EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 1
140-146
Year: 2018

ANALYSIS OF BACTERIAL SUSPENSIONS OF DIFFERENT CONCENTRATIONS AND SHAPES BY MEASURING ULTRASONIC VELOCITY

Adil Hamine[1]*, Bouazza Faiz[1], Ali Moudden[1], El Houssaine Ouacha[1], Mohamed Aghrouch[2] and Mustapha Faghire[3]

[1]Laboratory of Metrology and Information Processing. Faculty of Sciences. University Ibn Zohr, B.P 8106, Hay Dakhla, 80000, Agadir, Morocco.
[2]Regional Hospital Center, Hassan II, Route de Marrakech, Agadir, Morocco
[3]Laboratory of the Plant Biotechnology, Department of Biology, Faculty of Sciences. University Ibn Zohr, B.P 8106, Hay Dakhla, 80000, Agadir, Morocco

***Corresponding Author: Dr. Adil Hamine**
Laboratory of Metrology and Information Processing. Faculty of Sciences. University Ibn Zohr, B.P 8106, Hay Dakhla, 80000, Agadir, Morocco.

Article Received on 15/11/2017 **Article Revised on 05/12/2017** **Article Accepted on 26/12/2017**

ABSTRACT
Our study is based on the ultrasound method of ultrasonic velocity to measure the turbidity of bacterial suspensions. Turbidity is measured by an ultrasound system, which determines the absorbance-related decrease in the intensity of the well known frequency ultrasonic wave passing through each suspension. Several suspensions have been prepared with different bacteria, (*Escherichia coli*, *Klebsiella*, *Enterococci*, *Staphylococci*) and with different shapes, (Batonnier and concile). This first study aims to measure ultrasonic solute concentration of bacteria present in human urine. It is known that the urine is sterile, it must not contain any germ otherwise it indicates a urinary infection or other disorder to be diagnosed. For this, fecal bacteria will be sought in human urine samples, which could change the approach of prevention and treatment in several urinary disorders. The results obtained are encouraging and show that ultrasound can identify each bacterial suspension.

KEYWORDS: Urine analysis, bacteria in the urine, ultrasonic characterization of human urine.

INTRODUCTION
Urinary infections (UI) are the most common of all bacterial infections because urine has no property to resist microbes, and can therefore be an excellent growing medium. Normal urine is sterile because in its normal state it contains no microbes, viruses or fungi. A (UI) occurs when a microorganism, usually a bacterium from the digestive tract, enters the urethra and then the bladder and begins to multiply. (UI) in the presence of a pathogenic germ in the urine in the presence of compatible symptomatology. In general, these are the most common bacterial infections in humans. A bacterial infection is a disease caused by a bacterium, a unicellular organism lacking a nucleus. There are a very large number of bacterial infections that can affect humans. There are a very large number of bacterial infections that can affect humans, depending on the type of bacteria or region of the infected organism. The germs most commonly responsible for (UI) are community acquired as (Escherichia coli) between 75% to 85%, and other enterobacteria such as (Klebsiella spp and Proteus), between 4% to 25%, and Staphylococcus as (S. epidermidis and saprophyticus) is found in less than 4% to 15% of single (UI);[1] Alan Wolfe and Linda Brubaker, two American researchers in the Department of Microbiology and a doctor in the Department of Obstetrics, Gynecology and Urology at Loyola University in Chicago, have spoken of urinary microbiome to refer to the microbial flora present inside the bladder;[2] The set of bacterial genomes corresponding to this complex ecosystem so far largely ignored. The purpose of the basic urine analysis is to provide information for the diagnosis, control and prevention of:

- Kidney diseases,
- Diseases of the urinary tract,
- Diseases of certain organs or systems such as diabetes or liver diseases: in this case the renal function is often normal but an abnormal quantity of certain metabolites is excreted in the urine.

DIAGNOSIS OF URINARY TRACT INFECTIONS
The analysis is mostly done on urine:

- The Gram Positive germs suspected of causing urinary tract infection are: Staphylococci, S. Saprophyticus, Streptococcus haemoliticus, Enterococci.
- For Gram Negatives, there will be: E. coli, Klebsiella, Salmonella, Serratia.

