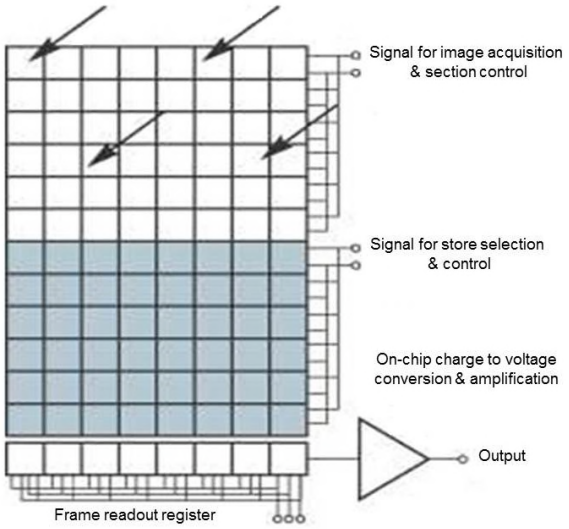

Figure 2. Block diagram of a CCD and its arrangement for the serial frame readout mode.

The serial shift is performed from top to bottom and directs the electron packets to the measurement electronics, which involve an analog to digital (A/D) converter to allow the measurement of the voltage created by the packet of electrons at the serial output and turn this into an electronic number that can then be digitally transmitted to and saved by a computer.

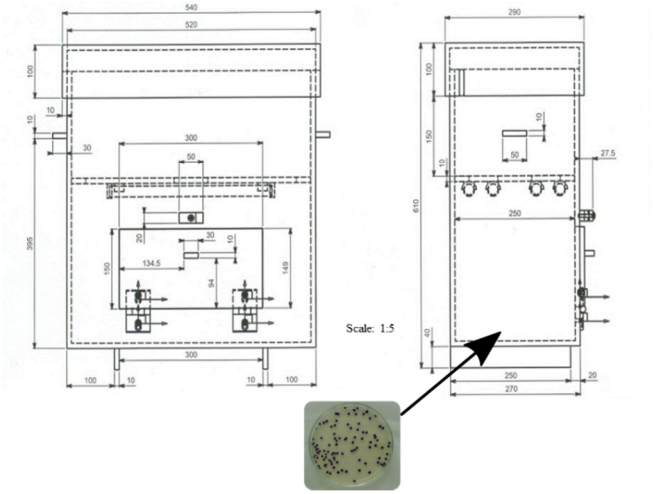

Figure 3. The schematic diagram with details for the illumination system, including information about the place for the location of the Petri dish, which is used for colonies bacteria growing.

In this schematic diagram, the following components of the illumination system are displayed: a box, the top cover, the front cover and built-in bracket for the lights and the CCD camera. Moreover, the arrow indicates that the Petri dish with the colonies is placed on the bottom center of box under electrical ballasts.

D. Hough Transform

The Hough Transform (HT) has been proposed as a method for detection of complex patterns in binary images by Paul Hough in 1962 in the form of a patent [16]. One of the goals Hough predicting was a method to recognize complex patterns in pictures; another goal was to provide a method and improved means for the recognition of particle tracks in photographs taken in a bubble chamber. The Hough Transform was first used in computer vision to detect parametric curves [16][17] and, more recently, for the widespread detecting of non-parametric forms [18][19].

In terms of circular objects, Duda and Hart (1972) suggested the use of the Hough Transform adapted circles. Whereas, the transform can be applied in the recognition curves, provided that the same can be described in parametric form are a circle may be provided by a parametric equation are that it is possible to adapt the Hough Transform for circles.

The property first defined that a point in the cartesian plane corresponds to a sine curve in the plane parameter. Extending this property, you can adapt it for circumference. In this case, a point on the cartesian plane corresponds to a circle in plan parameters. Thus, taking all the pixels of the cartesian plane and applying the circumference equation to have each pixel generate a circumference in the parameter space is represented by an accumulator array.

