DISINFECTION ACTION OF SOME ESSENTIAL OILS ON STAINLESS STEEL

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ABSTRACT

Bacteria can attach to different surfaces and form biofilm. Biofilms can cause a big problem in food industry by contamination of food items and reduction of the effectiveness of machines. In the biofilm bacteria are less exposed to the different disinfectants, than the free living cells. Essential oils (EO) with known antimicrobial effect can also inhibit biofilm formation. In our experiments minimal inhibitory concentrations (MIC) and minimal bactericide concentrations (MBC) of the investigated EOs: cinnamon, juniper and lemon were determined by macro-dilution method on Pseudomonas putida and E.coli. Cinnamon showed the best antibacterial effect with MBC values of 2mg/ml for*E. coli* and 4mg/ml forP.putida. Thebactericidaleffect of EOsdependedontheactingtime. We established 80 minutes for*P. putida* and 120 (cinnamon EO) and 240 (juniper EO) min for*E. coli*. The disinfection potential of the EOs were studied on *P. putida* and *E. coli* 1 and 7 days old biofilms formed on industrial stainless steel surfaces. Each of the EO was effective. The number of *P. putida* cells was reduced up to 99% and we had similar result by 1 day old *E. coli* biofilm. The cell number of 7 days old *E. coli* biofilm was reduced by 62.5% with cinnamon EO and juniper EO reduced it by 87%.

Keywords: biofilm, essential oils, MIC/MBC, stainless steel

1. INTRODUCTION

Bacteria can attach to different surfaces and form biofilm, which can contaminate equipment surfaces and food. In the biofilm bacteria are more resistant to disinfection, than as a single cell (Kumar and Anand, 1998). Biofilm formation can cause a big industrial problem mainly in the food industry (Van Houdt and Michiels, 2010), where accumulation of pathogenic bacteria leads to a food safety concern. The efficiency of processes can also be reduced (reduced membrane permeability, corrosion) (Simoes et al., 2009).
Biofilms can be reduced by chemical substances (sodium hypochlorite), natural materials (surfactant) (Simoes et al., 2009) or physical methods (ionizing radiation). By the disinfection EPS (extracellular polymeric substances) have to be removed, because the EPS is a barrier and protects the cells in the matrix. Survival bacteria can build a new matrix and will give the resistance to the other microorganisms. The disinfection action is depending on the temperature, pH, humidity, acting time and resistance(Van Houdt and Michiels, 2010). The used antimicrobials need to be safe, non-toxic and easily to remove from the surfaces(Simoes et al., 2009).
Most of the essential oils (EO) together with other plant extracts are well-known antimicrobials (Burt, 2004). The mechanism of action is coagulation of the cytoplasm, reduction of the integration of cell wall and membrane, leading toloss of cell components and death of the cell (Bakkali et al., 2008).

The aim of our study was to investigatethe disinfection effect of selected essential oils on stainless steel surface.

2. MATERIALS and METHODS

Materials

The biofilm forming Gram negative bacteria:*Escerichia coli* and *Pseudomonas putida*growing on LB (in g/l: NaCl 10; casein peptone 10 and yeast extract 5) or on TGE (glucose 10, peptone 5, yeast extract 2.5 g/l) medium were used. *E.coli* was incubated for 18-20 h, at 37°Cand *P.putida* at 25°C.

Cinnamon, juniper and lemon essential oils were purchased from the Aromax Natural Products Zrt. (Budapest, Hungary).

Stainless steel coupons in the size of 2x2cm from food industryequipments were defatted with EtOH and sterilized at 121°Cfor 20min.

Methods

Determination of MIC/MBC values was done by macro dilution method using essential oils in the concentration range of 1-60 mg/ml. Bacterial suspensions (10^5cfu/ml) were mixed with different concentration of essential oil, and 1% Tween 40 was added to aid dispersion of the oil. After incubation for 24hturbidity in the tubes was examined and clear tubes were declared having the MIC concentration. MBC values were determined by spreading100 µl suspension from the clear tubes on Petri dishes. After 24h incubation colony number was counted andthe concentration where no colony was foundrepresented the MBC value. Establishment of disinfection time: the effect of essential oils in MBC on bacterial cell suspension (10^5cfu/ml)was checked after 20, 40, 60, 120 minutes.