Physical analysis of urine

The physical examination consists of measurements of: density (solute concentration)- turbidity-color. No definitive diagnosis can obviously be carried out with this single parameter. Normal urine should be transparent. Thus a cloudy urine often accompanies a large quantity of leucocytes. Of course leukocytes can originate anywhere in the genitourinary tract if the sample is taken by natural urination, cystocentesis is the best option. Other causes of turbidity are the presence of suspended crystals, microorganisms (bacteria and yeasts) as well as natural secretions such as mucus, prostatic fluid and sperm. Microscopic examination of urinary sediment is essential for the explanation of turbidity.

Solute concentration (density of urine)

The measurement of the density of the urine makes it possible to evaluate the capacity of dilution and concentration of the kidney which is determining for the maintenance of the balance of the hydro-electric equilibrium (homeostasis). This corresponds to the proportion of dissolved elements in the urine relative to the volume of the sample. Very sensitive to temperature, the examination should be done at 20°C. The urinary density is very important, it must be performed during each analysis. It is defined as the ratio of the weight of the urine to the weight of an equal volume of pure water, the two liquids being at the same temperature,[3]; It makes it possible to determine the concentration and dilution capacity of the renal tubules and is absolutely necessary for the interpretation of the other analyzes performed on the urine. The solute concentration can be evaluated using an osmometer (osmolality), a urinometer (specific gravity) or a refractometer (refractive index);[4] The most used technique is that of the refractometer. The refractive index is defined as the ratio of the velocity of light in the air to the velocity of light in the solution. Urine contains solutes that will absorb light at different wavelengths that will change the path of the light beam. The angulation of this ray of light measured by the refractometer will give an estimate of the urinary density. Urinary density should be performed on the supernatant only. Thus the density will be falsely high if Dextran or contrast medium is present in the urine;[5] The presence of hemoglobinuria and myoglobinuria, increase the urinary density, which could distort the measurements. Normal urine has a density between 1.010 and 1.025, if the density is less than 1.010, we have low concentration urine and if the density is above 1.025, we have very concentrated urine.[5]

Bacteria size

Bacteria are the smallest living organisms capable of reproducing alone. The colibacillus Escherichia coli, bacterium of our intestinal flora, measures on average 1 µm by 2 µm. Some rare bacteria are longer, such as anthrax (6 µm) and giant spirochetes (500µ m), others a little smaller (0.2 µm).

Bacteria shape

A first classification (and the corresponding nomenclature) is based on the shape of the bacteria, a characteristic visible with a good optical microscope.

- Spherical bacteria, shape (shells):
- Bacteria grouped into unformed clusters: Staphylococci;
- Bacteria arranged like the pearls of a necklace: Streptococci;
- Non-spherical bacteria, shape (bars), ie cylindrical:
- Straight-grain bacteria in the shape of rice grain: Bacilli;
- Bacteria with curved axis, comma-shaped: Vibrios;
- Helicoidal axis bacteria: treponemes or spirochetes.

METHODS FOR MEASURING BACTERIAL CONCENTRATION

Among the measuring devices the bacterial concentration quoted in the literature is the DEN-1 density meter that was designed by Grant Bio Instruments to measure the turbidity of a solution in the range of Mc Farland units 0.3-5.0 ($100x10^6$-$1500x10^6$ cells/ml). DEN-1 is used to measure the turbidity of a solution in a wider range (5.0-15.0 Mc Farland units). It should be noted, however, that in this case the standard deviation values increase. The DEN-1 densitometer is used to determine the concentration of cells (bacterial and yeast cells that have larger sizes than bacteria), in the fermentation process to:

- determine the susceptibility of microorganisms to antibiotics,
- identify microorganisms using various test systems,
- measure the fixed wavelength optical density (λ= 550nm),
- evaluate quantitatively the solute concentration.

All these operation are based on the measurement of optical density.

Diffusion of light by bacteria, (turbidity effect)

The bacteria in suspension will generate a clouding effect invisible to the naked eye, they make the medium highly diffusive of light. The phenomenon of light scattering is characterized by the fact that a fraction of the light received at a given wavelength will be returned to the same wavelength in all directions. The physical theories of light scattering are made to distinguish diffusion phenomena by particles of dimension of the order of a few nanometers (in practice molecules) and phenomena of light scattering by particles of dimension 1/10 to 10 times the wavelength of illumination. The diffusion by the molecules is called Raleigh diffusion, and it is of low intensity, very dependent on the wavelength of illumination. The bacteria we are interested in are formed of particles whose dimensions are of the order of magnitude of the wavelengths of the visible and near the infra-red. This diffusion is of high intensity and it is little influenced by the wavelength of illumination. Fig.1.

