Rapid detection of *Escherichia coli* in milk and cream by impedance measurement

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SUMMARY. This work was focused on the rapid detection of milk and cream recontamination, *Escherichia coli* being used as a model microorganism. Milk, cream, impedance Medium 001A and Nutrient Broth were the media tested. Impedance of the medium was more suitable for measurement in milk, cream and Medium 001A (shorter detection time, lower standard deviations); only electrode impedance was usable for Nutrient Broth. Glucose added to milk and cream did not affect the growth of *E. coli*. Detection in Medium 001A was about 20 min faster, when Supplement S for spore-forming bacteria was added. Linear equations between log CFU.ml^{-1} (plate count method) and detection time were calculated. Residual standard deviations for milk, cream, Medium 001A and Nutrient Broth were $s_{y,x} = 0.1077$, $s_{y,x} = 0.1016$, $s_{y,x} = 0.2783$, and $s_{y,x} = 0.4144$, respectively, and the correlation coefficients were higher than 0.98 in all cases. Cell concentration (CFU.ml^{-1}) was retrospectively calculated from the detection time and thus the linear equation was confirmed. Duration of *E. coli* detection in the impedance Medium 001A was comparable to that of the direct determination in milk and cream.

KEYWORDS: impedance; *Escherichia coli*; milk; cream

Increasing requirements of dairy industry development are associated with improving of technologies, new continuous processes with high nutritious, sensory and microbial products quality requirements are established. The number of contaminating microorganisms present in raw food materials is reduced or eliminated by a pasteurisation or a sterilisation step.

Standard microbiological methods for the evaluation of microbial contamination are laborious and time- and material-consuming. This is a good reason to use alternative methods; one of them is the impedance measurement [1-3]. This method is based on changes in the electrical properties of the medium

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caused by the metabolism of the present bacteria [2-7]; it is faster, cheaper and more comfortable than conventional plate method [1, 6, 7].

Contamination of dairy products, mostly caused by gram-negative microflora, significantly reduces their shelf lives [8]. Presence of these bacteria in final products may be caused by recontamination or by underheating [9].

This work is focused on a comparison of the rapidity of coliform bacteria detection in different media by impedance measurement. Non-selective media were used, growth properties of coliform bacteria were presumed to be similar (optimal temperature, redox potential etc.) and *Escherichia coli* was chosen as a model microorganism. Its generation times in different media were evaluated. The linear functional relationships between detection times and initial bacterial numbers were calculated.

Material and methods

Principle of the impedance method

Microbial metabolic processes producing electrically measurable changes in the growth medium were utilized to detect bacteria, which metabolize high-molecular-weight nutrients into smaller charged ionic compounds, thereby increasing the electrical conductivity of the medium [1-3, 5, 10-12]. The impedance of the growth medium, which is a complex quantity composed of conductance and capacitance [5, 8, 12], will subsequently decrease [1]. The medium impedance (M-impedance) is useful for media with low conductivity [5, 10, 13]. In addition to M-impedance, changes in ionic layers in the vicinity of the electrodes, so-called electrode impedance (E-impedance), can be detected. An increase in mobile ions cause a decrease of E-impedance [1, 10, 13]. E-impedance is suitable for media of high salt contents [5]. The used measuring system BacTrac 4100 (Sy-Lab, Neupukersdorf, Austria) records the changes in M- or E-impedance in 10 min intervals and expresses the values as percentages of the initial value (*%M*, *%E*):

$$M\,[\%] = \frac{Z_{M0} - Z_M}{Z_{M0}} .100$$

where Z_{M0} [Ω] is the initial impedance of the medium and Z_M [Ω] is the measured impedance value of the medium at a given time. Data collection starts 1 h after the insertion of measuring cells with the inoculated medium into the instrument. The measuring cells are equipped with two stainless steel electrodes and their capacity is 10 ml.

As soon as the change in the impedance (*%M* or *%E*) reaches the arbitrarily selectable threshold, the computer registers detection time (*DT*), which is inversely proportional to the initial bacterial count (fig. 1) [2, 5, 6, 10, 12-14]. Microbial counts at *DT* for different inoculum rates of individual strains are constant and reproducible [2, 10, 11, 13].

