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Rapid evaluation of proteins by turbidimetry: Application to some agro-alimentary effluents treated by electrocoagulation

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ABSTRACT

This study provides a turbidimetric method, recognized for its rapid and precise evaluation, of flow dispersion of proteins contained in agro-alimentary effluents, before and after clarification treatment by continuous electrocoagulation (EC). Different wastewater effluents were studied such as those of dairy, abattoir and cereals. The principal aims of this study are: the existence and the variability of the correlation between turbidity and proteins; in order to implement the measure in continuous and in situ of turbidity to estimate, by extrapolation, proteins concentrations. The results obtained were able to highlight possible extrapolation between these two parameters but within the tested limits of concentrations; the correlation coefficients obtained are 0.96, 0.94 and 0.74 for the dairy, the slaughterhouse and the cereal effluents, successively. Extrapolation has been validated with satisfaction on effluents treated by EC. It is therefore possible to monitor the effectiveness of this technique via protein parameter and optionally perform turbidity sensor automation; thus reducing time and cost related to the analysis of proteins.

Key words: automation; electrocoagulation; extrapolation; proteins; turbidity.

INTRODUCTION

The increase of the world population and the over-industrialization are the main causes of various kinds of environmental pollution, especially in developed countries. Among these types of pollution, the wastewaters coming from different industries discharged without preliminary treatment into different mediums such as sea, rivers and soils, cause a deterioration of the physicochemical and biological qualities of the medium which leads to generate many environmental and health problems [1]. Actually, the main objective of research works is to treat these industrials wastewaters prior to reject them into the environment by the use of several simple and economical technologies.

Usually, before accomplishing an appropriate treatment of an effluent, it is necessary to characterize it in order to identify its composition. Therefore, it is important to choose the adequate method for measuring of each parameter as presently the chemistry and physico-chemistry offer numerous analytical techniques such as volumetric, gravimetric, electrochemical, and optical [2]. However, most of these techniques have some analytical drawbacks related to the measurement accuracy, the equipment used and its automation and also the cost of the technique adopted [3].

The judicious choice of analytical technique for the measurement of a given parameter depends on a compromise often made from these limits. In the present study, the turbidimetry was used to analyze the proteins in wastewater. This method was chosen for the main reasons such as simplicity, speed, accuracy and cost [4, 5]. In addition, the turbidity is already applied by the use of turbidity sensors in drinking and wastewater treatment stations [6, 7]. For the quantitative determination of proteins, there are numerous methods have been used such as methods involving the degradation of proteins by chemical means (Kjeldahl method, Dumas, Kofranyi, dosage of sialic acids), titration of formaldehyde, fixation of colorant, colorimetric, electrophoretic, chromatographic, immunologic and by exopeptidases action (Carboxypeptidase A) [8]. However, these methods if they are not long and expensive, most of them cannot be easily automated [9]. In the present study, it was tried to relate the physical measurement of turbidity to the chemical one of proteins (Bradford), the latter was chosen for its performance and low detection limit; it is actually the most commonly used and cited method in the scientific literature [10, 11, 12]. The wastewater coming from different waste stations located in city of Annaba (Algeria) such as dairy, abattoir and cereals were used. The determination of proteins content in these wastewaters before and after the treatment by electrocoagulation (EC) process was achieved using a turbidimeter. The contribution of organic pollution charge in urban effluents, the nutritional and technological importance of proteins in agricultural and food products, make their analysis are undeniable analytical priority as well as in research laboratories that those of control.