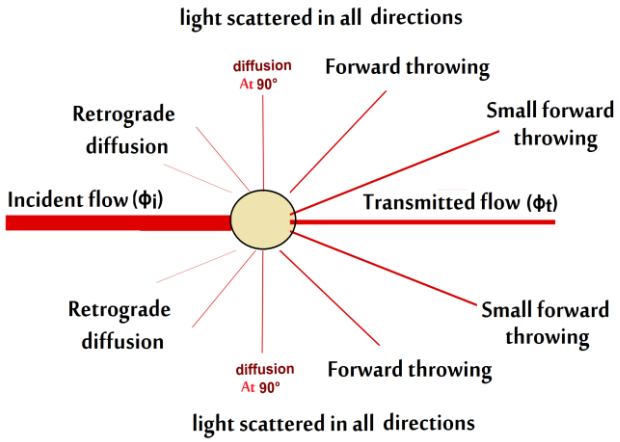

Figure 1: Diffusion of a monochromatic light by a spherical particle, (Particle 10 times the size).

When the diffusers are spherical particles (concile bacteria) and their size are the order of d=0.05µm larger than the wavelength used for illumination at λ= 500 nm, (d/λ=1/10), the scattering before is fairly homogeneous in all directions. When the diffusers have a size d= 5 µm smaller than the wavelength used for illumination at λ= 500 nm, (d /λ=10), the diffusion is very weak. Fig.2.

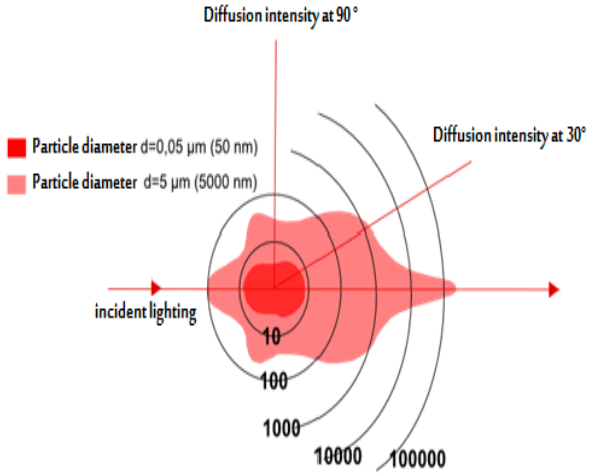

Figure 2: Example of light scattering by large or small size diffusers in front of the illumination wavelength (λ= 500µm).[6]

It can be concluded that the scattering of light depends very little on the wavelength of illumination in the visible range, therefore the choice of the wavelength does not matter a priori between 400 and more than 900 nm. The measurements of the optical density with a visible or UV spectrophotometer. It is possible to use a visible spectrophotometer or UV as an opacimeter to measure bacterial concentrations. The measuring device displays the decimal logarithm of the ratio of the reference flow to the transmitted stream. The relationship of optical density and bacteria in suspension is linear up to a certain limit (usually 0.5-0.7), above this value the linearity is lost, Fig.3.

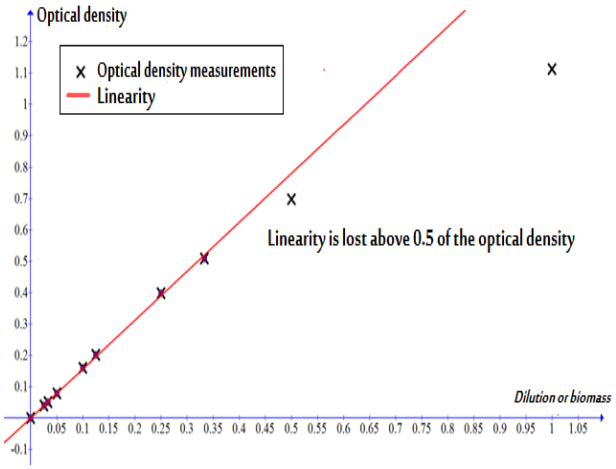

Figure 3: Optical density at λ= 600 nm, due to the bacterium "E. coli".[7]

Measurements of bacterial turbidity by scattered light measurements

Conventional nephelometer turbidimeters which measure the light diffused at 90 ° are devices conventionally intended for turbidity measurements in the water field. Turbidimeters used for biomass measurements (bacteria and yeasts) generally use scattered light measurements from several angles (eg, 90° and a forward angle). Fig.4. The devices also measure transmissive light and the displayed value is the result of calculating a ratio of light scattered and transmitted. The current light sources are almost always diodes emitting in the IR towards 950 nm.