Microorganism used and the plate method

As a model microorganism of dairy products recontamination, *Escherichia coli* DMF 7504 (stock culture of Department of Dairy and Fat Technology, Institute of Chemical Technology, Prague, Czech Republic) was used. The strain was grown for 18 h at 37 °C in UHT milk or sterilized cream (Bohušovická mlékárna, Bohušovice nad Ohří, Czech Republic). For cell concentration determination, the inoculum was diluted in steps 1:9 [15]. The number of microorganisms in this inoculum was determined by the plate count method (Nutrient Agar, Oxoid, Basingstoke, Great Britain; 37 °C, 24 h).

Sample preparation for impedance measurement

The impedance cells were first steamed for 15 min at 100 °C and then sterilized for 15 min at 121 °C. After inoculation and mixing, the media were

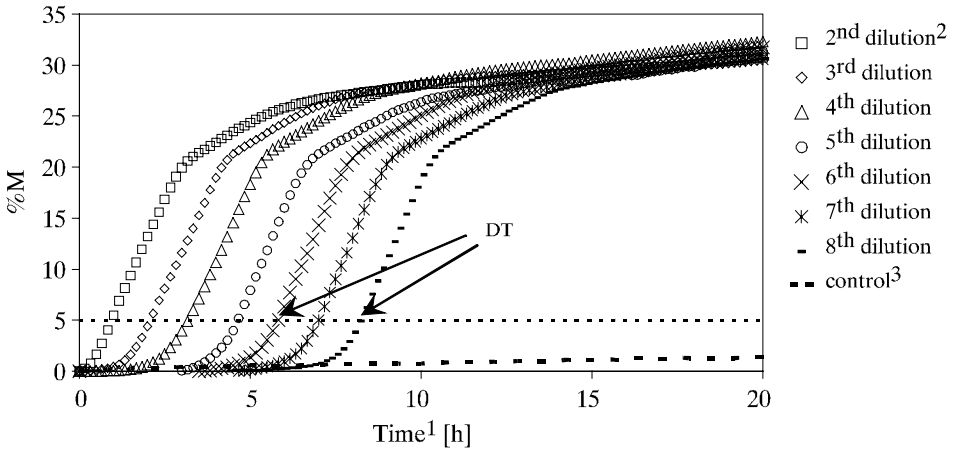

FIG. 1. Dependence of M-values on time for several dilutions of *E. coli* culture (inoculum 6.0×10^8 CFU.ml^{-1}; Medium 001A).
OBR. 1. Závislost M-hodnot na čase pro řady ředění suspenze *E. coli* (inokulum $6{,}0.10^8$ JTK.ml^{-1}, Médium 001A).
1 - čas, 2 - ředění, 3 - kontrola.

distributed to at least three measuring cells (10 ml) and incubated at 37 °C in the BacTrac thermostatic blocks. The used media were: UHT milk (1.5 % fat) and sterilized cream (10 % fat; both Bohušovická mlékárna, Bohušovice nad Ohří, Czech Republic); Nutrient Broth (Oxoid, Hampshire, Great Britain); Medium 001A designed for electrical measurement with and without Supplement S for spore-forming bacteria (Sy-Lab, Neupukersdorf, Austria). The effect of glucose addition to milk and cream was observed. Constant volume of different stock glucose solutions to milk or cream was added to obtain a final concentration range of 0; 0.10; 0.25; 0.50; and 1.00 % (w/v).

Evaluation of the measured data

The impedance analyses were carried out in at least three parallels and average M-, E-values and detection times were calculated. The relationship between the logarithm of colony forming units (log *CFU*) and detection times (*DT*) was calculated for each of the used media [5, 11]. The coefficients *a, b* of the linear calibration equation

$$\log CFU = a + b.DT$$

were determined by the least squares method. The reliability of a calibration curve was described by the residual standard deviation $s_{y,x}$ and by the correlation coefficient *R*, which is dependent on the accuracy of the methods. Generation times (*GT*) of tested bacteria were calculated from the slope *a* [5]:

$$GT = \frac{\log 2}{[a]}$$

The standard deviations *s* of $DT_{\%M}$ and $DT_{\%E}$ impedance for each of media were calculated [16]:

$$s = \sqrt{\frac{1}{N-k} \cdot \sum_{j=1}^{k} \sum_{i=1}^{n} (DT_{ij} - \overline{DT_j})^2}$$

where *k* represents the number of different samples (i. e. number of dilutions), *N* - number of all results, *n* - figure of repeating, DT_{ij} - detection time of *i* parallel tests of *j* sample, $\overline{DT_j}$ - average detection time of *j* sample.

