FACULTY OF CIVIL
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POLISH ASSOCIATION
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„ENVIRONMENTAL ENGINEERING – THROUGH A YOUNG EYE”

VOLUME 19

ENVIRONMENTAL ENGINEERING SYSTEMS

Edited by
Iwona Skoczko
Janina Piekutin
Magdalena Horysz

Białystok 2015

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The analysis of factors influencing the choice of radiators

Key words: *radiator, heating system, heat transfer*

Abstract: Nowadays all new and modernized buildings are equipped in heating systems. The most popular are water installations, although in single family houses they are often assisted by air systems. Proper design and execution of heating system is very important. Choice of main elements of HVAC system is often difficult. This paper discusses the main factors that should be taken into account before making decisions about the type radiators. They should be characterized not only by high efficiency, compact design and small water capacity, but should also work well with elements of automatic regulation like thermostatic valves to adapt to changes in the demand of heat. The analyze included also economic parameters (price of radiator and its equipment).

1. Introduction

Recently consumers can find a great number of different kinds of heat emitters which differ in materials, styles, price, color and design. The main selection of radiators is connected with type of heat transfer. The units which more than 50% of heat emit by convection are [1, 2, 3]:

- Column radiators,
- Panel radiators,
- Natural convectors,
- Floor trench convectors,
- Forced convectors,
- Bathroom tube radiators.

Column radiators (Fig.1) were the most popular for years. Nowadays during modernization of heating system they are often replaced by other kinds of radiators. They are composed of the same elements, so it is possible to change their power by adding or elimination of some modules [2]. The material used to produce column radiators is cast iron, cast steel and aluminum [3]. They emit approximately 75% of heat by convection [1].

Figure 1. Column radiator

Source: Author's photo

Panel radiators (Fig.2) started to be the most popular kind of heaters few years ago. They are light, have low water capacity and are made in different configuration regarding to their length, high and number of panels. To achieve highest heat power and efficiency most of them is equipped in metal convection fins between panels.

Figure 2. Panel steel radiator

Source: Author's photo

Recently many new designs of radiators used in bathrooms or halls appeared for example towel rails (Fig.3). They are available in many colors and models, therefore every custumer can find a heater suitable for himself. The only problem in rooms with high value of heat losses is low efficiency of these units and in many cases another radiator is indispensable to satisfy the heat requirements of the bathroom [1, 3]. Most towel radiators is constructed from high grade, low carbon steel, which is both: durable and resistant to corrosion. Many factories offer radiators covered with a triple layer of chrome or a thick layer of white powder.

Figure 3. Bathroom towel rail

Source: Author's photo

Convectors are made in three types: natural or forced units and floor trenches. They are mostly more expensive than other types of radiators, but have high efficiency and small size. In natural units cold air circulate near the finned heating element from the floor to outlet in the top of radiator. According to [1] the high efficiency could be achieved when the temperature is above 82^{o}C. Forced convectors (Fig.4) have another construction which does not create a stack effect in them so in this case additional fan is necessary, what makes some noise in the room. The fan could be controlled by the room thermostat or switch on/off bottom in the unit. Whereas floor trench convectors are used in buildings where it is not possible to install normal radiators for instance car showrooms (Fig.5). They are located in floor ducts near windows or doors. The warm air which is going though the top grille could collect some parts of dust together so it is extremely important to keep the floor clean in this type of heating.

Figure 4. Convector

Source: Author's photo

Figure 5. Floor trench convector

Source: Author's photo

2. Description of the analyzed building and variants

The analysis was performed for a semi-detached building located in Białystok. The building consists of two apartments. In the basement there is a garage and boiler room on the first storey, there are halls, toilets, lounges, laundry rooms, kitchens, dining rooms and on the second storey there are bathrooms, bedrooms, hall. Each apartment has a separate central heating system, with a separate gas boiler. The heating system was design like a water with pump and a closed water circuit. Parameters of the system are 80/60^{o}C. To maintain proper indoor temperature the thermostatic valves in all rooms and a central regulator installed in the boiler room were selected. The heating medium is fed to the vertical lines on the floor distributors and horizontal carrier.

The heat losses were calculated according to standard PN-EN 12831:2006 [4] using computer program KAN OZC 4.01. The results are shown in table 1.

Table 1. Design heat losses in the building

Name of the room	Indoor temperature θ_{int} [°C]	Heat loss Φ [W]
Garage	5,0	-
WC	20,0	182
Hall	20,0	540
Living room	20,0	1408
Kitchen	20,0	558
Hall2	20,0	429
Bathroom	24,0	627
Bedroom 1	20,0	815
Bedroom2	20,0	511
Bedroom3	20,0	843

Source: Authors calculations

For all rooms different variants of radiators were selected: panel, column and convectors, whereas in bathrooms various heaters dedicated to such kind of areas were chosen [5-15].

3. Results and discusion

After selection of radiators their costs were estimated and technical parameters were compared.

3.1.Column radiators

In group of column radiators the thermal power achieved from 1 meter of unit was estimated for a range 1030-1437W/m (Fig.6). It is worthy to note that the radiator with the highest unitary power had the lowest cost (Fig.7) what is connected with the material (aluminum).

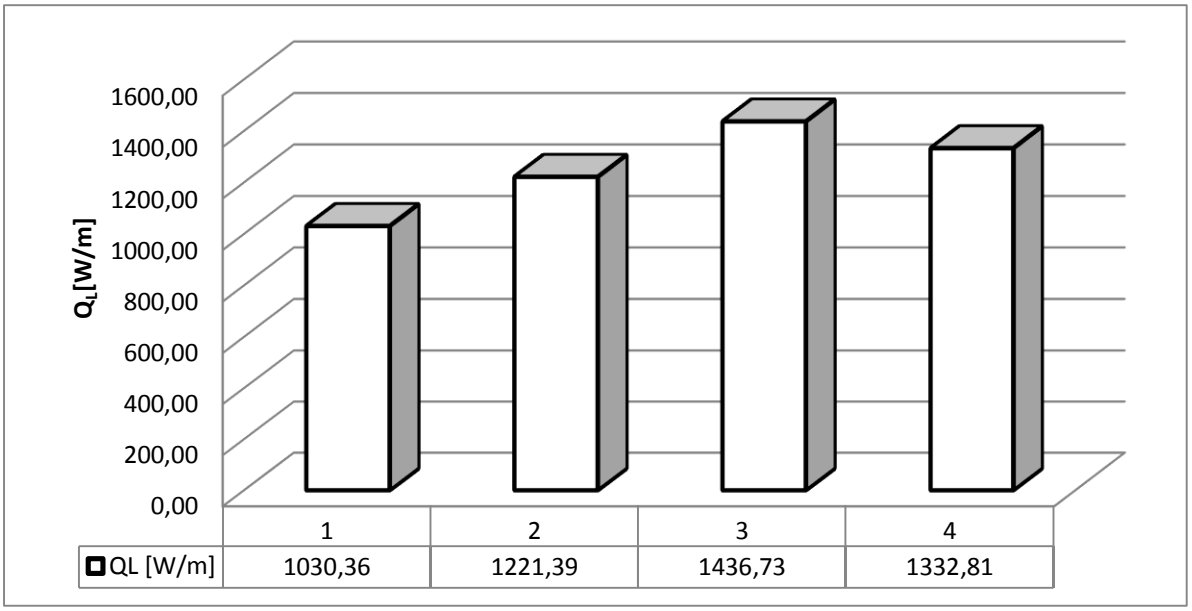


Figure 6. Thermal power obtained from the column radiator in nominal conditions from the unit length