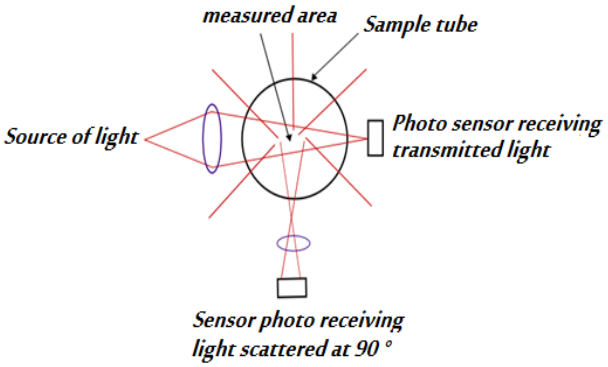

Figure 4: Diagram of the principle of a conventional nephelemeter turbidimeter.

Manufacturers generally report linearity of biomass concentration measurements from 4 to 100 times higher than that of optical density measurements with a spectrophotometer at 600 nm; (ie from 2 to more than 100 equivalent of optical density in the spectrophotometer).

Disadvantage of optical methods

From the literature review conducted in this work on optical measurement methods of bacterial suspensions, we concluded that optical methods have limitations in the choice of wavelength used. It is advantageous to choose a wavelength for which the phenomena of

absorption of light by the medium containing the cellular biomass are minimal. This is why most of the work is done most often for conventional media beyond 600 nm. All modern cellular biomass sensors using diffusive effect use near infrared illumination at 850-950 nm.

ULTRASONIC METHODS

The effects of ultrasonic waves on cells can be divided into two categories, thermal and mechanical, although both types of effects can occur simultaneously. Thermal effects are limited to an increase in cell temperature as a result of the absorption of ultrasonic wave energy, while mechanical effects may vary in manifestation and severity.

Sample preparation method

To measure bacterial turbidity or absorbance, we will prepare suspensions fairly rich in bacteria, it is necessary that the concentration is between 10^6 and 10^7 bacteria/cm^3. For example, the preparation of bacterial suspensions is made from a stock solution of Eshircia coli (5 colonies per 100 ml of solution), Klebsia (3 colonies per 100 ml). The bacterial suspension prepared is colorless, it is made with physiological water, because it is the medium most suitable for this kind of bacterial suspensions. A suspension can be prepared in a colored medium, which may distort the ultrasonic measurements. The suspensions are prepared using bacterial fences containing several colonies with which new bacterial suspensions are prepared in physiological water diluted 1/10. Dilutions of 1/10 are made in test tubes containing 1 ml of the stock suspension in 9 ml of sterile physiological water, and then stirred to homogenize the solution.

Choice of measuring vessel

The choice of measuring vessel was the major difficulty of this study because the amount of bacterial suspensions was very small. Different plates of the measuring vessel have been tested and studied. The first container was designed so that the first plate is Plexiglas and the second in glass (Pl=3 mm/Ver=4mm). An example of the signal acquired by measuring vessel 1, containing the distilled water using a 20 MHz transducer is shown in Fig.5. A copy of the Lab*view* software screen is given in this figure.

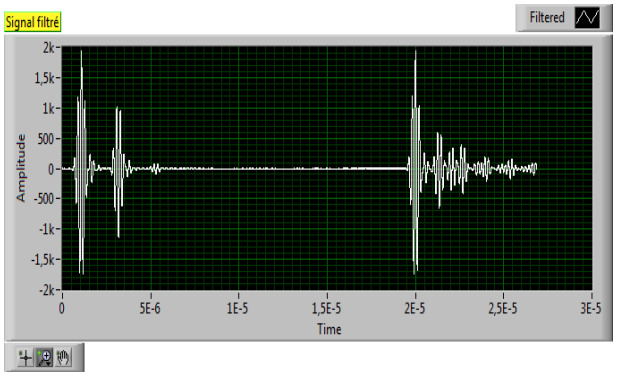

Figure 5: Signal acquired by the first container.