TAB. 1. Standard deviations of M- and E-values for the determination of *E. coli* by the impedance method in tested media; evaluation of the calibration curves.
TAB. 1. Standardní odchylky M- a E-hodnot pro stanovení *E. coli* impedanční metodou v testovaných médiích; vyhodnocení kalibračních křivek.

Medium[1]	$s(DT_{\%M})$ [h]	$s(DT_{\%E})$ [h]	Used impedance[2]	a	b	s_a	s_b	$s_{y,x}$	R	GT [min]	n
Milk[3]	0,14	1.07	M	–0.810	9.077	0.0064	0.0401	0.1077	–0.999	23.3	35
Cream[4]	0,21	0.87	M	–0.784	8.889	0.0072	0.0640	0.1016	–0.999	23.0	26
Medium 001A[5]	0,06	0.84	M	–0.840	7.691	0.0129	0.0729	0.2783	–0.994	21.5	52
Nutrient Broth[6]	unusable[7]	0.38	E	–0.782	8.622	0.0310	0.2524	0.4144	–0.986	23.0	25

$s(DT_{\%M})$, $s(DT_{\%E})$ - standard deviations of %M and %E values; a, b - regression coefficients; s_a, s_b - standard deviation of coefficients a and b; $s_{y,x}$ - residual standard deviation; R - correlation coefficient; GT - generation time; n - number of determinations.
$s(DT_{\%M})$, $s(DT_{\%E})$ - standardní odchylka stanovení hodnot %M a %E; a, b - koeficienty regresních vztahů; s_a, s_b - standardní odchylky koeficienů a a b; $s_{y,x}$ - reziduální standardní odchylka; R - korelační koeficient; GT - generační doba; n - počet stanovení. 1 - médium, 2 - použitá impedance, 3 - mléko, 4 - smetana, 5 - Médium 001A, 6 - Nutrient bujón, 7 - nepoužitelná.

Results and discussion

Milk and cream with and without glucose, Medium 001A and Nutrient Broth were the media tested in this work. Effect of the addition of Supplement S for spore-forming bacteria to Medium 001A was examined for non-selective application on real samples.

The first step of this study was to choose more suitable signal (M- or E-impedance) for each of the tested media. M-impedance was better for deter-

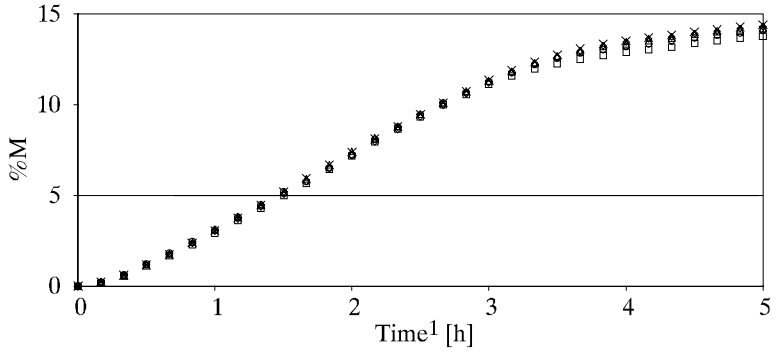

FIG. 2a. Repeatability of M-values determination in milk
(*E. coli* 7.2×10^7 CFU.ml^{-1}; 5 parallel determinations).
OBR. 2a. Opakovatelnost stanovení M-hodnot v mléce
(*E. coli* 7,2.10^7 JTK.ml^{-1}; 5 paralelních stanovení).
1 - čas [h].