EXPERIMENTAL SECTION

Data about wastewaters studied, parameters and analytical conditions

Three kinds of wastewaters originating from different agro-industries located in Annaba city (Algeria) were used: dairy wastewater (DW), abattoir wastewater (AW), cereal products wastewater (CPW).The dairy which produces essentially reconstituted milk (water, milk powder, more fat) and the municipal abattoir, are located at 6 km in the south of the Annaba city; these are the only companies that discharge their effluents into the common receivers (Oued Meboudja and Oued Seybouse). For the case of cereal unit that produces particularly pasta, it is located in an industrial area of about 30 km in the south of Annaba city. This industrial unit discharges its waste effluents produced from the production and washing procedures into the neighboring ravines of the city namely in east of Oued Seybouse, northwest of Oued Meboudja and south of Oued El-Rassoul. View to the enormous use of drinking water by these agro-industries, this is lead to produces huge quantities of wastewaters. Dairy factory uses it for the production of milk and milk products as well as for the usual acido-basic cleaning of the installations for their disinfection. In the abattoir, water is used for the washing of the by-products (offal) and the waste removal (fecal contamination, remains of paunch and blood) as well as the global disinfection of the abattoir. For the industry of pasta products, water is employed especially for cleaning production facilities at the end of each workstation. These effluents untreated with in particular organic load constitute a menace of pollution for these valleys or aquatic receptors. For each company, the sampling point chosen is the main collector. It is the meeting place of all the wastewaters: in particular workshops of production with their water of cleaning, disinfection and water of valves. Each sampling was performed on an average sample of 50 liters of waste water, spread out over a whole day with full activity of the workshops of production. For reasons of multiplicity of the analyses and samples, the samples were performed in the same week in May 2013. Other specific samples were performed during all this year until May 2014, with strong and weak load of production of the workshops even at the times of cleanings and disinfections. The parameters chosen for the characterization of pollution are those which make it possible to appreciate better the quality of water to know their potential action about the aquatic receptor and the environment such as pH, temperature, Chemical Oxygen Demand (COD) and the Biological Demand Oxygen for 5 days (BOD_5) …etc [13]. The pH and the temperature were analyzed on site using a mobile multi-parameters analyzer (CONSORT C535, Belgique) .The other parameters such as turbidity, total suspended solids (TSS), BOD_5 and COD were measured in the laboratory by respecting all the rules of samples conservation during their transport [14, 15]. The turbidity of wastewaters or their degrees of transparency [4] is measured using a turbidimeter (Phywe 2100N). TSS are determined by the classical technique which applied for the separation by direct filtration or centrifugation AFNOR T90: 105 (1979) [16]. The content of proteins in the wastewater samples was analyzed by the chemical method of Bradford (1976) [17], and using a spectrophotometer (Jenway 7315) at a wavelength fixed at 595 nm for a concentration range between 0 to 1 g/L. The measurement of proteins by the Bradford method was used initially in order to compare its results with that of turbidimetry which will subsequently be followed continuously. The analyses of the BOD_5 during 5 days and the COD are performed on original wastewaters according to the experimental protocols of APHA (2005) [18].

Equipment and working conditions of dynamic EC treatment process

Description of the EC process

The electrocoagulation (EC) is an innovative technology that can be applied for treating of different industrial wastewaters containing organic and mineral pollutants. It is based on the principle of soluble anodes by producing metallic cations such as Al^{3+} and Fe^{3+} in wastewater under a direct current between electrodes (iron, aluminum or alloy). These cations will react the role of coagulant and allow the destabilization by discharge of suspended particles and colloids. The Formation of hydroxides (iron or aluminum) and the particular geometry of electrocoagulation reactor cause flocculation phenomena. During treatment, the electrolytic reactions at the electrodes surface can produce micro-bubbles. After that, fine and insoluble matters like suspended solids, hydrocarbons, oils, fats and colloids of dimensions < 10^{-2} mm will rise to the surface which indicates that the wastewater has been treated. At the end of the process, treated water or decontaminated water for the category of the pollution by toxic substances is obtained [19, 20, 21]. The flock of formed sludge is recovered by decantation or flotation depending on the application kind. The uses of this process for water treatment are numerous since it has several advantages such as that a little maintenance, little follow-up by the operator and low energy demand. The energy expenditure is depended each application which is often varied between 0.5 and 4 kWh per m³ of treated water. The follow-on costs of this procedure are usually less than the majority of other technologies and it can also ensure significant results [22]. In fact, the EC can also be used properly in rural areas or, if electricity is not available, by applying of solar panels attached to the EC device [23, 24]. Since to the dissolution of the sacrificial electrodes, various species are generated in relation with pH of the solution and in the presence of various chemical species [25, 26]. In the case of aluminum electrode which has been used in the present work, the main reactions that can take place are the following (Equations 1-2-3):

Anode: $Al(s) \rightarrow Al^{+3}(aq) + 3e^-$ (1)

Cathode: $3H_2 (l) + 3e^- \rightarrow 3OH^-(aq) + 3/2\ H_2 (g)$ (2)

In the solution: $Al^{+3} (aq) + 3H_2O (l) \rightarrow Al (OH)_3 (s) + 3H^+ (aq)$ (3)