The parameter space is generated from the transformation of the Cartesian plane through the circular Hough Transform, where image points correspond to circles within the parameter and, therefore, the a and b coordinates are stored in the accumulator array. The crossing of the circumferences

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and the accumulated value in this cell defines how many pixels belong to the circle. Besides, the use of the technique known as backmapping provides means to reduce the false peaks, which are usually found in the Hough Transform [20]. In this work, we used a three dimensional accumulator array, in which a third dimension, representing the possible radii of circumference, can be detected. In this way, the definition of the new arrangement should be obtained by:

$$\sum_{y}^{Y_{max}} \sum_{x}^{X_{max}} \sum_{r=R_{min}}^{R_{max}} array(a,b,r) = \begin{cases} 1, if\ f(x,y) = 255 \\ 0, otherwise \end{cases} \quad (2)$$

where $array(a,b,r)$ is the accumulator arrangement filled by Hough Transform, Y_{max} is the height of the image, X_{max} is the width of the image, r is the radius of the pattern to be recognized in the interval $[R_{min}, R_{max}]$, $a = x - \cos(\theta)$, $b = y - \sin(\theta)$, and $f(x,y)$ is the gray level of a pixel on the (x,y) coordinate whose value is in the range from 0 to 255.

The dimensions of the accumulator arrangement should be defined to avoid loss information, and it can be the same height and width of image. However, if one pixel on the image is near to the edge, the circle drawn exceed the limits in the arrangement. Thus, to avoid loss of information the dimensions of the accumulator array is defined by:

$$array[X_{max} + 2 * radius][Y_{max} + 2 * radius] \quad (3)$$

where $radius$ means a value between interval $[R_{min}, R_{max}]$, Y_{max} is the height and X_{max} is the width of the image.

III. THE AGGREGATION OF COMPUTER INTELLIGENCE

The aggregation of intelligence was based on the use of a customized computer with 256MBytes of RAM, 1GHz processors, and a Windows© operational system. The algorithm to aggregate the decision-support system was developed using the object-oriented programming language C++. Additionally, the tool platforms were based on the Borland© C++ Builder. The flowchart of the algorithm is illustrated in Fig. 4 and consists of five modules, namely as: acquiring information module; pre-processing module; processing module; post processing module; module for analysis and decision making support.

The acquisition module includes a lighting system developed in order to maintain uniformity in luminance to obtain the process of capturing images suitable for analysis. The capture is achieved by means of a CCD sensor for higher pixel counts and miniaturization. The ICX452AQ, having a diagonal 9.04 mm (Type 1/1.8) 5.13M pixel, was used to respond to these needs, i.e., unit cell size equal to 2.775 µm (Horizontal) × 2.775 µm (Vertical) square pixels.

The pre-processing module contains a set of techniques to prepare the information collected from the bacteria for processing and is responsible for preparing to organize in a matrix to the stage of processing. This set includes a simple global threshold [21], the threshold of Otsu [22][23], conversion of color images (RGB) to shades of gray levels and detection of edges using the Laplacian filter [24][25].

The processing module uses circular Hough Transform to detect circular bacterial colonies from the pre-processed image, containing the edges of possible bacterial colonies with a circular primitive, and for each edge pixel, a circumference is generated and stored in the accumulator array.

