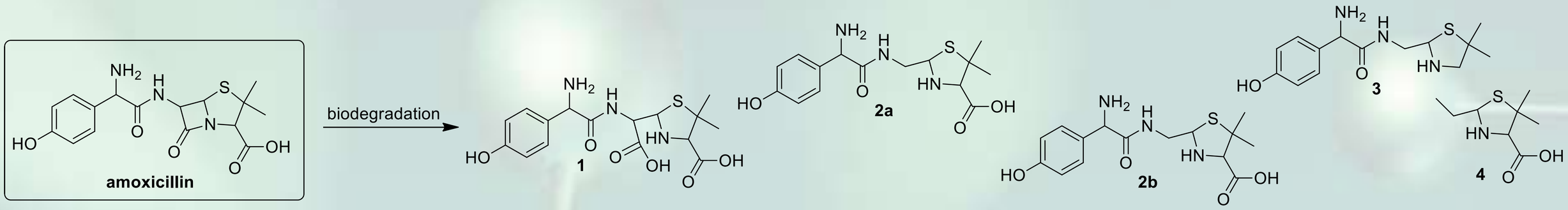
BIORAZGRADNJA AMOKSICILINA
BIODEGRADATION OF AMOXICILLIN

S. Kovačić[1], V. Lovrinčević[2], D. Vuk[2], M. Vuković Domanovac[3]

[1]Pliva Croatia Ltd., Prilaz baruna Filipovića 25, 10000 Zagreb, Croatia
[2]Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, 10000 Zagreb, Croatia
[3]Department of Industrial Ecology, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

The antibiotic residues in the environment is a huge problem nowadays, since they represent one of the main discoveries in a medicinal field. Therefore, the usage of antibiotics is increasing rapidly but it is getting out of control also. Sometimes unnecessary and improper consumption has increased the levels of residual antibiotics in ecosystems and some bacterial communities, which results with antibiotics losing their primary activity, bacterial function changing and finally bad effects on human health.

Environmental antibiotic contamination should be diminished by regulating the causes of occurrence and antibiotic discharge by detection and quantification of the antibiotics and the characterization of their behavior in the environment, which should all be summarized in risk assessments.[1] Meanwhile, the removal of the antibiotic residues from the nature is also very important. The accumulation with the highest concentration of the residues in the environment is in the places where the antibiotics are most commonly used and thus are the greatest source of pollution. Such places are livestock farms, hospitals and pharmaceutical factories. Antibiotics eventually end in the ground; soil and aquatic environment through the waste water, sludge and manure used in agriculture, and finally in the food we are eating.[2]



Scheme 1. Products of the amoxicillin biodegradation.

By this work we present a possibilities of the amoxicillin biodegradation (two different concentrations) in laboratory scale (Figure 1.) by adding microbiological cultures, *Pseudomonas* (Gram-negative) and *Bacillus* (Gram-positive) which are a type of bacteria that are commonly found in the environment, like in soil and water. The focus on amoxicillin took place since it is an antibiotic quite often proscribed in disease treatment nowadays and therefore is not a rare residual antibiotic found in environment.

Figure 1. Chromatogram of amoxicillin standard solution.

Figure 2. Chromatogram of amoxicillin biodegradation products.

Figure 3. Results in area % for amoxicillin and biodegradation products.

Biodegradation was monitored by UHPLC method with gradient elution and reversed phase column Gemini C18 (Figure 2. and 3.). Identification of degradation products was also done by LC-MS method, where the focus was on scanning the masses of the main peaks which grow and are changing the most through the sampling time.