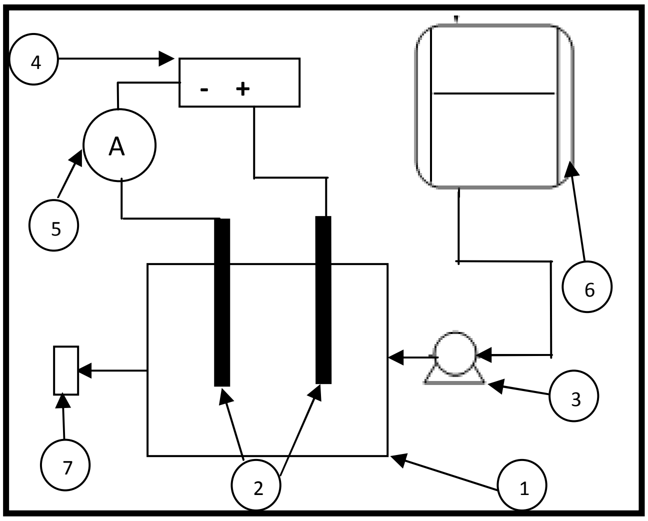


Fig. 1: Reactor of EC in dynamique

1- Electrochemical reactor, 2 - Aluminium Electrodes, 3 - Peristaltic pump, 4 - Potentiostat, 5 - Ammeter, 6 - Supply reservoir, 7 - Exit reactor (sampling point)

Set up of EC experimental used

Experiments of EC were performed on different agro-industrial wastewaters at temperature of laboratory (20 ± 2°C) in an electrochemical reactor glass of a capacity of 1 liter. It was chosen for its simplicity of only two electrodes and its ease of use, wherein two plates of aluminum electrodes are located and between them the wastewater effluent is flowed for treating **(Fig. 1)**. The two electrodes were approximately equal in size of 15 cm in length 3 cm in width with an effective area of 45 cm^2. The spacing between these electrodes is 1 cm. This low value was chosen in order to limit not only the ohmic voltage drop, but also to avoid the clogging. In the reactor, the fluid from the waste container was upwardly pumped through a peristaltic pump (Master degree Flex L/S Model 77202-60) in order to work with low flows and to avoid the gas bubbles. By the use of a potentiostat (Metrix–AX-502), the electrodes were connected to a direct current power supply for an electrical current (15 mA/cm^2) which allows a uniform dissolution of the metal at the anode and a regular hydrogen releasing at the cathode. The control of the current power was simultaneously maintained on this potentiostat and on an amilliammeter (SKY-Sronic-600-527) connected in series. Potassium chloride (KCl 2×10^{-2} mol/L) is added to increase the solution conductivity and the applied circulation flow is fixed at 1.75 ml/min. The electrolysis was chosen as 2 h, because beyond this duration the effectiveness of the treatment gets to its maximum until a period of 55 h when it would be decreased due to the accumulation of produced sludge. The adopted experimental conditions were derived from the results of a study on the effectiveness of pre-treatment of effluents coming from an industrial dairy using coagulation-flocculation and electrocoagulation dynamics in our laboratory [27]. At selected time intervals, samples of treated water were collected at the outlet of the reactor to analyze proteins by turbidimetry until an optimum efficiency was reached. The turbidity, which was the key parameter in the monitoring of the wastewater to be treated, presented an error average of approximately ± 3%. All other parameters: TSS, BOD_5, COD, pH etc were measured in a batch-wise manner. Between each test**,** the electrodes were removed from the reactor, rinsed thoroughly with dilute hydrchloric acid (10^{-2} M) and then with distilled water to remove the salt deposits from the solution. All the chemical compounds used have an analytical grade (Sigma®-Aldrich, U.K.), and all the solutions were prepared with ultra-pure water with a resistivity 18 MΩcm. Whether for the proteins parameter or any other parameters followed in continuous and discontinuous successively, the abatement rate of a given parameter X, expressed as a percentage TX (%), is calculated using following formula (Equation 4):

$$TX(\%) = (\, C_i X - C_f X \,)\, 100/C_i X \quad (4)$$

C_iX and C_fX: Value of a parameter successively before and after treatment.