Biofilm inhibition:bacteria were growing on sterile stainless steel coupons in culture medium (*P. putida* TGE broth, *E. coli* LB broth) at 24h and 168h. After incubation coupons were rinsed with distilled water to remove non-attached bacteria from the surface. Coupons were transferred to a disinfectant solution containing essential oils in MBC concentration. After disinfection time established in previous experiments biofilms on the coupons were scrapped off by sterile applicator swab which was transferred to a tube with sterile peptone water. After one hour cotton swabs were gentle pushed out and cfuwas determined by spreading 100 µl cell suspension on Petri dishes.

3. RESULTS and DISCUSSION

All the essential oils had good antibacterial effect (Table 1).In our experiments cinnamon EO had the lowest MIC and MBC values.

Table 1 MIC/MBC (mg/ml) values of the investigated essential oils

Essential oil	*P. putida*		*E. coli*	
	MIC	MBC	MIC	MBC
Cinnamon	2	4	1	2
Juniper	27	54	13,5	27
Lemon	27	54	>27	>54

Lemon EO was excluded from further investigations on *E. coli*, because of the very high MBC.

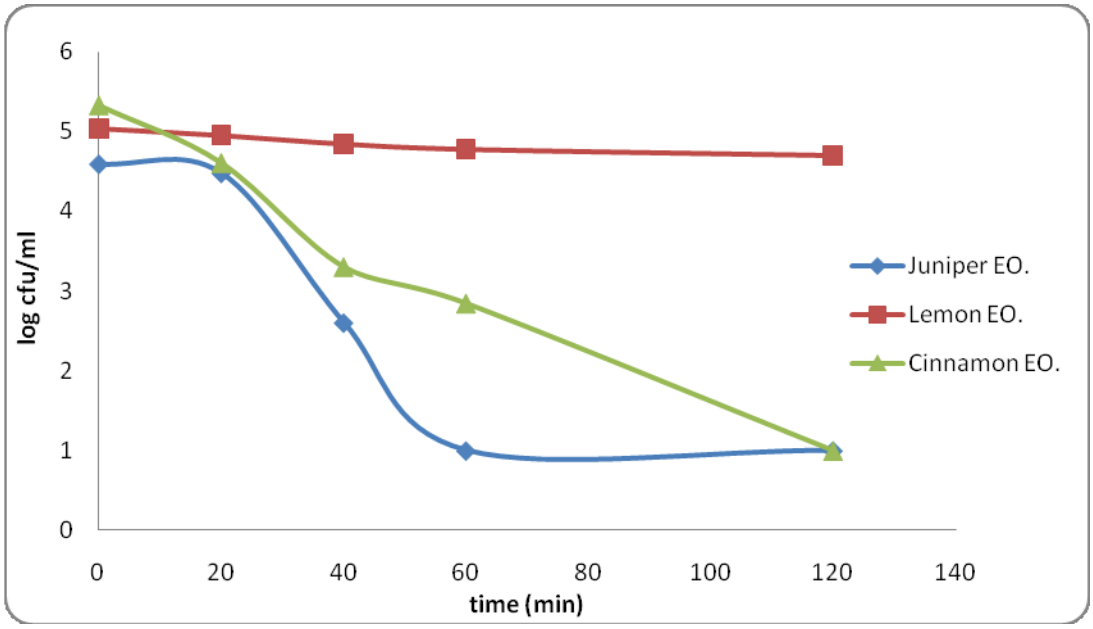

Figure 1 Time killing curves for *P. putida*

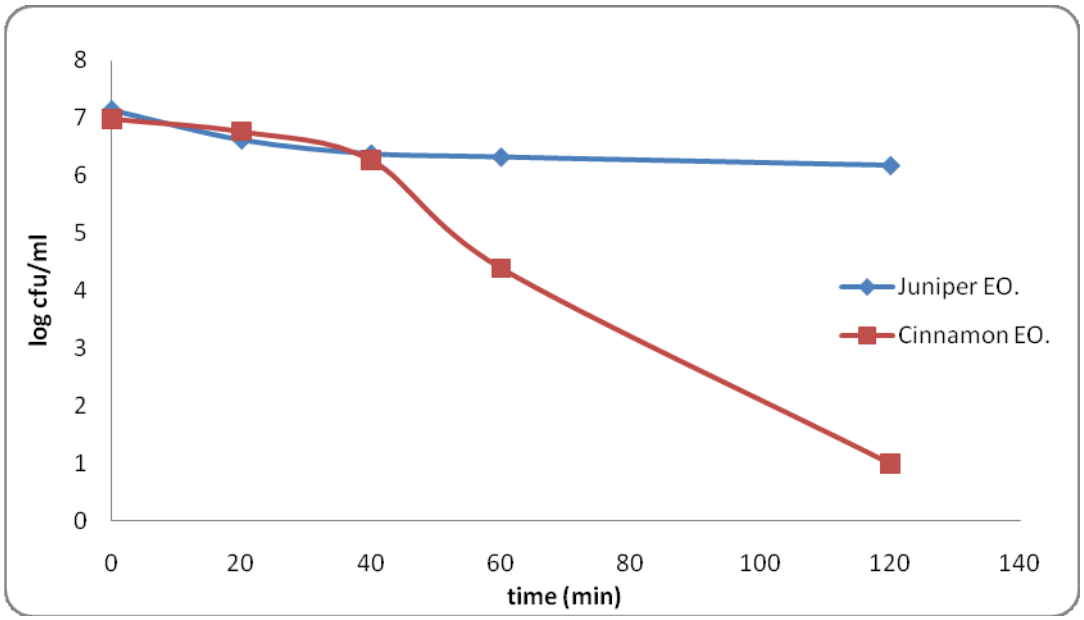

Figure 2 Time killing curves for*E. coli*

On the Figures 1 and 2 you can see the time killing curves for *P. putida* and *E.coli.* It can be seen that juniper and cinnamon EO had the best effect causing a fast linear degradation of the living cell number while lemon EO had only limited effect during the time of investigation on *P. putida.*