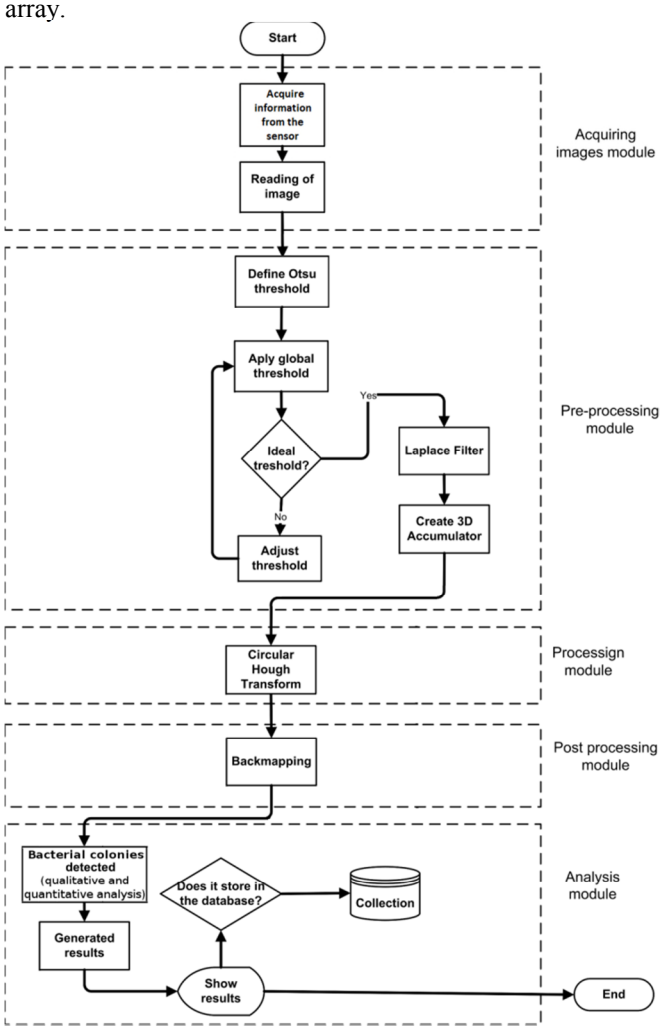

Figure 4. Flowchart of the algorithm which allows the aggregation of intelligence to support decision makers.

The post-processing stage is responsible for preparing the processed image for analysis and generation of results. The technique of backmapping is applied to the circular Hough Transform, i.e., in order to remove possible noise due to false peaks generated during the processing process.

Fig. 5 shows the class diagram for the sensor reading and computational vision and intelligence aggregation for the recognition of the formation and colony count of live bacteria.

The `CHoughCircle` generates and fills accumulator array implemented by `CMatrix` class. The `CSensor` is responsible for acquiring the image from the CCD sensor and preparing it for analysis. The `CFilter` class implements the essential functions to the automated method as laplacian filter.

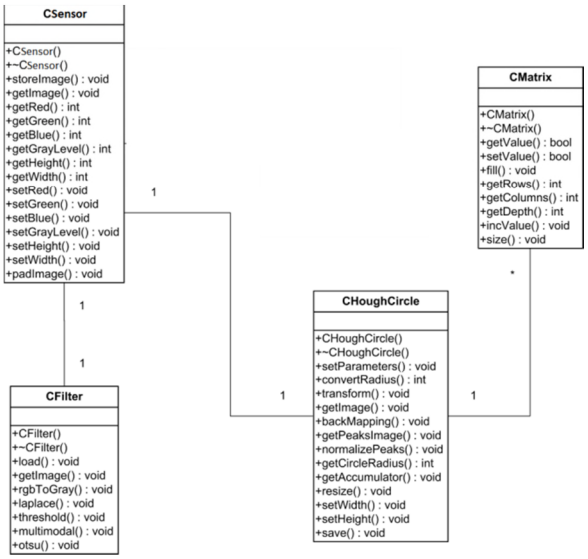

Figure 5. The class diagram of the system for counting the colony formation of live bacteria in an intelligent manner.

Furthermore, there is a stage for the analysis and decision making support that allows for extracting the number of the count and colony formation of live bacteria from the processed image. Information can also be extracted and stored in a collection for post-analysis of the growth of bacterial species.

IV. RESULTS AND DISCUSSIONS

The illumination system allowed for regular illuminance of 1200 lumen/m² over the image. Outside, the 750 lumen/m² was the illuminance observed. Fig. 6 shows Petri dish information acquired, i.e., having bacteria in both cases, that means, inside and outside the illumination system and their respective histograms. The value of illuminance does not depend on the material properties of the surface being illuminated. However, since the information depends on how much is being reflected from other surfaces around it, it does depend on the color and reflectance of the surfaces that surround it.

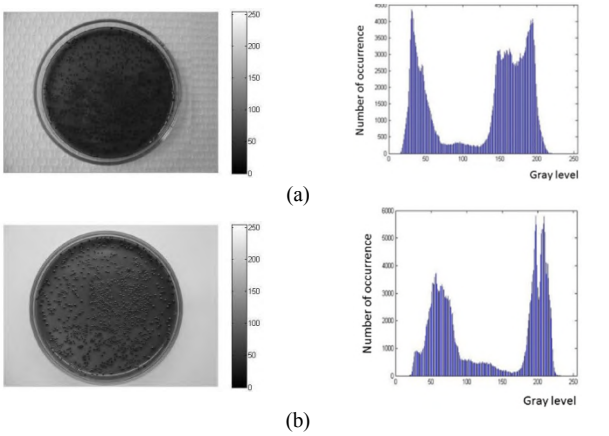

Figure 6. Culture of *Escherichia Coli*. (a) Information acquired in 256 gray levels without additional illumination and respective histogram. (b) Information acquired in 256 gray levels with additional illumination and respective histogram.