RESULTS AND DISCUSSION

Analyses of wastewater before treatment by EC

As mentioned above, the studied wastewaters have enormous amounts of different contaminations with an irregular manner, containing biodegradable matters essentially organic, which can have negative consequences to the aquatic receptors. The characterization of these wastewaters by analysis of selected parameters is essential which allows appreciating their potential actions on the environment in general. The toxicity parameters are not taken into account since as a rule the primary materials such as milk powder, fat (dairy), sheep and cattle in particular (abattoir), durum wheat (cereals) are previously analyzed by the exporter and the importer in officially approved laboratories **(Table 1)**, includes all parameters analyzed, thus characterizing the wastewater pollution studied, in full production activity (average values) taking into account to the same parameters, specific measures (extreme values). The values recorded for parameters TSS, COD, BOD, and in some cases the temperature and pH, greatly exceed the limit values from relating Algerian standard to industrial discharges liquid [28]. For each kind of wastewater studied, found results are of the same order of level as those of wastewater from dairy "ORLAC" of Bir Khadem in Algeria [29] of six samples of abattoirs in Canada [30] and cereals [31]. The mean values of temperature and pH of wastewater from the main collectors are acceptable in view of the standard. However, it is important to notice that extreme occasional values over 30°C and 3 to about 10 in pH units. High temperatures are mainly due to the warm waters of flushing from heating appliances. These temperatures accelerate the acidification process by fermentation of sugars contained in the various products release and enhance the formation of bacterial biomass and algae causing unpleasant odors. The high temperature also inhibits the aquatic life; many organisms with no thermal control mechanisms will have their vital activities slowed [32, 33]. Extreme pH variations would be related to overdose in acid-base cleaning products, insufficient rinsing of production equipment and also to natural bacterial acidification of certain sugars contained in waste. For example as in the case of lactose that turns into lactic acid by an enzymatic pathway. Such amplitude of pH is detrimental to the environment and to the concrete network; it would also have negative consequences for flora and fauna aquatic whose growth of pH is between 6 and 7.2 [32]. The modifications in pH would also entail a discount by solution of mineral salts in the aquatic receptors. The excess of TSS in all studied wastewaters, can significantly affect the operation of the sewer system.

Large particles decantable and colloidal can cause nuisances such as sludge deposits and clogging aquatic receptors funds. The settled sludge is noxious to the maintenance of the natural biological edifices [32, 33]. Often, the TSS is identified by turbidity [4]. However, the relation between turbidity and the concentration of TSS remains a difficult issue to treat [34]. Indeed, it depends on several parameters, including the geometric and optical characteristics of suspended particles that are heterogeneous and variable in wastewater. For this reason, the explanation of the results of the differences between these two parameters is not too easy to put en relation. For all the studied waters, measured turbidity is high and irregular. Releases are constantly turbid; which would cause a very difficult light scattering in the aquatic receiving environment and compromise the growth of photosynthetic organisms. As an Indication, water inlet to the production units are very clear, turbidity are less than or equal to 4 NTU. Moreover, colloidal particles or non decantable matters can also represent the turbidity parameter; the protein is an example of these particles [4].

The results of proteins found by the Bradford method, after prior calibration **(Fig. 2)**, are all relatively low compared to other studies where values between 1988 to 3213 mg/L are achieved in an abattoir for example [35]. The dilution effect, where frequent washings at the level of the production workshops, could explain the results in protein found. For the results of COD or BOD, the values found in full activity of production units or grab samples are all high and superior to the standard due to the organic load of wastewater studied. Reports COD/BOD of wastewater from dairy, abattoir and cereals, are of 4.0, 3.4 and 5.2 respectively. They indicate an eminent biodegradability of release. This one would be more complete if the bacterial metabolism were not inhibited, by moments, by the excessive presence of detergents. These reports are comparable to those of the majority of agro-industries with average organic load [36, 37, 38].