The Fourier transform of the entire signal of Fig. 5 is illustrated in Fig. 6.

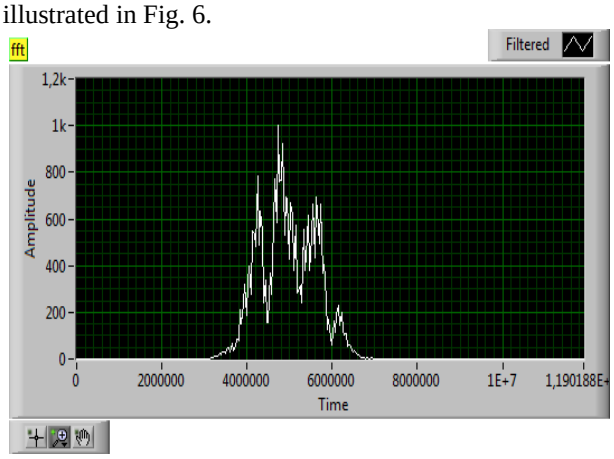

Figure 6: FFT of the signal acquired by the container 1.

The second container was designed in the same way as the first except this time the Plexiglas plate has a thickness of 4mm, (Pl=4mm/Ver=4 mm). An example of the signal acquired by the container 2 containing the distilled water using a 20 MHz transducer. A copy of the Lab*view* software screen is shown in **Fig.7**, which shows the form of the signal acquired by the second measuring vessel, containing the water using a 20MHz transducer.

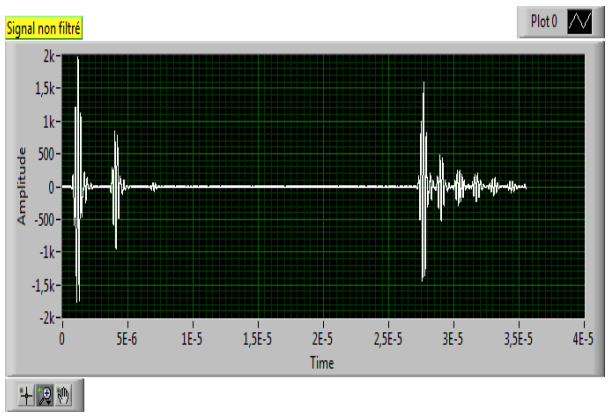

Figure 7: Signal acquired by the second container.

The Fourier transform of the entire signal of Fig. 7 is illustrated in Fig.8.

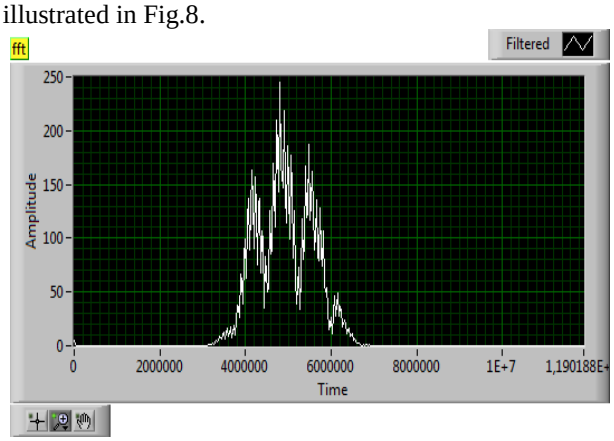

Figure 8: FFT of the signal acquired by the second container.

From a brief comparison between the Fourier transforms, of the Figures 6 and 8, our choice fell on the container 2, because it represents less overlap and resonance and the ultrasonic waves seem less attenuated in the thick Plexiglas 4 mm.

RESULTS AND DISCUSSION

Ultrasonic velocity measurement of bacterial suspensions

From ultrasonic velocity measurements of bacterial suspensions, we found that ultrasound can be used to identify, shape and concentration of bacteria. Indeed, according to the results obtained, it is found that the suspensions of rod-shaped bacteria, such as (Escherichia coli, klebsiella) remain in suspension for a long time, Fig.8; While bacterial suspensions of concile forms, such as (Enterococcus, Staphylococcus) sediment very quickly, Fig.9.