Source: Authors' calculations

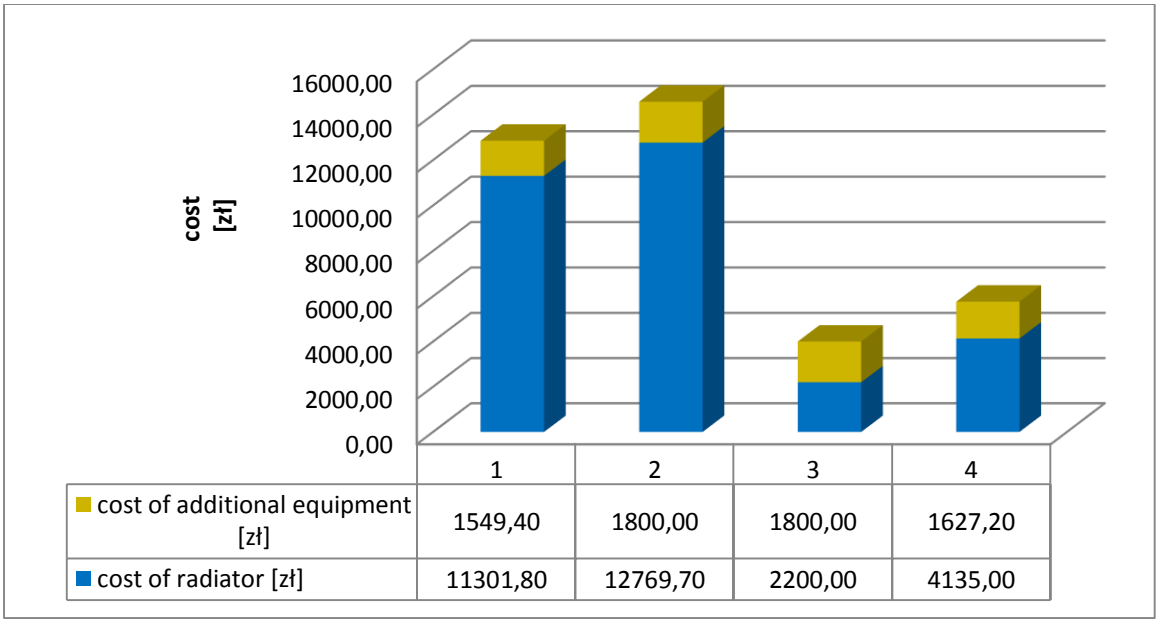


Figure 7. The total cost of column radiators with equipment

Source: Authors' calculations

3.2.Panel radiators

Four different kinds of panel radiators were analyzed and the comparison of their power possible to obtain from unit length was shown in fig.8. In this case the unitary power is less differentiated than in column radiator group. Moreover to show the difference in their cost for typical units Fig.9 was presented. The higher cost was estimated for radiators with smooth

plates which are more hygienic than the other ones. Most producers offer radiators in many colors, which are 20-33% more expensive than white units (Fig.10).

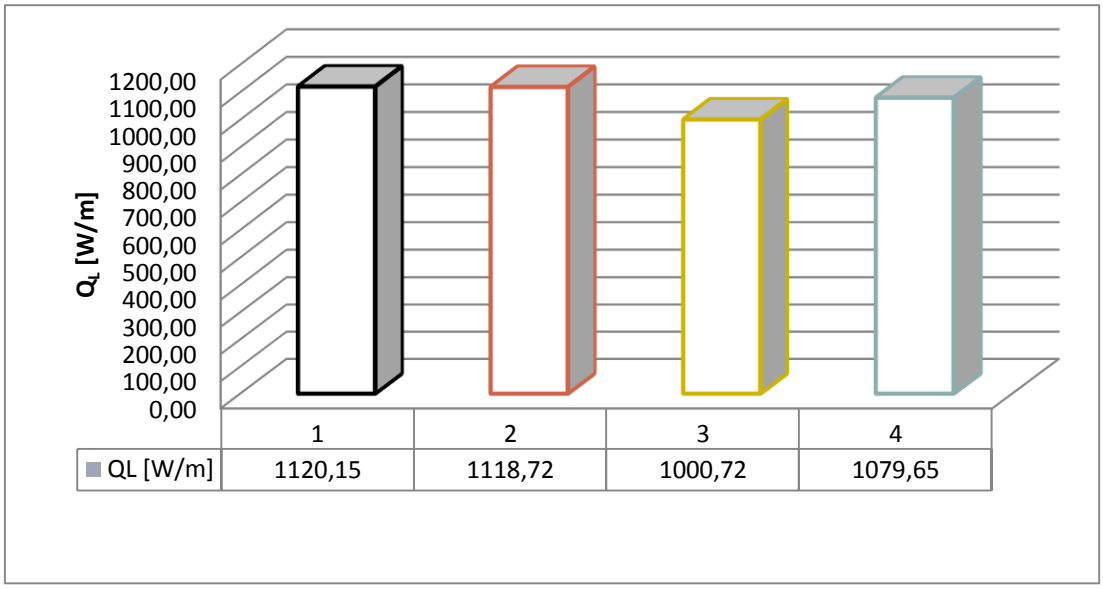


Figure 8. Thermal power obtained from the panel radiator in nominal conditions from the unit length