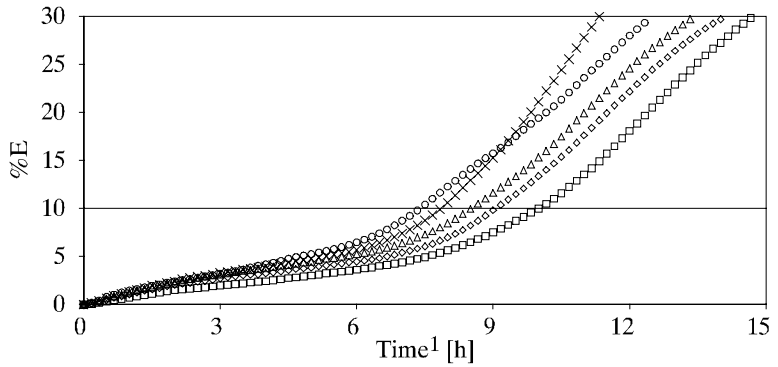

FIG. 2b. Repeatability of E-values determination in milk
(*E. coli* 7.2×10^7 CFU.ml^{-1}; 5 parallel determinations).
OBR. 2b. Opakovatelnost stanovení E-hodnot v mléce
(*E. coli* 7,2.10^7 JTK.ml^{-1}; 5 paralelních stanovení)
1 - čas [h].

mination in milk, cream and Medium 001A because of about 7 h lower *DT*s (data not shown) and lower standard deviations of *DT*s (tab. 1). During the determination in milk and cream, a decrease in pH caused by the microbial metabolism resulted in the coagulation of casein and gas production took place as well. Individual E-impedance curves, and thus also *DT*s, differed in response to the distribution of casein coagulate, whey and gas in the vicinity of the electrode surfaces, and therefore the repeatability of E-values was low (fig. 2). E-impedance only was usable for determination in Nutrient Broth, owing to its high salt content; the changes in M-values caused by microbial activity were consequently minimal and the sample as well as control curves were practically identical.

Formation of lactic acid from lactose is of crucial importance for the determination of microbial contamination directly in milk or cream using the impedance method. Contribution of proteins and peptides to milk conductivity is negligible [17], thus addition of glucose to milk or cream could facilitate determination of other microorganisms that do not utilize lactose. Although *E. coli* is lactose positive, the possibility that *DT* could be reduced by glucose addition was examined. The concentration range of 0.10; 0.25; 0.50 and 1.00 % (w /v) of glucose was tested. However, neither addition of glucose nor glucose in milk or cream had any effect on the *DT*s of the M--values (data not shown).

The effect of the addition of Supplement S to Medium 001A was evaluated. Despite being designed for spore-forming bacteria, *DT* of more suitable M-values was reduced by 20 min on an average (fig. 3).

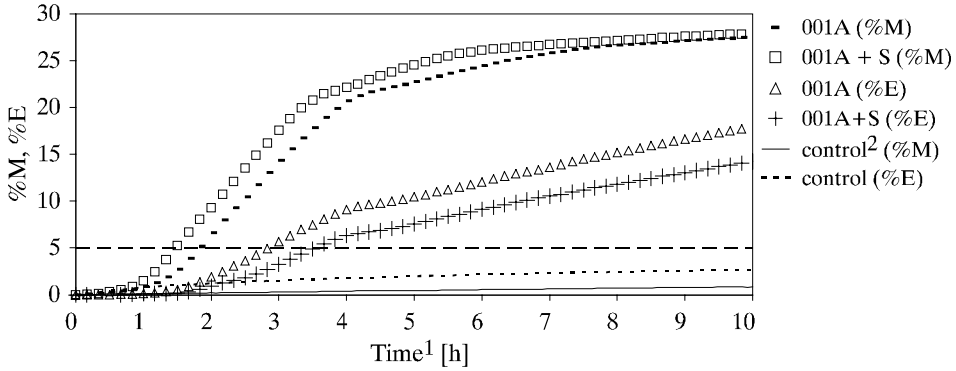

FIG. 3. Influence of the addition of Supplement S to Medium 001A on the growth of *E. coli* (inoculum 8.0×10^7 CFU.ml^{-1}).
OBR. 3. Vliv přídavku Suplementu S k Médiu 001A na růst *E. coli* (inokulum 8,0.10^7 JTK.ml^{-1}).
1 - čas [h], 2 - kontrola.