A set of twenty-six samples having bacteria was analyzed and divided into two groups, i.e., Group #1 and Group #2. For each group, computational and manual analysis was performed by experts. The values of absolute error, relative error and percentage error were calculated for each analyzed sample. The Fig. 7 shows the sample #22 of the Group #1, i.e., having *Escherichia Coli* samples.

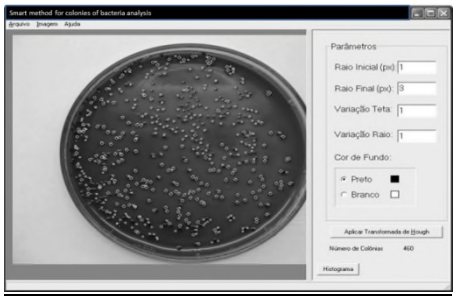

Figure 7. Example of a sample prepared with *Escherichia Coli* from the Group #1. The white circles have show the processed information with the detected colonies (Above, on the left side, one may find the commands <file>, <image>, and <help>; on the right side, one may find, from top to the bottom, the fields for setting the parameters for analysis: initial radius, final radius, angle variation, background color selection (black or white), and also the command for <Application of the Hough Transform> and <Histogram>. Additionally, the number of reached colonies of bacteria/sample is also shown in the interface).

The results obtained with the smart method for the identification and colony count of the bacteria or even microorganisms identified 460 colonies, while for this exampled sample, the manual count realized by experts identified 472 colonies. Table II presents the results for the analysis carried out for Group #1, which had five samples of the bacteria *Escherichia coli* in the solid culture.

TABLE II. RESULTS OF THE ANALYSIS OF SAMPLES HAVING THE BACTERIA *Escherichia coli* IN SOLID CULTURE.

Sample #	Absolute error	Manual error		Smart method error	
		Relative	%	Relative	%
22	12.00	0.03	2.54	0.03	2.61
23	14.00	0.04	3.72	0.03	3.87
24	40.00	0.08	7.78	0.08	8.44
25	9.00	0.04	4.31	0.05	4.50
26	109.00	0.17	17.41	0.21	21.08

For this Group #1, one may observe that the percentage error was smaller than 10%, except for sample # 26, for which the error rate exceeded such percentage. However, it is possible to observe that the number of colonies per sample was greater than 300 in most cases and, for the smart method of counting, the errors remained small.

Fig. 8 shows the result of values dispersion, and the linear correlation coefficient was equal to 0.98, i.e., when plotting results obtained by measurements realized by experts versus those obtained with the smart method.

This graph presented in Fig. 8 shows that the absolute errors, which are associated with error-bars, are low in the majority of the measurements. In the sample which the error rate was greater than 10%, number of colonies were higher than 600. In such cases, the manual analysis by experts is usually performed by estimation of the occupied area on the

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plate and not actually counted, and such situation may explain these results.

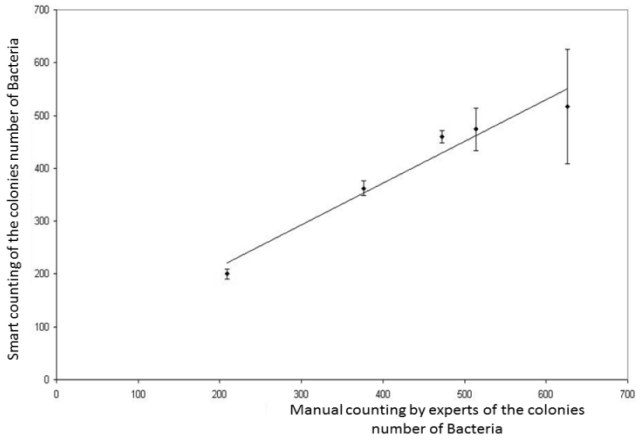

Figure 8. Correlation between the sensor-based recognition method and the manual by experts for the Group #1,which represents the *Escherichia coli* samples.