Source: Authors' calculations

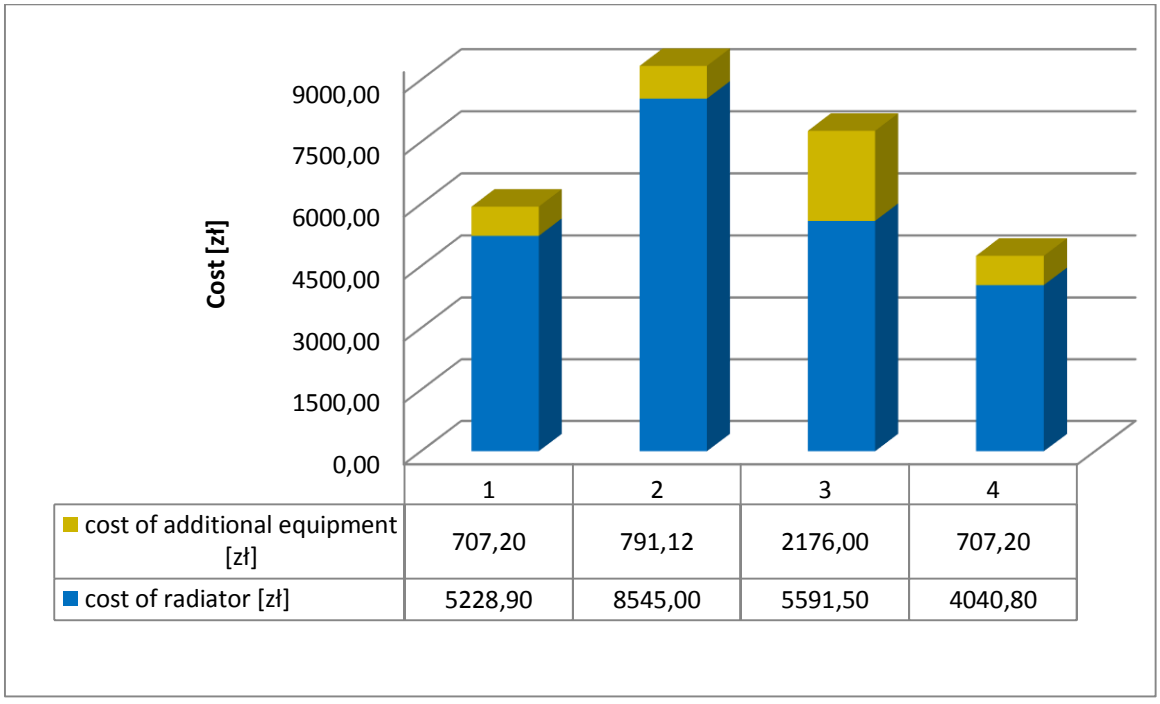


Figure 9. The total cost of panel radiators with equipment

Source: Authors' calculations

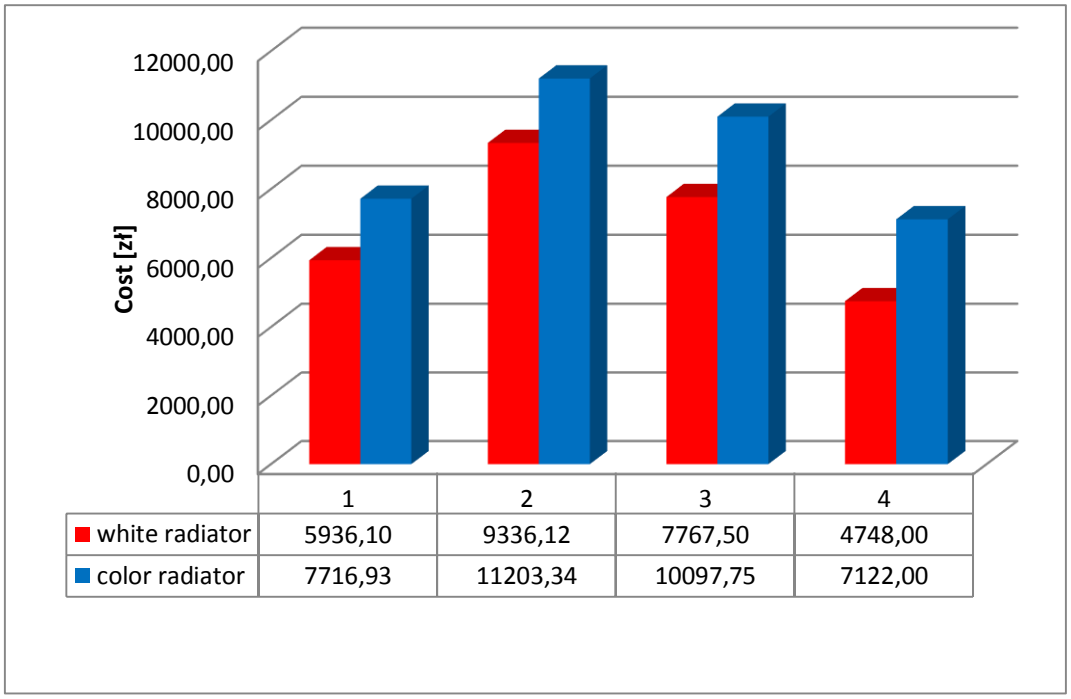


Figure 10. The total cost of panel radiators in comparison to color ones

Source: Authors' calculations

3.3.Convectors

In group of convectors 14% in unitary power difference between types was calculated (Fig.11).

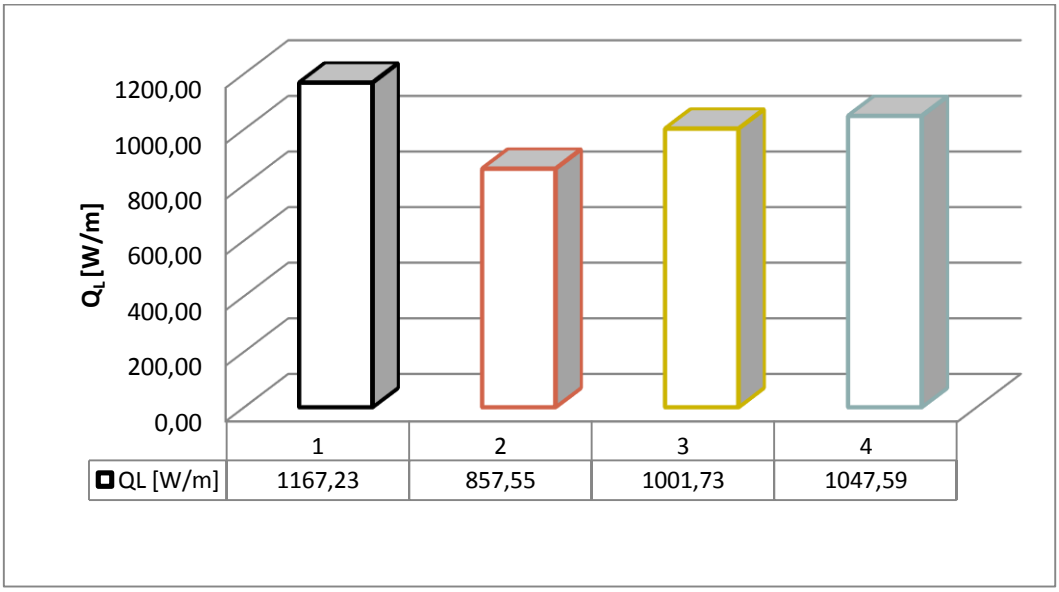
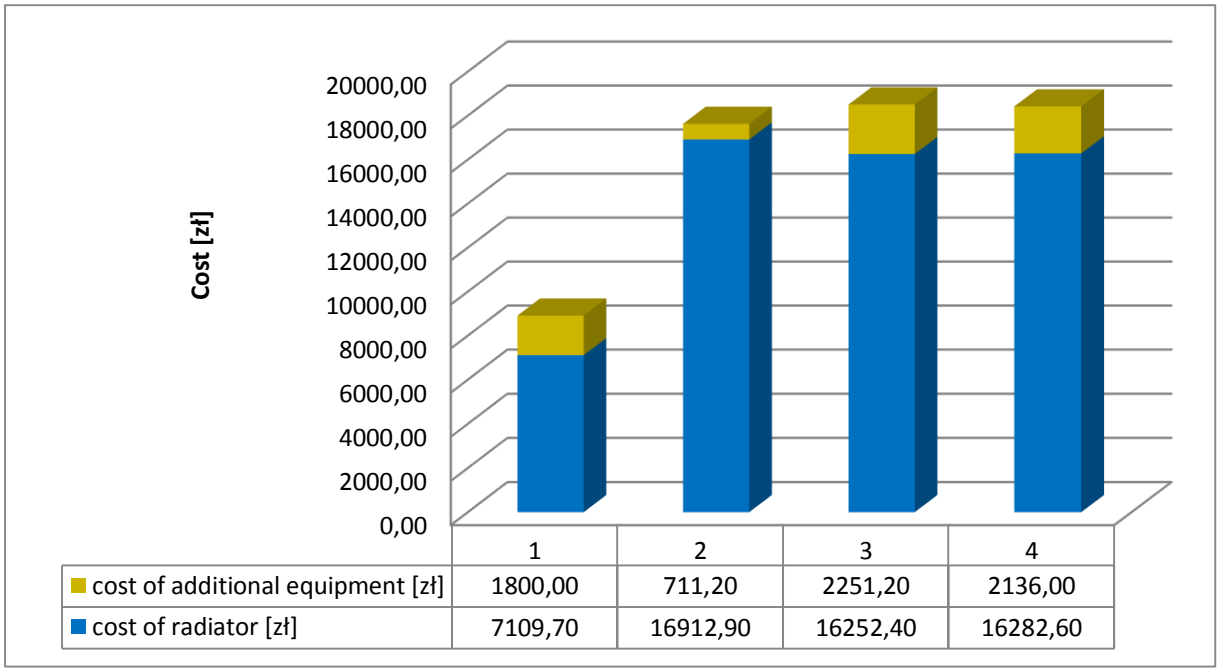