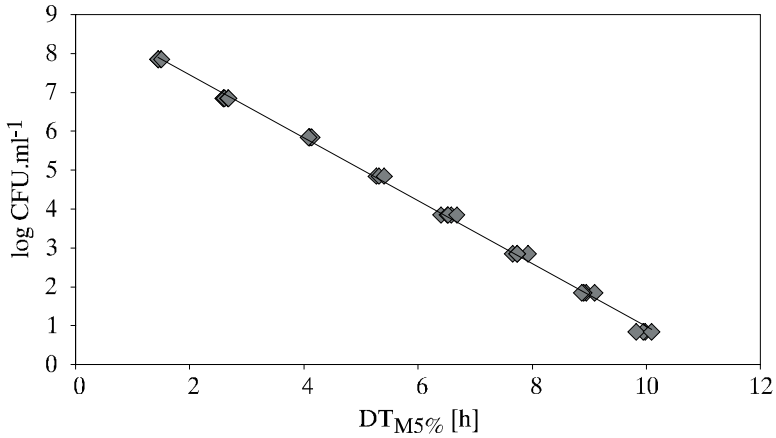

FIG. 4. Calibration curve for the determination of *E. coli* by the impedance method in milk.
OBR. 4. Kalibrační křivka pro stanovení *E. coli* impedanční metodou v mléce.

To study the relationship between log CFU and *DT*, the inoculum of *E. coli* was diluted in steps 1:9 in the given medium and at least three samples of each of them were cultivated in BacTrac 4100. The regression equations of the calibration curves (one example is given in the fig. 4) were calculated by the least square method. Coefficients of these equations as well as the residual standard deviations $s_{y,x}$ and generation times of *E. coli* in all media tested are shown in tab. 1. The validity of the regression relationships was proven roughly by calculation of the cell concentration from *DT*s determined in independent samples and their comparison to cell number determined by the plate count method. The results obtained by both methods were comparable (tab. 2).

The average values of *DT*s, given in tab. 2, were 13.15 h, 10.07 h and 12.05 h for cream, Medium 001A and Nutrient Broth, respectively. Confidence intervals of the estimated cell concentrations ($CFU.ml^{-1}$) were calculated (0.034–0.042, 0.158–0.184 and 0.084–0.297 for cream, Medium 001A and Nutrient Broth) and from the aspect of the uncertainty of measurement, the impedance method seemed to be slightly better than the plate method (assumed error 30 %; [18]) when the M-values were used (cream and Medium 001A); the confidence interval for E-values (used for Nutrient Broth) was wider.

The growth of *E. coli* was fastest in Medium 001A (generation time was shortest; tab. 2). However, despite being seemingly the most suitable medium for rapid *E. coli* detection, the necessary dilution step in the case

Tab. 2. Comparison of impedance method and plate count method.
Tab. 2. Porovnání impedanční metody s plotnovou metodou.

Medium[1]	Detection time[2] [h]	Impedance method[3]		Plate method[4]
		Concentration of *E. coli*[5]		
		log CFU.ml^{-1}	CFU.ml^{-1}	CFU.ml^{-1}
Cream[6]	13.0	–1.37	5.0×10^{-2}	2.0×10^{-2}
	13.3	–1.56	2.8×10^{-2}	2.0×10^{-2}
Medium 001A[7]	10.0	–0.74	1.8×10^{-1}	2.1×10^{-1}
	10.3	–0.65	2.2×10^{-1}	2.1×10^{-1}
	9.9	–0.94	1.5×10^{-1}	2.1×10^{-1}
	10.5	–1.16	7.0×10^{-2}	2.1×10^{-2}
Nutrient Broth[8]	12.0	–0.72	1.9×10^{-1}	1.1×10^{-1}
	12.1	–0.84	1.4×10^{-1}	1.1×10^{-1}

CFU - kolonie tvořící jednotku. 1 - médium, 2 - detekční čas, 3 - impedanční metoda, 4 - plotnová metoda, 5 - koncentrace *E. coli*, 6 - smetana, 7 - Médium 001A, 8 - Nutrient bujón.

of Medium 001A (1 ml of sample + 9 ml of Medium 001A) is to be considered. So there would be ten-fold lower initial bacteria level in Medium 001A in comparison to direct determination in milk and cream and thus the detection times would be comparable. In case of an illustrative sample, *DT* was by about 45 min lower for Medium 001A than for a direct determination in milk and cream (tab. 3). However, it is also important to take simpler sample preparation and lower material costs into account, when milk and cream are directly analyzed.