The contamination by *Escherichia coli* can be very complex and involves all aspects of human and the agricultural production, products and their interactions with ecosystem. The epidemiology of each pathotype varies with the reservoir host, levels of community sanitation and hygiene, and agriculture and food production systems. Prevention and control require a multidisciplinary approach in which the transducers and sensors, as well as intelligent systems and computer vision, plays an important role to evaluate the risk-based approaches and to support decision makers, i.e. involving from the producers up to the consumers. Group #2 of samples referred to the cultivation of *Acidithiobacillus ferrooxidans* on solid culture. Fig. 9 illustrates one of the results obtained with the intelligent counting process. For this exampled sample, the manual count by experts identified 82 colonies, while by the automatic method, the result returned 84 colonies for such sample.

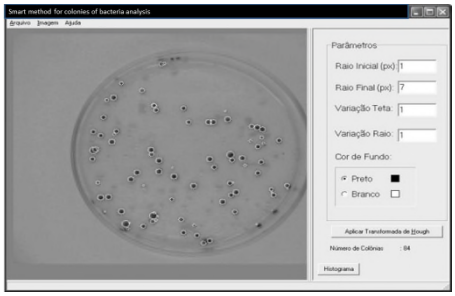

Figure 9. An example of one sample with the cultivation of the *Acidithiobacillus ferrooxidans*. The white circles have show the processed information with the detected colonies (Above, on the left side, one may find the commands <file>, <image>, and <help>; on the right side, one may find, from top to the bottom, the fields for setting the parameters for analysis: initial radius, final radius, angle variation, background color selection (black or white), and also the command for <Application of the Hough Transform> and <Histogram>. Additionally, the number of reached colonies of bacteria/sample is also shown in the interface).

Table III presents results, which were performed with the Group #2, having 21 samples prepared with the *Acidithiobacillus ferrooxidans* on solid culture. In this table the absolute, relative and percentage error found for each sample are presented.

TABLE III. RESULTS OF THE ANALYSIS OF SAMPLES HAVING THE BACTERIA *ACIDITHIOBACILLUS ferrooxidans* IN SOLID CULTURE.

Sample #	Absolute error	Manual error		Smart method error	
		Relative	%	Relative	%
1	2.00	0.02	2.44	0.02	2.38
2	1.00	0.02	1.52	0.02	1.54
3	6.00	0.11	10.71	0.12	12.00
4	3.00	0.08	8.33	0.09	9.09
5	1.00	0.02	2.38	0.02	2.44
6	3.00	0.03	2.63	0.03	2.56
7	1.00	0.01	0.77	0.01	0.76
8	10.00	0.18	17.86	0.15	15.15
9	3.00	0.04	4.17	0.04	4.00
10	2.00	0.02	2.44	0.03	2.50
11	1.00	0.02	2.27	0.02	2.22
12	5.00	0.03	2.86	0.03	2.78
13	1.00	0.02	2.13	0.02	2.08
14	1.00	0.01	1.06	0.01	1.05
15	21.00	0.10	9.50	0.11	10.50
16	17.00	0.12	11.89	0.13	13.49
17	3.00	0.07	7.14	0.07	6.67
18	12.00	0.06	6.32	0.06	5.94
19	2.00	0.06	5.71	0.05	5.41
20	4.00	0.05	5.48	0.05	5.19
21	4.00	0.07	7.41	0.07	6.90

From analysis of the Group #1, one may observe that the absolute error remained smaller than 10%. However, there are results which show a relative error rate above 10%, particularly sample #3, sample #8, sample #15, and sample #16. These results are linked to external factors inherent in the process of sample preparation. Examples of external factors of errors occur due to the provision of culture medium on the Petri dish that can face problems related with wrinkle or bubble, i.e., may find the growing of the colonies near the edge of the plate. Fig. 10 shows the result of dispersion of the values found in both the manual by experts and the automatic counting for Group #1.