Figure 11. Thermal power obtained from the convector in nominal conditions from the unit length

Source: Authors' calculations

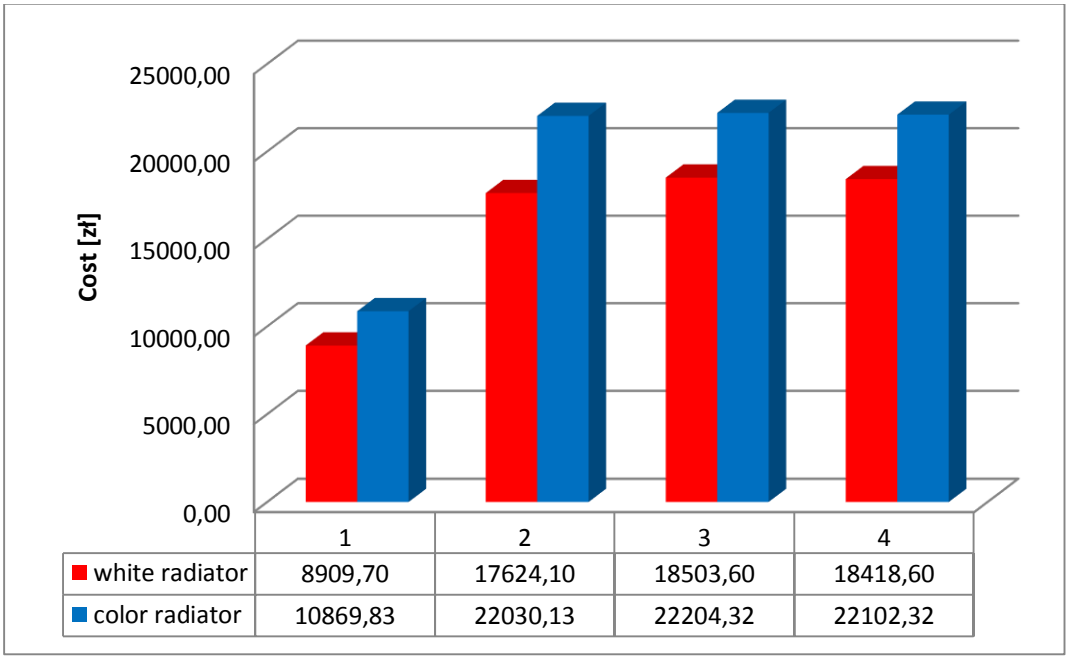
In a group of convectors units with Cu-Al heater are nearly 50% cheaper than radiators with steel heaters (Fig.13). When we compare white and color convectors (Fig.14) the differences in costs are similar for all units: 20-25%.



	1	2	3	4
cost of additional equipment [zł]	1800,00	711,20	2251,20	2136,00
cost of radiator [zł]	7109,70	16912,90	16252,40	16282,60

Figure 12. The total cost of convectors with equipment

Source: Authors' calculations



	1	2	3	4
white radiator	8909,70	17624,10	18503,60	18418,60
color radiator	10869,83	22030,13	22204,32	22102,32

Figure 13. The total cost of panel radiators in comparison to color ones

Source: Authors' calculations

3.4. Floor trench convectors

During the analysis three natural convectors and one additionally equipped with a ventilator were compared (No 3). The highest power from 1 meter was achieved for a radiator with ventilator while the other three had similar unity values (Fig.14). The cost in case of radiator

no. 3 was also highest then for the others (Fig.15).

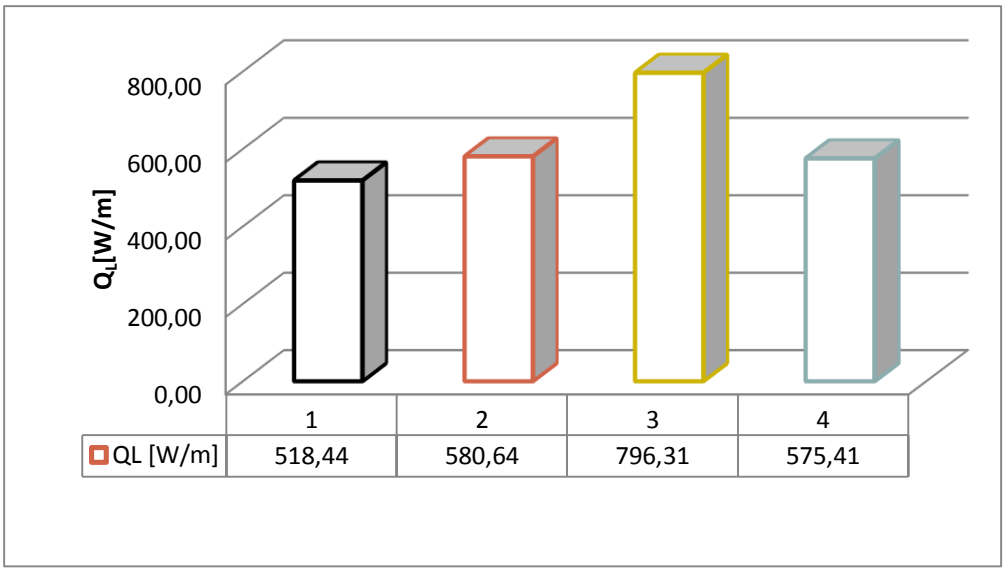


Figure 14. Thermal power obtained from theunderfloor convectors in nominal conditions from the unit length