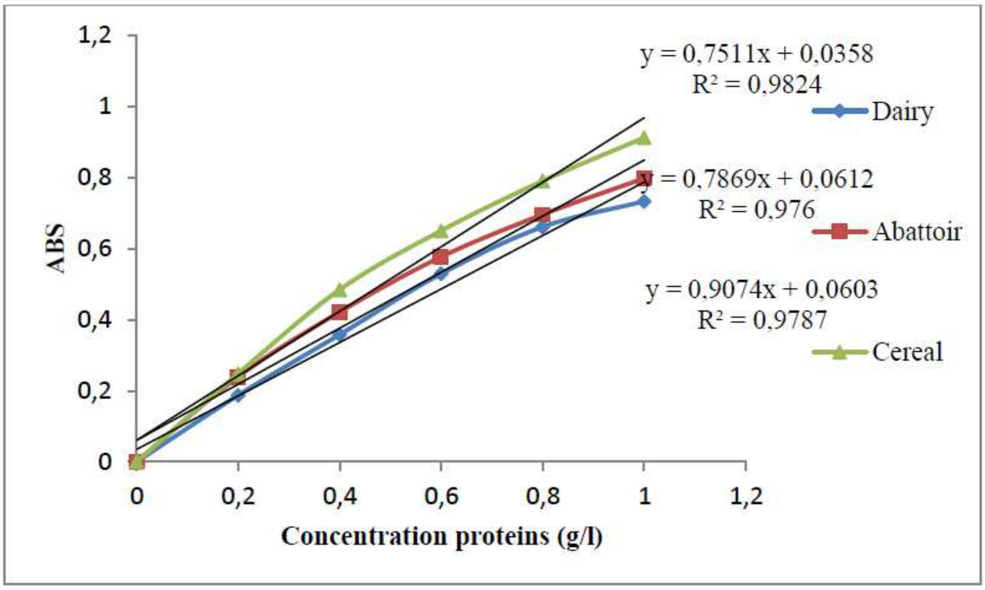


Fig. 2: Calibration curve of Bovine Serum Albumin (BSA) for the proportioning of protein by the method of Bradford (calibration range 0 to 1 g/L)

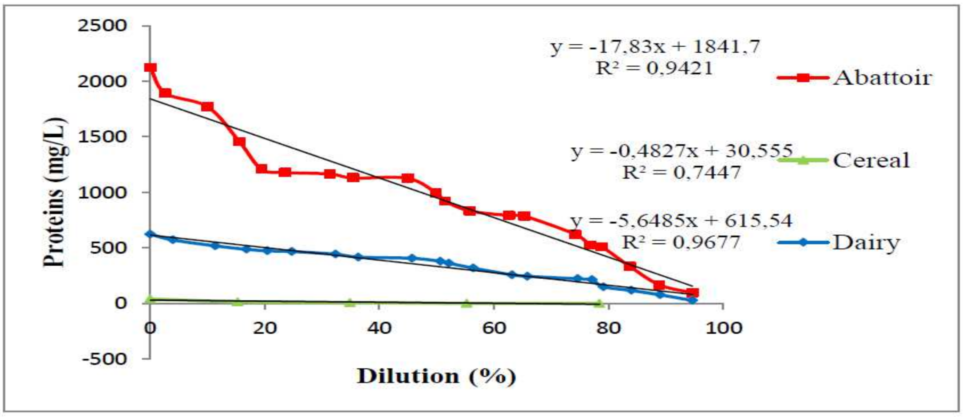
Table 1: Main characteristics of wastewaters before to treatment by EC

Parameters	Result wastewater						Algerian standard industrial waste
	dairy		Abattoir		Cereals		
	Vave	VpExt	Vave	VpExt	Vave	VpExt	
Temperature (°C)	25	20 to 34	21	20 to 32	22	19 to 31	lower than 30°C (ocean discharge)
pH	6.9	3.0 to 9.9	6.5	6.0 to 7.9	5.3	4.9 to 7.8	6.5 to 8.5
TSS (mg/L)	430	260 to 640	250	150 to 401	218	110 to 350	40
COD (mgO_2/L)	5118	2300 to 6375	9442	4330 to 11530	952	566 to 1570	120
BOD_5 (mgO_2/L)	1270	1093 to 3197	2810	1820 to 3162	183	120 to 392	35
Turbidity (NTU)	1035	990 to 1050	1020	650 to 1512	255	35 to 260	-
Proteins (Bradford) (mg/L)	624	590 to 1000	2215	1600 to 3200	42	30 to 80	-

Influence of the dilution of wastewaters studied on the results of analyses of turbidity and proteins

This experiment of the influence of dilution on the results of proportioning of turbidity and proteins was performed while analyzing first for each of the parameters, the wastewater on the outlet of the collector of each unit production studied. Progressive dilution with ultrapure water of these wastewater and the systematic analysis of proteins and turbidity at each dilution rate, made it possible to represent the results of determination of protein **(Fig. 3A)** and turbidity **(Fig. 3B)**. These results show a very good distribution or correlation of the values of proteins and turbidity with the rate of dilution of wastewater; correlation coefficients given by the curves are satisfactory for the waters of dairy and abattoir. However, the cereal unit presents an R^2 of 0.74 for proteins, that would be due to the low initial concentration of protein (0.04 g/L), which is low compared to other studied water samples; the dilution effect further reduces this concentration and makes the measurement absorbsometric of protein inaccurate. For this case, the use of the method of Bradford becomes uncertain and the dilution effect for the cereal unit is not convincing for a broad extrapolation.