For the reduction of young and matured biofilms we used 80 minutes exposure timefor*P. putida* and 120 (cinnamon EO) and 240 (juniper EO) min for *E. coli* (Table 2).

Table 2 Reduction of living cell number of *P. putida* and *E. coli*biofilms [cfu/ml/cm^2]

Bacteria	Biofilm age	control	cinnamon EO	juniper EO	lemon EO
P. putida	1 day	8.8×10^3	7.5×10^1	<10	<10
	7 days	2.5×10^4	1.4×10^2	2×10^2	2.5×10^2
E. coli	1 day	2.6×10^3	<10	<10	-
	7 days	5×10^3	1.9×10^3	6.5×10^2	-

Table 2 shows the biofilm disrupting potential of essential oils.It can be seen that matured biofilmshad limited sensibility to the EOs.*P. putida* biofilm (24h and 168 h old) was reduced by 99% after 80 min disinfection time. We got smaller percent by 168h old *E. coli* (cinnamon EO 62.5%; 120min, juniper EO 87%; 240min treatment), but the younger biofilm had same reduction, than *P. putida*.

Our result was compared with the other study, where *Listeria monocytogenes* biofilm was reduced by lemongras EO. 3h old biofilm colony decreased by 40.28% after 15 min treatment, after 60 min this percent was 44.58, by 240h old biofilm this number was lower (26.59% 15min), but after 60min it was 72.51% (de Oliveira et al., 2010).

In the future we will extend our investigations on mixed culture biofilms and it is also planed to use essential oil mixes instead of single oil. In this way we can establish the interaction between different EOs or between EOS and other plant-derived components.

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