Source: Authors' calculations

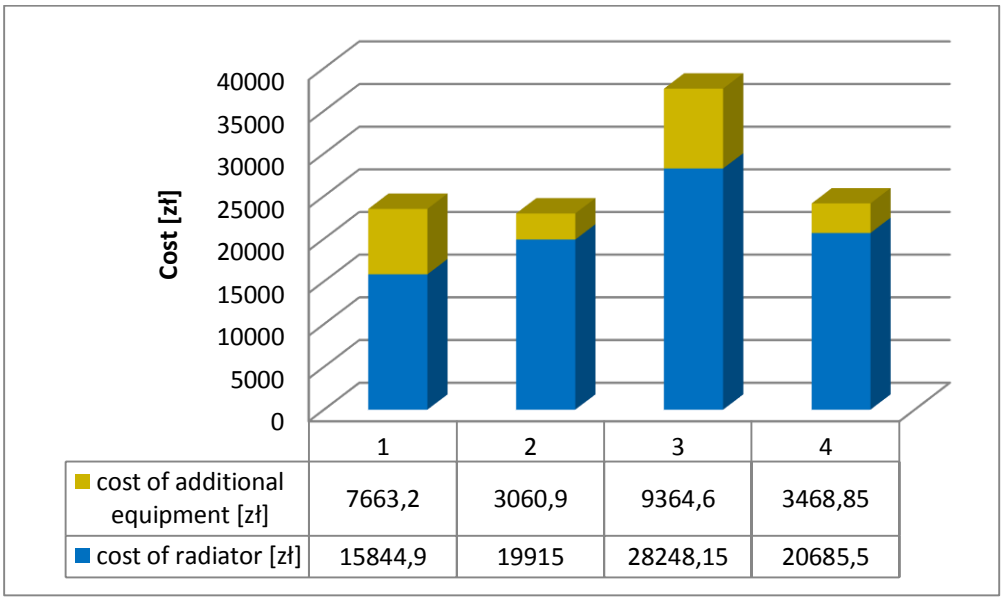


Figure 15. The total cost of floor trench convectors with equipment

Source: Authors' calculations

3.5.Bathroom radiators

Two groups of bathroom radiators were analyzed: for toilets (Fig.16-18) and bathrooms (Fig.19-21). The difference in unity length power in case of radiators in toilets (Fig.16) was estimated for 36%, whereas in bathrooms 27% (Fig.19). Total cost of typical radiators differed significantly. The most expensive units cost even 8 times more than the cheapest ones, what

was the result of their material, design etc. In case of electric heater installation cost rises about 13-35% in comparison with typical radiator.

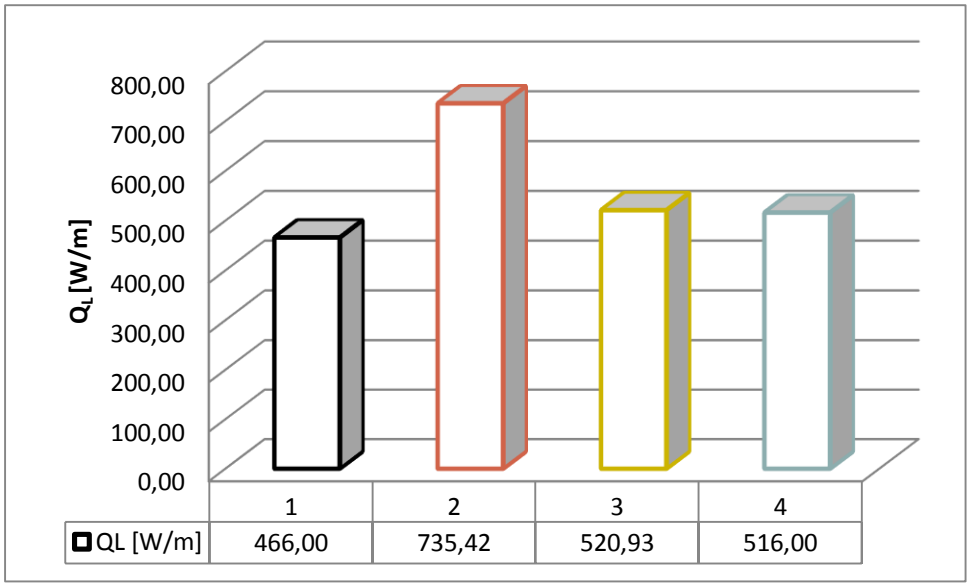


Figure 16. Thermal power obtained from thebathroom radiators in nominal conditions from the unit length