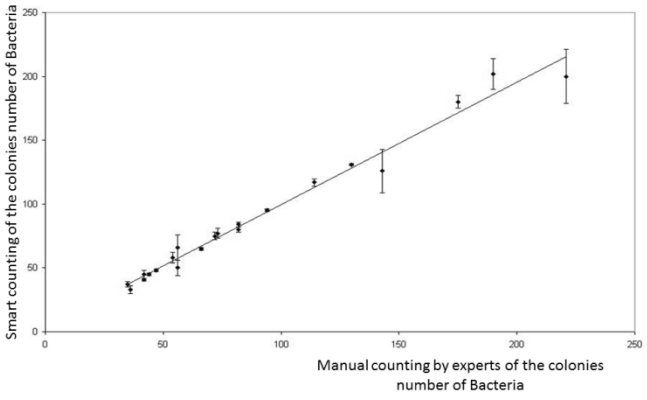

Figure 10. Correlation between the sensor-based recognition method and the manual by experts for the Group #1, which represents the *Acidithiobacillus ferrooxidans* samples.

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In the graph, the bars of errors are associated with the absolute errors of each sample. The coefficient of linear correlation found was equal to 0.99. For this group, the number of colonies per sample was smaller than 200, and in this case, the results obtained by automatic analysis were very close to the manual one.

The Sulfur (S (symbol), Z (atomic number) = 16, ρ (density) = 2.07g/m³) deficiency in soils is becoming common in many areas of the world, as a result of agricultural practices. Therefore, the development of a smart method to support decision making, especially in the processes related to the identification and count of colonies of microorganisms in soils, in particular those of the genus *Thiobacillus*, is very much required.

V. CONCLUSION

The method presented allowed intelligent recognition and both the qualitative analysis and the quantitative count of the bacteria colonies, i.e., using a customized computer and a CCD sensor, as well as the Hough Transform for circular microorganisms.

Groups of samples were analyzed to get the information regarding to the bacterial colonies growing, its formation and its number in the solid culture, all prepared in Petri dishes. Results have shown high linear correlation, i.e., when compared with the manual analysis executed by expert people. In both cases, the linear correlation coefficients were equal to 0.99, i.e., for the *Escherichia coli* samples (Group #1), and for the *Acidithiobacillus ferrooxidans* samples (Group #2), respectively.

In the future, based on qualitative and quantitative analysis on the bacteria and its colonies, additional methods will be designed and that will have the possibility to be used directly in the agricultural field for in-situ analyses. Additionally, this method will be able to be embedded and ported to Android smartphones for online processing.

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REFERENCES

[1] MAPA, "Ministry of agriculture, livestock and food supply. Normative Instruction n 62 (original in portuguese language)," 2003.

[2] CONAMA, "National Health Surveillance Agency, in the DRC 275: Technical regulation of microbiological characteristics for natural mineral water and natural water (original in portuguese language)," 2005.

[3] W. K. Durfee, "Designing smart machines.- teaching mechatronics to mechanical engineers through a project based, creative design course," Mechatronics, vol. 5, 1995, pp. 775–785.

[4] B. F. Spencer, M. E. Ruiz-Sandoval, and N. Kurata, "Smart sensing technology: opportunities and challenges," Structural Control and Health Monitoring, vol. 11, no. 4, 2004, pp. 349–368.

[5] M. C., J. E. Cohen, H. Setl, J. Bloem, and A. M. Breure, "Bacterial traits, organism mass, and numerical abundance in the detrital soil food web of dutch agricultural grasslands," Ecology Letters, vol. 8, 2005, pp. 80–90.

[6] F. O. Bendez´u and P. A. J. de Boer, "Conditional Lethality, Division Defects, Membrane Involution, and Endocytosis in mre and mrd Shape Mutants of Escherichia coli," Journal of Bacteriology, vol. 190, no. 5, March 2008, pp. 1792–1811. [Online]. Available: http://dx.doi.org/10.1128/JB.01322-07

[7] H. Ates and O. N. Gerek, "An image-processing based automated bacteria colony counter," 24th International Symposium on Computer and Information Sciences, 2009, pp. 18–23.

[8] S. Kothari, Q. Chaudry, and M. Wang, "Automated cell counting and cluster segmentation using concavity detection and ellipse fitting techniques," IEEE International Symposium on Biomedical Imaging: From Nano to Macro, 2009, pp. 795–798.