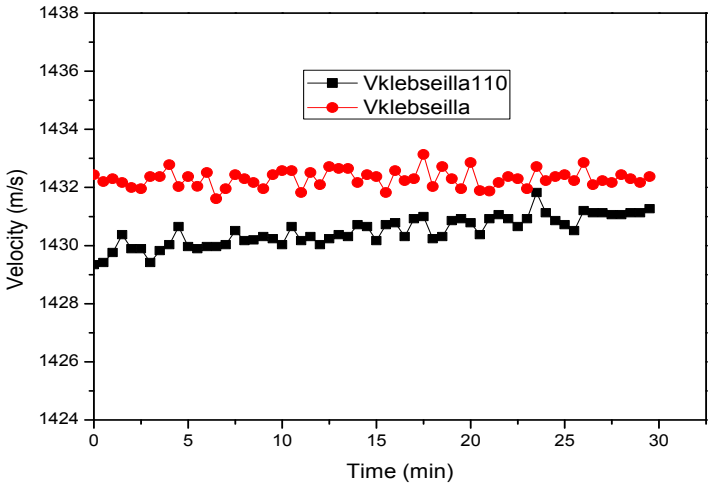

Figure 8: Ultrasonic velocity in the klebsiella suspension. (Transducer 20MHz).

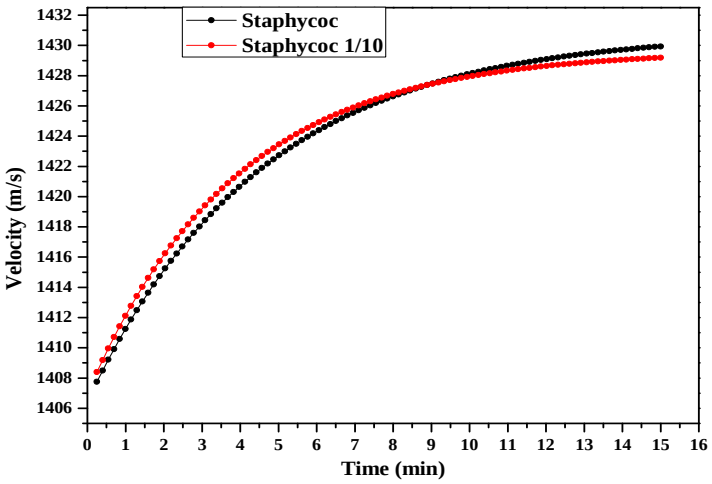

Figure 9: Ultrasonic velocity in the suspension Staphylococcus. (Transducer 20MHz).

We note also that the klebsiella suspension is stable during ultrasonic bombardment and there is a slight difference of about 2m/s between the diluted 1/10 suspension and the parent suspension. Whereas the Staphylococcus and Enterococci suspensions sediment rapidly, as the ultrasonic velocity increases from 1390 m/s to1412m/s (ultrasonic velocity in distilled water); From 1387 m/s to 1412 m/s for Staphylococcus. It is also noted that no difference between the two diluted suspensions and mother solution. Fig.10.

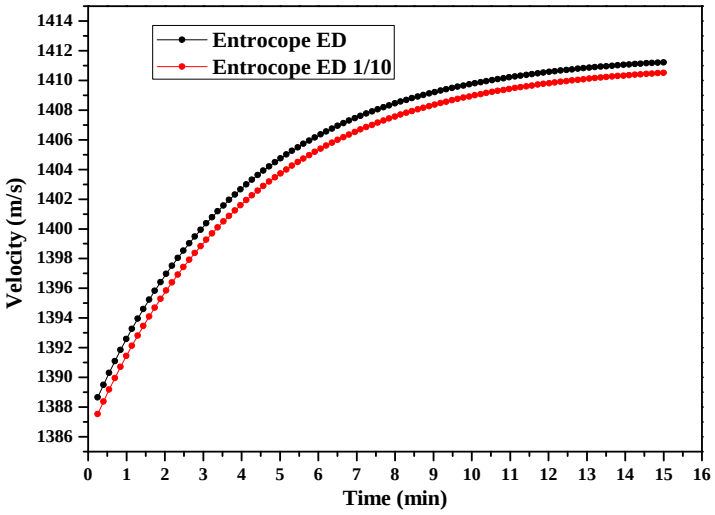

Figure 10: Ultrasonic velocity in the suspension Enterococcus. (Transducer 20MHz).