Source: Authors' calculations

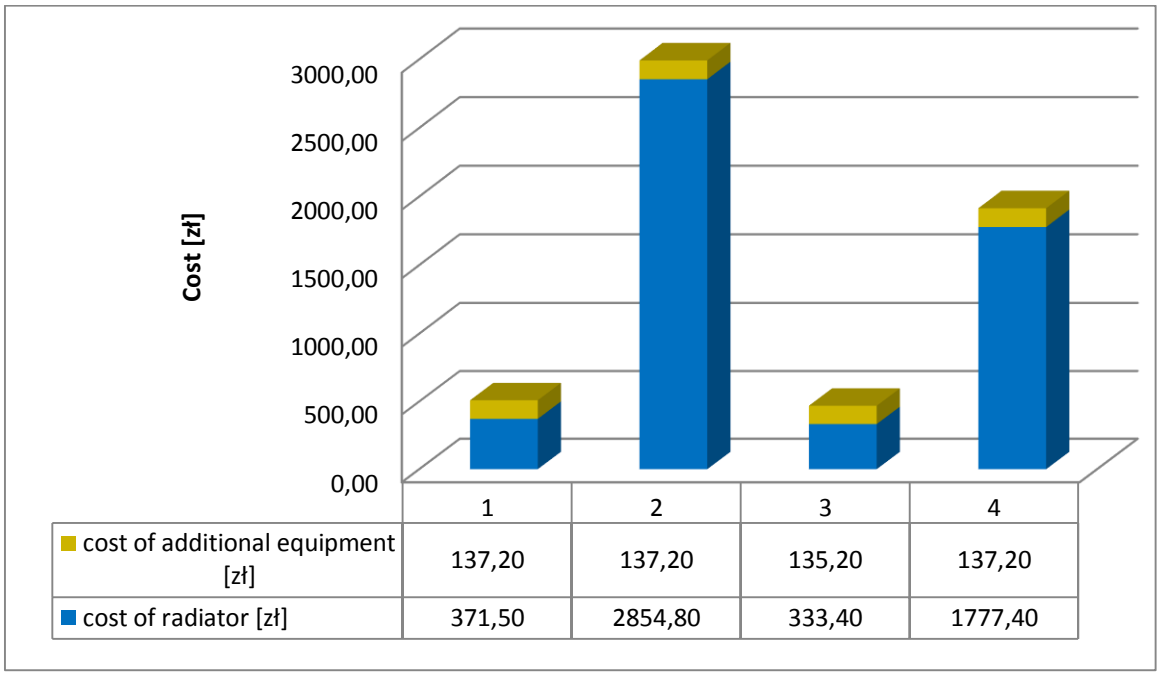


Figure 17. The total cost of bathroom radiators with equipment

Source: Authors' calculations

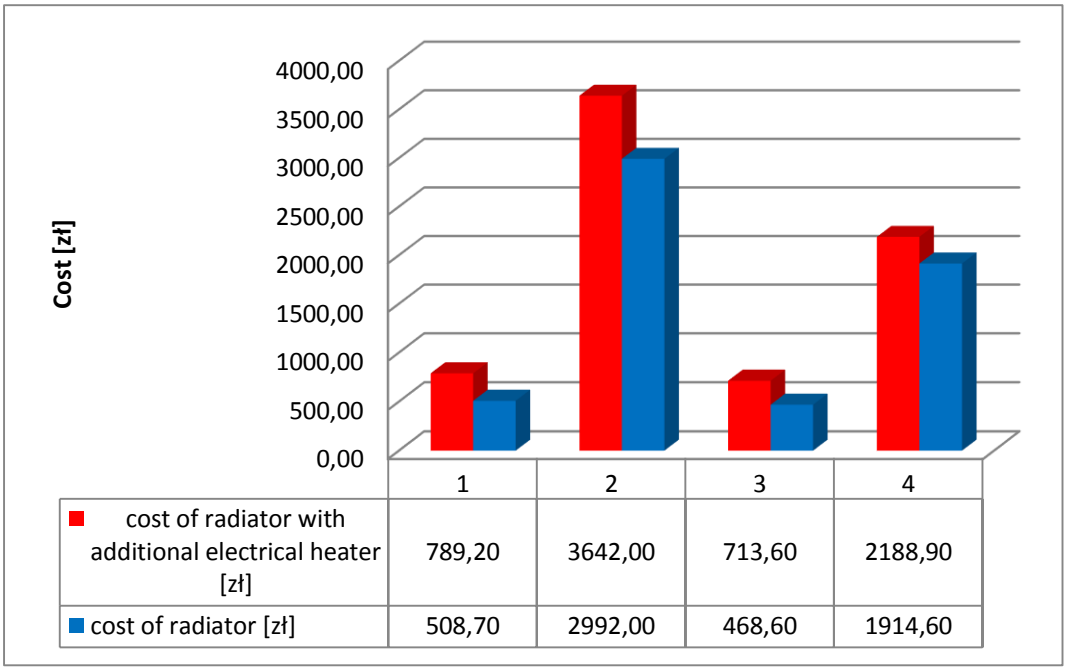


Figure 18. The comparison of radiators with and without electric heater

Source: Authors' calculations

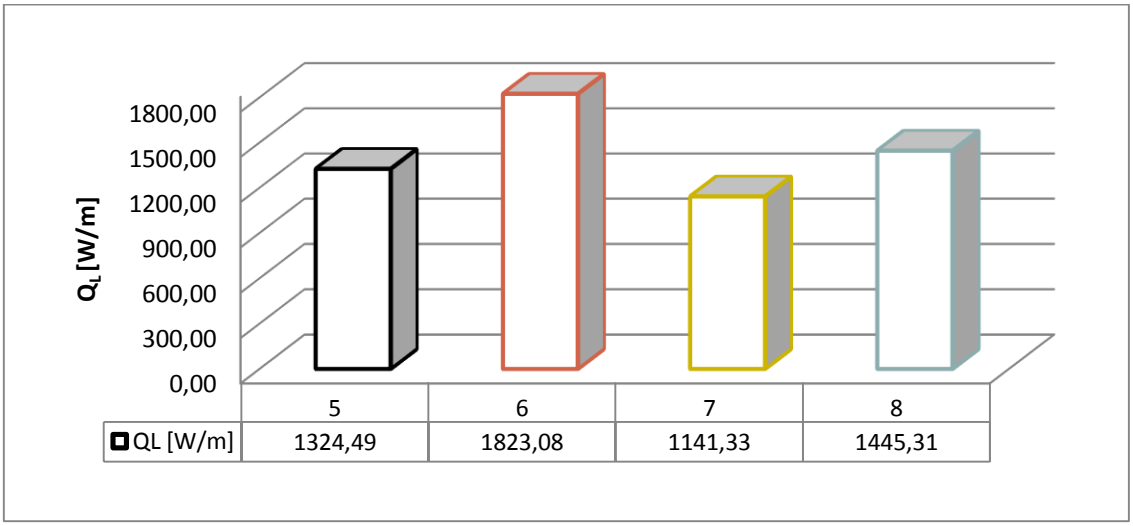


Figure 19. Thermal power obtained from the bathroom radiators in nominal conditions from the unit length