[9] M. Park, J. Jin, Y. Peng, P. Summons et al., "Automatic cell segmentation in microscopic color images using ellipse fitting and watershed," IEEE/ICME International Conference on Complex Medical Engineering, 2010, pp. 69–74. [Online]. Available: http://dx.doi.org/10.1109/ICCME.2010.5558869

[10] R. Hazan, Y.-A. Que, D. Maura, and L. G. Rahme, "A method for high throughput determination of viable bacteria cell counts in 96-well plates," BMC Microbiology, vol. 12, no. 1, 2012, pp. 1–7. [Online]. Available: http://dx.doi.org/10.1186/1471-2180-12-259

[11] G. M. Alves, P. E. Cruvinel, and H. A. F. Nascimento, "Automated method for cfu counting in petri dishes," Revista SODEBRAS, vol. 9, no. 101, May 2014, pp. 44–49.

[12] L. R. Trabulsi and M. R. F. Toledo, Microbiologia, 2nd ed. Rio de Janeiro: Editora Atheneu, 1991.

[13] M. J. J. Pelczar, E. C. S. Chan, ̃ and N. R. Krieg, Microbiologia: Conceitos e aplicac¸ o ̃ es, 2nd ed. Pearson Education do Brasil, 1997, vol. 1, traduc¸ a ̃ o Sueli Fumie Yamada, Tania Ueda Nakamura, Benedito Prado Dias Filho; revis ̃ao t ecnica Celso Vataru Nakamura.

[14] J. Mullikin, L. Vliet, H. Netten, F. Boddeke et al., "Methods for CCD camera characterization," Proc. SPIE Conference (San Jose CA, Febr.9-10, 1994), SPIE, vol. 2173, 1994, pp. 73–84.

[15] D. G. Abdelsalam, M. Stanislas, and S. Coudert, "CCD or CMOS camera calibration using point spread function," Proc. SPIE 9234, International Conference on Experimental Mechanics 2013 and Twelfth Asian Conference on Experimental Mechanics, 2014, pp. 92 340Z–92 340Z–4. [Online]. Available: http://dx.doi.org/10.1117/12.2048991

[16] P. V. C. Hough, "Method and means for recognizing complex patterns," 1962, u.S.Patent 3.069.654.

[17] R. O. Duda and P. E. Hart, "Use of the Hough transformation to detect lines and curves in pictures." Graphics and Image Processing, vol. 15, no. 1, 1972, pp. 11–15.

[18] M. Smereka and I. D. Eba, "Circular object detection using a modified hough transform," Int. J. Appl. Math. Comput. Sci., vol. 18, no. 1, 2008, pp. 85–91.

[19] B. Gorte and G. Sithole, "Lookup table hough transform for real time range image segmentation and featureless co-registration," Journal of Sensor Technology, vol. 2, no. 3, 2012, pp. 148–154.

[20] G. Gerig and F. Klein, "Fast Contour Identification through Efficient Hough Transform and Simplified Interpretation Strategy." IEEE, New York, NY, USA, 1986, pp. 498–500, cited By 47. [On-line]. Available: http://www.scopus.com/inward/record.url?eid=2-

 ISBN: 978-1-61208-523-4

s2.00022957510partnerID=40md5=af10f41984977b2fb5394f41a5c42a67

[21] M. Luessi, M. Eichmann, G. M. Schuster, and A. K. Katsaggelos, "Framework for efficient optimal multilevel image thresholding," Journal of Electronic Imaging, vol. 18, no. 1, 2009, pp. 013 004–013 004–10. [Online]. Available: http://dx.doi.org/10.1117/1.3073891

[22] N. Otsu, "A Threshold Selection Method from Gray-Level Histograms," IEEE Transactions on Systems, Man and Cybernetics, vol. 9, no. 1, January 1979, pp. 62–66.

[23] M. Sezgin and B. Sankur, "Survey over image thresholding techniques and quantitative performance evaluation." Journal of Electronic Imaging, 2004, pp. 146–165.

[24] L. Chen, ". laplacian embedded regression for scalable manifold regularization," Neural Networks and Learning Systems, IEEE Transactions, vol. 23, 2012, pp. 902–915.

[25] G. Shrivakshan and C. Chandrasekar, "A comparison of various edge detection techniques used in image processing," IJCSI International Journal of Computer Science Issues, vol. 9, no. 1, September 2012, pp. 269–276.

 ISBN: 978-1-61208-523-4