(A)



(B)

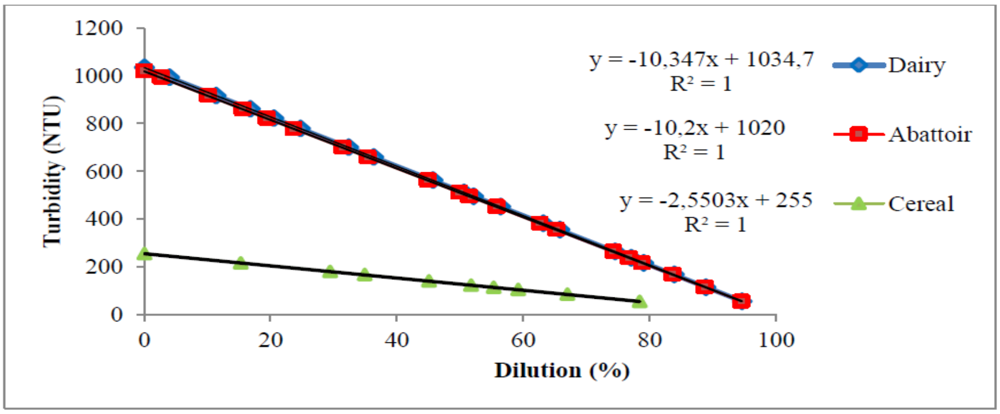


Fig. 3: Graphical Representation of the influence of dilution of wastewater on the measurement of proteins (A) and (B) turbidity
Initial Turbidity wastewater of dairy 1035 NTU; Initial protein concentration 624 mg/L
Initial Turbidity wastewater of abattoir 1020NTU; Initial protein concentration 2215 mg/L
Initial Turbidity wastewater of cereal 255 NTU; initial Protein concentration 42 mg/L

Extrapolation of turbidity in protein measurements

Given that the correlation of measurements of turbidity and proteins of the effluent to the identical dilutions reported in **Figure 3** is satisfactory, you can with the same measures, to, establish then, an expression linking these two parameters. **Figure 4** gives linear functions with coefficients of correlation of 0.96, 0.94 and 0.74 for wastewaters of dairy, abattoir and cereals respectively. As for the effect of dilution, an extrapolation turbidity protein is also limited for the unit of cereal because of the low proteins initial concentrations and turbidity. These results of extrapolation will dispense to systematically perform measurements of proteins, which are costly in reagents and waste of time. This allows being up and quickly operational because measurements of turbidity are fast.

It is obvious that the correlation and extrapolation are possible only for the ranges of concentration of protein and tested turbidity values that are usually encountered in agro-food wastewaters [39, 30, 38].

To confirm and observe the possibility to generalize the extrapolation to effluents of different nature and composition, the equation of the curve was taken to strong correlation coefficient of the dairy ($y = 1.772\ x - 72.75$), then to calculate starting from this equation estimated turbidity and compare it to real turbidity or measured at the exit of the main collector of five agro alimentary samples including wastewaters of abattoir and cereals. All water samples were collected in the locality, in one day of full production activity. The results are presented in **Table 2**. It can be noticed that the results are far from conclusive to the views of significant relative deviations. It therefore seems a priori that such an equation applies only to a single type of effluent. A hypothesis on the causes of variation between the effluents agro-alimentary and the effluents of dairy can be the proteins known as "soluble". Indeed, the soluble organic matters very present in the agro-alimentary effluents cannot appear by turbidimetry. Water containing a low concentration out of proteins, limiting of advantage extrapolation as well as the method of measure adopted. However the number of samples tested, remaining low it is undoubtedly hasty to draw now this kind of conclusion at least for the samples from the unit poultry abattoir, silos washing of wheat samples and the drinks unit, juice.