Tab. 3. Detection times recalculated from the calibration equations for the same degree of sample contamination.
Tab. 3. Detekční časy spočtené z kalibračních vztahů pro stejný stupeň kontaminace vzorku.

Medium[1]	Concentration of *E. coli*[2]			Detection time[5] [h]
	in sample[3]	in measuring tube[4]		
	CFU.ml^{-1}	CFU.ml^{-1}	log CFU.ml^{-1}	
Milk[6]	2.0×10^{2}	2.0×10^{2}	2.00	8.7
Cream[7]	2.0×10^{2}	2.0×10^{2}	2.00	8.8
Medium 001A[8]	2.0×10^{2}	2.0×10^{1}	1.00	8.0
Nutrient Broth[9]	2.0×10^{2}	2.0×10^{1}	1.00	9.8

CFU - kolonie tvořící jednotku. 1 - médium, 2 - koncentrace *E. coli*, 3 - ve vzorku, 4 - v měřicí nádobce, 5 - detekční čas, 6 - mléko, 7 - smetana, 8 - Médium 001A, 9 - Nutrient bujón.

Conclusion

Impedance method is suitable for rapid detection of *E. coli* recontamination of milk and cream. It is also possible to use this method for estimation of *E. coli* numbers in dairy products. The presence of these bacteria can be detected in up to 14 h even in very low numbers. Medium impedance was more suitable for milk, cream and Medium 001A, only electrode impedance was usable for the determination in Nutrient Broth. Low numbers of *E. coli*, which cannot be detected by the plate method without a cultivation step, can be detected by the impedance method.

List of symbols and abbrevitations - Seznam symbolů a zkratek

%M, %E - relative values of M-impedance and E-impedance
relativní hodnoty M-impedance a E-impedance

DT - detection time
detekční čas

$DT_{\%M}$, $DT_{\%E}$ - detection time of %M and %E
detekční čas %M a %E

E-impedance - electrode impedance
elektrodová impedance

GT - generation time
generační doba

M-impedance - impedance of medium
impedance média

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Rychlá detekce *Escherichia coli* v mléku a smetaně impedanční metodou

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SOUHRN. Práce je zaměřena na rychlost detekce rekontaminace mléka a smetany. Modelovým mikroorganismem byla *Escherichia coli*. Jako růstové medium bylo použito mléko, smetana, impedanční Médium 001A a Nutrient bujón. Pro stanovení v mléce, smetaně a Médiu 001A byla vhodnější impedance média (kratší detekční čas, nižší směrodatné odchylky stanovení), pro stanovení v Nutrient bujónu lze použít jedině elektrodovou impedanci. Glukosa přidaná k mléku a ke smetaně neovlivní růst *E. coli*. Supplement S pro sporulující bakterie v Médiu 001A urychlil stanovení v průměru o 20 min. Byly stanoveny lineární vztahy mezi log JTK.ml^{-1} (plotnová metoda) a detekčním časem pro každé médium. Reziduální stan-

dardní odchylky byly pro mléko $s_{y,x}$ = 0,1077, pro smetanu $s_{y,x}$ = 0.1016, pro Medium 001A $s_{y,x}$ = = 0.2783 a pro Nutrient bujón $s_{y,x}$ = 0.4144. Korelační koeficienty byly ve všech případech vyšší než 0,98. Zpětným výpočtem JTK.ml^{-1} z detekčního času byla ověřena platnost kalibračních vztahů. Rychlost detekce v impedančním Médiu 001A byla srovnatelná s přímým stanovením v mléce a smetaně.

KLÍČOVÁ SLOVA: impedance; *E. coli*; mléko; smetana