Source: Authors' calculations

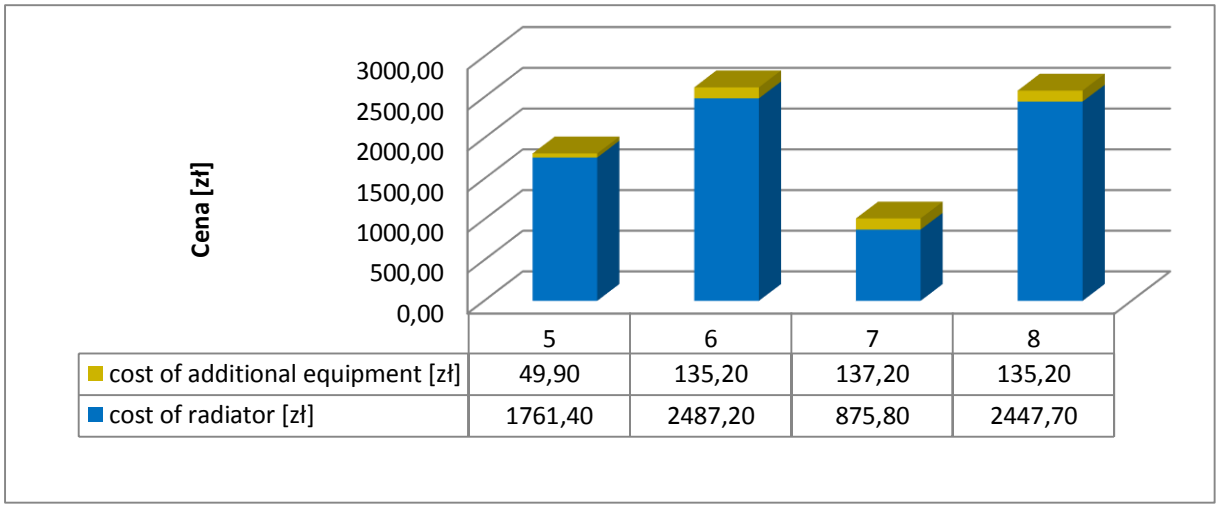


Figure 20. The total cost of bathroom radiators with equipment

Source: Authors' calculations

3.6.Comparative analysis of all types of radiators

Moreover the power achieved from 1 kg of radiator was analyzed (Fig.21). The highest value was achieved for aluminum column heater, whereas the lowest for steel column ones. On the other hand, this type of radiators, has twice bigger power of one meter length in comparison with underfloor convectors, and about 18-26% higher than normal convectors, panel or steel column radiators. (Fig.22). The analyze also contained the comparison of thermal power obtained in the nominal conditions from the unit water capacity of radiator (Fig. 23). The best value in this comparison to the others was achieved for underfloor convectors, twice value less was achieved for aluminum column radiators, almost four for panel radiators.