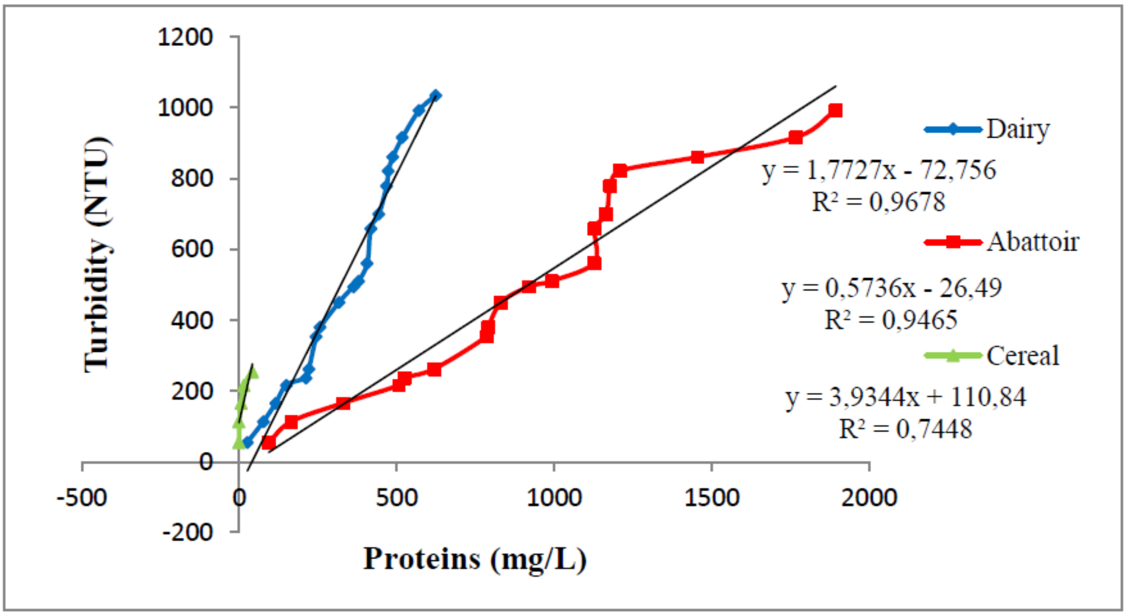


Fig. 4: Extrapolation of measurements of turbidity to proteins for the different wastewaters studied

Table 2. Application of the relationship between correlations of the dairy to other effluents

Wastewater nature	Proteins (mg/L)	Turbidity reels (NTU)	Turbidity estimate (NTU)	Ecart (%)
Unit demolition, poultry cutting.	1090	920	1859	-50
silos washing of wheat	49	90	14	84
Gas unit drinks, Juices	3440	1063	6025	-82
Local abattoir	2215	1020	3853	-73
Unit cereals	42	255	1.7	99

Control continuously of proteins by extrapolation of turbidity measurements: application in treatment by EC

Current research aims to limit the contaminations of industrial origins by proposing simple and less expensive techniques of treatment. From this point of view the EC is particularly interesting especially for its aspect non-polluting and its facility of automation which adapts well to the desired turbidity/protein extrapolation. The study of the pretreatment of clarification by EC proposed, is designed to reduce the pollution matters of wastewaters of dairy, abattoir and cereal which is essentially organic more precisely proteinic. The reduction of organic matter and mineral wastewater by EC consists in destabilizing the colloidal substances of negative overall charge, in an insoluble particulate form which will be eliminated by decantation. This reduction passes by mechanisms of electrooxidation, complexation of surface, electrostatic attraction and chemical modification, has been largely studied [40].

The results obtained by this process operating conditions already applied to the laboratory when a study on the effectiveness of pre-treatment of the effluents from an industrial dairy by coagulation-flocculation and electrocoagulation in Dynamics [27] are presented in **Figure 5**. Reducing of protein values come from the extrapolation of measurements of turbidities in the treatment of EC based on **Figure 4** and Equation (4). From the results of extrapolation of measurements of turbidities on the protein concentrations **(Fig. 4)**, can then easily find concentrations of proteins at any time of the treatment and consequently can be able to control the effectiveness of the process compared to the parameter protein and proceed in the end to a possible automation in turbidity sensor. For what is the effectiveness of the process of EC, 30 min time of electrolysis are sufficient to reduce the organic load of the waters studied at considerable rates ranging between 90 and 100% at pH between 6 and 7.5 favorable to the formation of flocks precipitant aluminum hydroxyl.