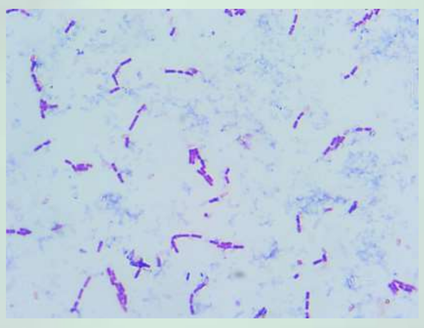
sample	days	Amox	1	2a/2b
PS-2 (A0,5)	0	96,94	2,49	0,31
	1	90,66	4,51	0,32
	3	91,27	5,26	0,50
	5	88,45	7,45	0,64
	7	65,51	17,32	0,82

sample	days	Amox	1	2a/2b
PS-1 (A0,2)	0	94,35	2,62	0,16
	1	93,00	3,41	0,55
	3	90,95	5,12	0,75
	5	50,55	17,22	1,17
	7	55,68	16,80	0,59

sample	days	Amox	1	2a/2b	2a/2b	3	4
BS-4 (A0,5)	0	80,99	14,00	0,73	0	0,33	0,14
	1	60,33	23,86	3,00	2,68	0,60	0,66
	3	56,64	18,48	5,95	5,29	0,96	1,94
	5	57,63	11,37	6,94	6,32	2,05	3,48
	7	51,98	9,91	7,36	6,44	2,40	3,89

sample	days	Amox	1	2a/2b	2a/2b	3	4
BS-1 (A0,2)	0	55,30	36,19	1,26	0	0	0,25
	1	6,30	64,04	7,50	0	1,19	1,51
	3	2,13	44,77	13,23	12,57	2,12	4,59
	5	0,28	43,16	19,79	15,49	3,92	3,91
	7	2,78	22,15	19,02	13,10	4,46	10,32

Table 1a-1d. Amoxicillin 0,2 mg/mL biodegradation with *Bacillus*.



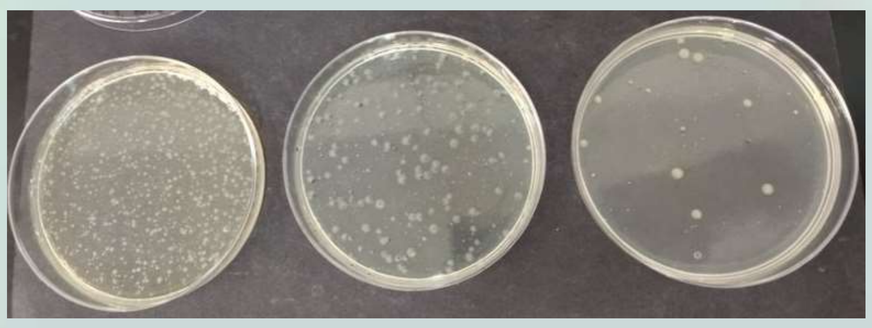


Figure 5. Results of CFU determination and sensitivity test for *Pseudomonas*.

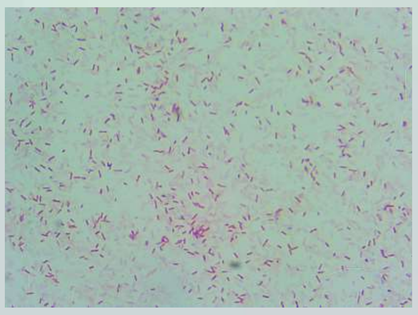


Figure 4. Photomicrographs of *Pseudomonas and Bacillus*.

Figure 6. Results of CFU determination and sensitivity test for *Bacillus*.

The results of the microbiological analysis show that the values obtained by the determination of colony-forming units (CFU) were maintained throughout the experiment and there was no significant decrease. The results of the sensitivity tests show that no new toxic products were formed. The bacterial cultures of *Pseudomonas* and *Bacillus* examined showed the biodegradation potential of amoxicillin.

REFERENCES:
[1] Svetlana Iuliana Polianciuc, Anca Elena Gurzău, Bela Kiss, Maria Georgia Ștefan, Felicia Loghin; Antibiotics in the environment: causes and consequences, Medicine and Pharmacy reports Vol.93, No.3, 2020: 231-240.
[2] Du L, Liu W. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agron Sustain Dev. 2012;32:309–327.

međunarodni znanstveno-stručni skup
19. Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
21. – 23. rujna 2022. | Vukovar, Hrvatska