Study of a bacterial multi suspension

By analogy with the urine infected by bacteria, we have prepared a multi bacterial suspension composed of several bacteria of different shape. The ultrasound technique allowed us to draw the curve of **Fig.11**, which shows that each bacterium has a unique acoustic signature, except in the case of Staphylococci and the Entrococci are indistinguishable. The only difficulty that arises in the ultrasound study is that the urine contains different crystals, the latter will screen the different bacteria in the urine, and this may infect the urinary inspection by ultrasound.

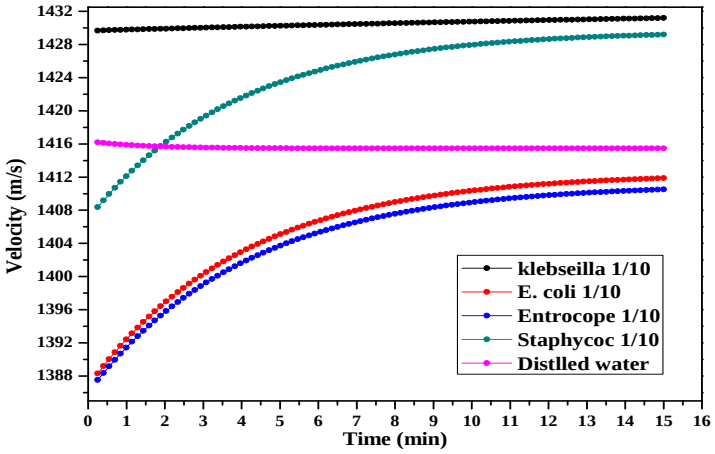

Figure 11: Ultrasonic velocity in the different suspensions using a 20MHz transducer.

CONCLUSION

From the literature review conducted in this work on optical measurement methods of bacterial suspensions, we concluded that these optical methods have limitations in the choice of wavelength used. Indeed, it can be concluded that light scattering depends very little on the wavelength of illumination in the visible range, therefore the choice of the wavelength does not matter a priori between 400 and more than 900 nm. Since the reflected and scattered light intensity is neglected, it can be said that the absorbance is proportional to the bacterial mass. But the optical methods in this kind of suspensions are complex and their implementations prove difficult from the point of view of the choice of the wavelength. The ultrasound technique has identified each bacterial suspension, and each of them has a unique acoustic signature. The only ultrasound difficulty that arises is that the urine contains different crystals. The presence of crystals in suspension, as well as natural secretions such as mucus, prostatic fluid, sperm and yeasts will screen the different bacteria present in the urine, and this may infect ultrasound urinary inspection.

REFERENCES

1. François, H. Brandstätter, A.-C. Bréchet, A. Huttner. Urinary Infections, HUG «DMCPRU». Department of primary care, University Hospitals of Geneva, 2013; 3-4.
2. Alan J. Wolfe, Linda Brubaker. Sterile Urine and the Presence of Bacteria. Eur Urol, 2015; 68: 173-4.
3. C.A. Osborne, D.R. Finco. Canine and feline nephrology and urology. Baltimore. Lea & Febinger, x-y, 1995; 135.
4. C.A. Osborne, J.B. Stevens. Handbook of canine & feline urinalysis. Saint-Louis, Ralston Purina Co, 1981; 148.
5. Willard, M.D., Tvedten H., Turnwald G.H. Small animal clinical diagnosis by laboratory methods.
6. Montreal, W.B. Saunders Co, 1989; 121-153.
7. Seppo I. Niemelä. Uncertainty of quantitative determination derived by cultivation of microorganisms, Centre for metrology and accreditation MIKES Publication J4, 2003; 5-6.
8. JF Perrin, Measurement of the bacterial concentration of a suspension of a pure E. coli K12 strain by counting colony-forming units (CFU) on a solid medium. Study of the correspondence between biomass disturbance (optical density at 600 nm) and the concentration of microorganisms, 2014; 3-4.