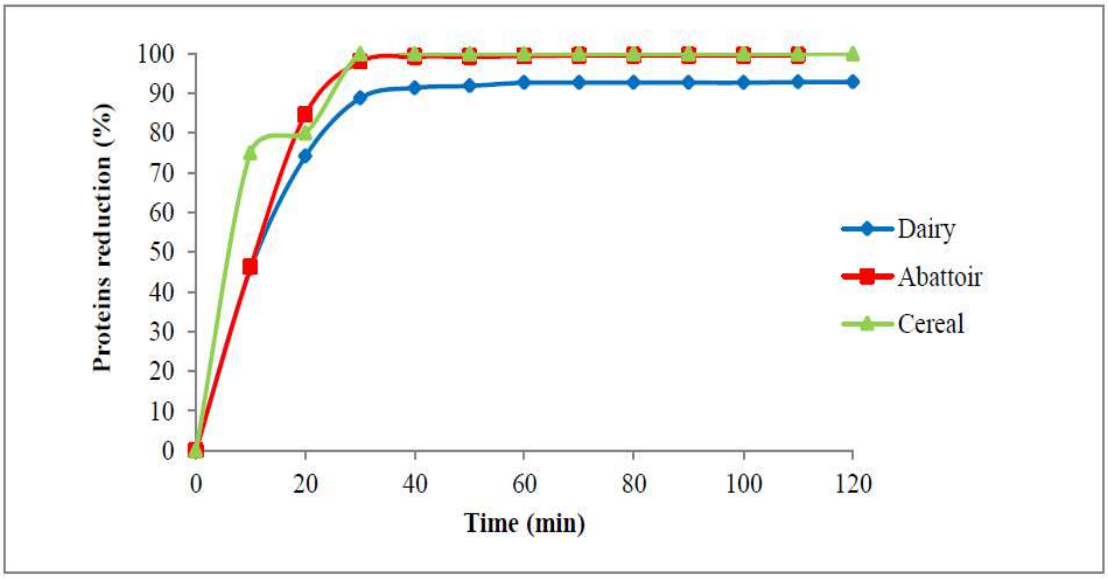


Fig. 5. Reduction rate of proteins by extrapolation of the turbidity after treatment by EC measurements

Which: common operating conditions are: current density = 15 mA/cm^2, electrode gap = 1 cm, Flow rate = 1.75 mL/min, electrolyte Support KCl 2×10^{-2} mol/L, special operating conditions:Dairy: initial pH 6.9; initial turbidity 1035 NTU; proteins 624 mg/L
Abattoir: initial pH 6.5; initial turbidity 1020 NTU; proteins 2215 mg/L
Cereal: initial pH 5.3, initial 255 NTU turbidity; proteins 42 mg/L

In addition, the other parameters controlled punctually before and at end of treatment, namely: TSS, BOD, COD undergo also considerable abatements **(Table 3)** but their content remain high if one refers to the Algerian standard of industrial waste [28]. Although the good clarification of water, complementary treatment is in this case necessary to eliminate the dissolved organic matter, before to a rejection of the treated water into the surrounding aquatic environment or their reuse.

Table 3. Quality of wastewater before and after the treatment by EC

Parameters	Dairy wastewater			abattoir wastewater			Cereal wastewater		
	Raw water	Treated water	(%) of reduction	Raw water	Treated water	(%) of reduction	Raw water	Treated water	(%) of reduction
Temperature (°C)	24		-	23		-	23		-
pH	6.9	8.8	-	6.5	8.7	-	5.3	7.9	-
TSS (mg/L)	430	47.3	89	250	70	72	218	34	84.4
COD (mgO$_2$/L)	5118	588.6	88.5	9442	2143	77.3	952	174.2	81.7
BOD$_5$ (mgO$_2$/L)	1270	99	92.2	2810	533.9	81	183	54.3	70.3
Turbidity (NTU)	1035	5.1	99.5	1020	4.1	99.6	255	2.2	99
Proteins (mg/L)	624	44.9	92.8	2125	11.3	99.46	42	0	100

CONCLUSION

The implementation of turbidimetry to evaluate proteins from wastewater of dairy, abattoir and cereal, appeared positive. It proves to be effective in terms of time of analysis and precision, thus avoiding the disadvantages of the use of classical methods for the determination of proteins which are essentially: long and costly. The correlation and extrapolation in this context, first required an update analytically which consists in measuring in different waters, the proteins by the method of Bradford for a range standard going until 1g/L. The experience of the extrapolation of turbidity in protein measurements is possible between these two parameters, even at high dilution of samples. However the cereal unit presents a low correlation coefficient (R^2 ~ 0.74) compared to other waters. Extrapolation has been validated successfully on an application of treatment of these waters by EC. Of course, extrapolation is possible only for the ranges of concentration of proteins and tested turbidities values. These results make it possible to control the effectiveness of this technique compared to the protein parameter and to possibly perform turbidity sensor automation. On the other hand, it was found that enlargement of the extrapolation to effluent of different nature is to be taken with caution.

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