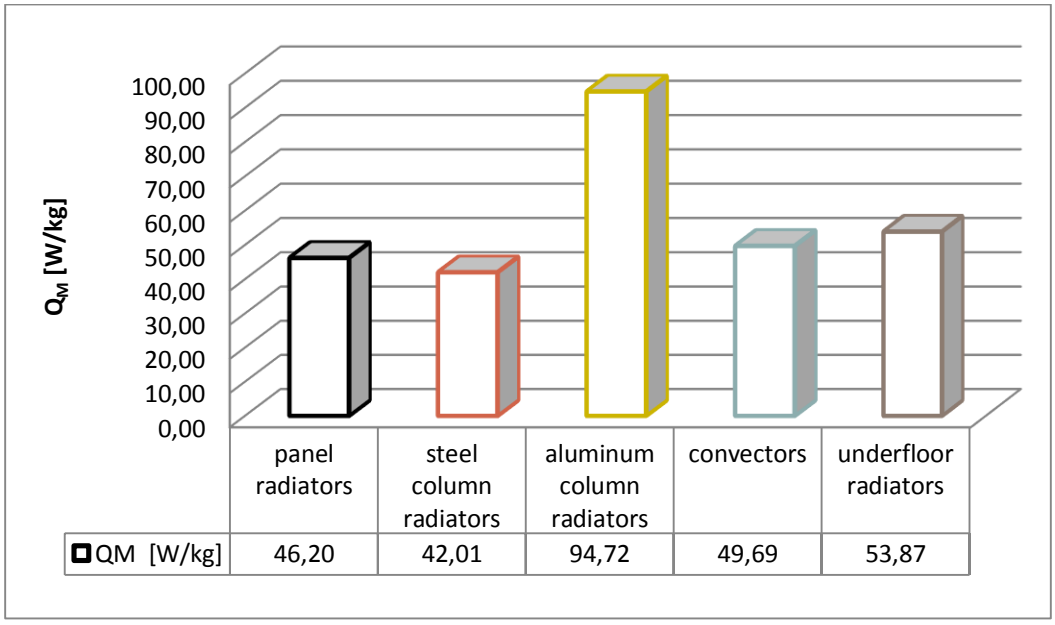


Figure 21. The comparison of thermal power from 1 kg of radiator

Source: Authors' calculations

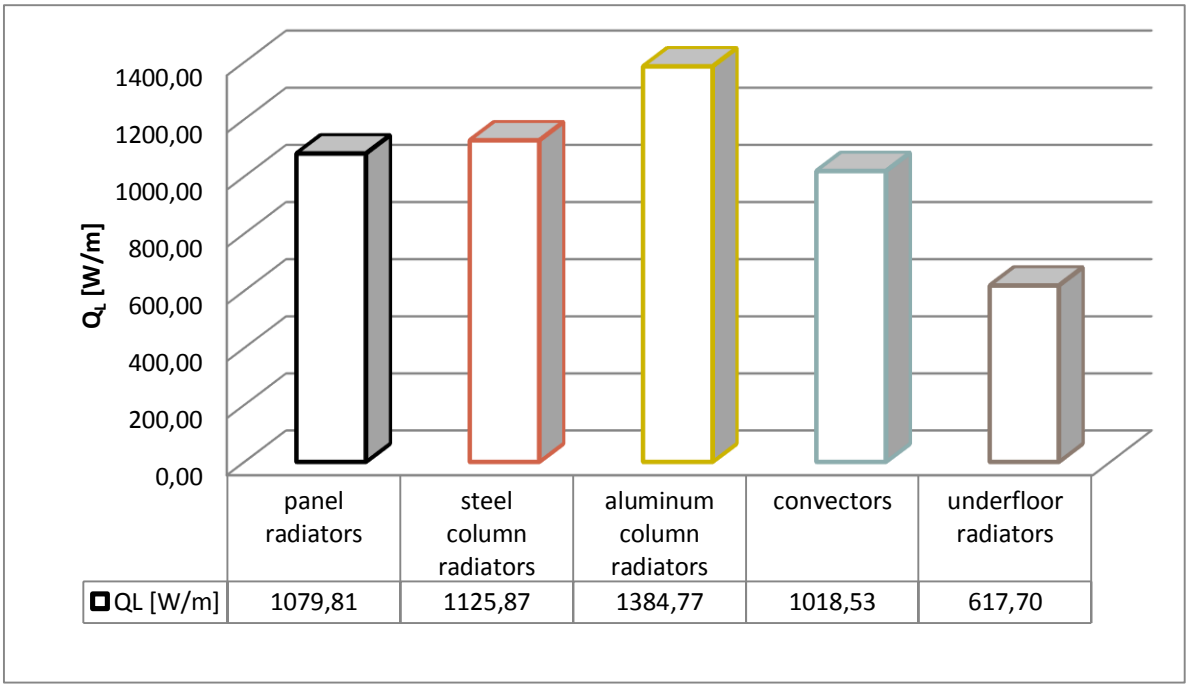


Figure 22. The comparison of thermal power from 1 m of radiator

Source: Authors' calculations

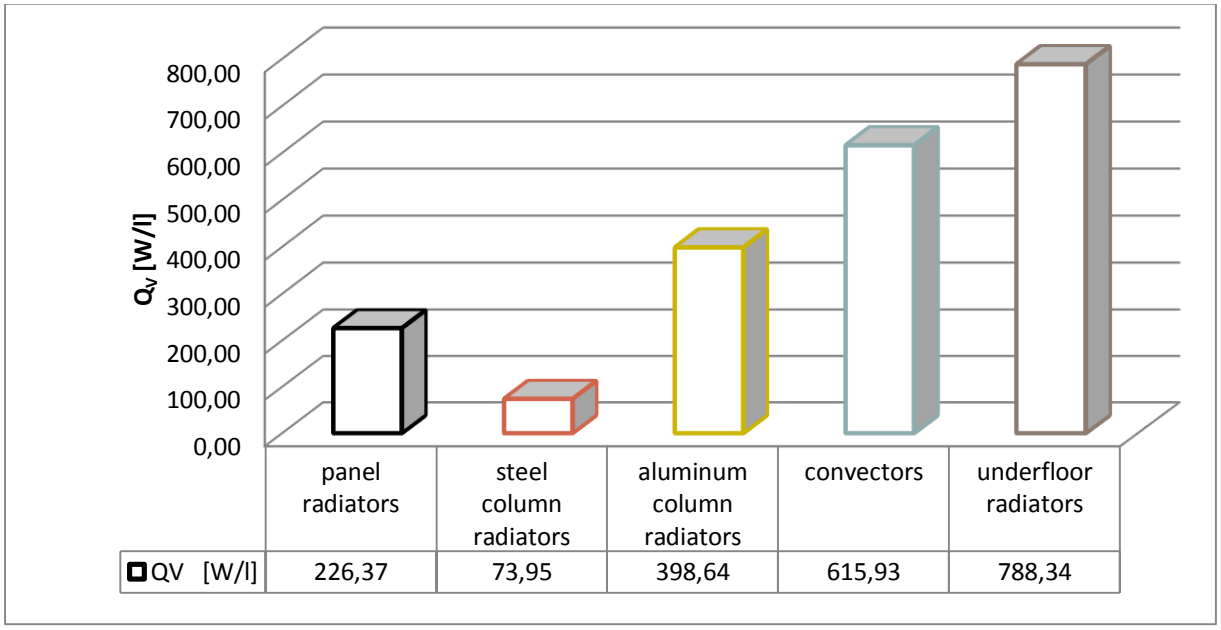


Figure 23. The comparison of thermal power from 1 l of water in the radiator

Source: Authors' calculations

The most important factor for the investors in the selection of heaters is their cost. When we compare all types the most expensive are underfloor and normal convectors, whereas the cheapest ones – aluminum column radiators (Fig.24).

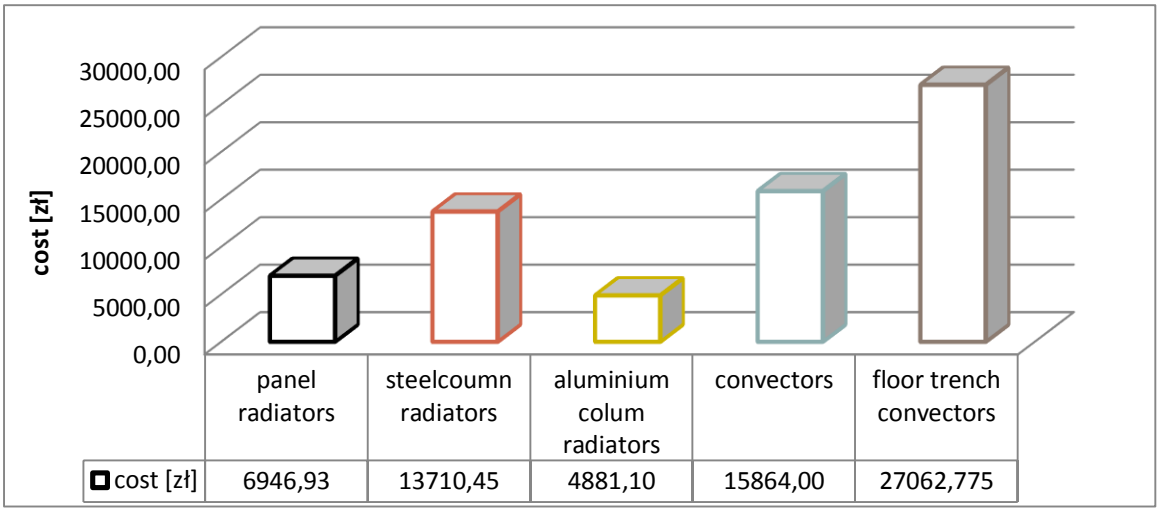


Figure 24. The comparison of costs for all types of radiators

Source: Authors' calculations

4. Conclusion

This paper presents results of technical and economical comparison of different kinds of radiators for a semi-detached house which could be generalized and used for other kinds of buildings. The economical analyze showed a huge diversification of radiators' prices, even when the same type of heaters was considered. The cheapest were column radiators made of aluminum and steel panel radiators, while the most expensive proved to be floor trench and normal natural convectors. Many factors influence the total cost of heaters: material, type of radiator, color, additional equipment (thermostatic valves, electrical heater, mirrors or towel pegs etc). Moreover radiators differ in place they need to be installed and give required heat value: the best are convectors with their high unit power, whereas the lowest power area is achieved from unit area of the bathroom tube radiators. During technical analyze more parameters like maximum operative temperature, weight, chemical and physical resistance, water capacity must be considered. For most people design and clean-ability are also important. Summarizing the choice of radiators depends on many technical factors, individual preferences and economical possibilities.

References

1. Watkins A.E.: Heating Services in Buildings. Wiley-Blackwell,Chichester 2011.
2. Nantka Marian (2006), Ogrzewnictwo i Ciepłownictwo tom 2, Wydawnictwo Politechniki Śląskiej, Gliwice.
3. Pieńkowski K., Krawczyk D., Tumel W.: Ogrzewnictwo tom 1, Wydawnictwo Politechniki Białostockiej, Białystok 1999.

4. PN-EN 12831:2006.
5. Katalog techniczny Instal Projekt, Edycja I/2013.
6. Katalog techniczny; grzejniki kanałowe, PURMO Polska 2012.
7. Katalog techniczny; grzejniki elektryczne, PURMO Polska 2013.
8. Katalog techniczny; Honeywell.
9. Katalog techniczny Brugmann 2013.
10. Katalog techniczny Vogel&noot, 2013.
11. Katalog techniczny Zehnder, 2013.
12. Katalog techniczny Jaga, 2013.
13. Katalog techniczny Fondital, 2013.
14. Katalog techniczny Alukal, 2013.
15. Katalog techniczne Radson, 2013.

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Zeolite mass activation with MnO_2

Keywords: *Zeolite, Aluminosilicates, Iron, Manganese.*

Abstract: The subject undertaken thesis was mainly concerned the removal of iron and manganese from the water sample. For this purpose there prepared a fillers for filters from two types of zeolite : fine-grained (with grain size from 0.5 to 1.0 mm) and coarse (with grain size from 4.0 to 8.0 mm. At first, zeolite was covered by manganese. After that, all of analyzed samples was left for three, fife, seven and nine weeks (it was two types of zeolite for each one of the weeks). Next, all of analyses probes were dried and activated. Zeolite mass activation was consisted from using several methods. Research were carried out to select the best from all methods of activation of the zeolite for the removal of impurities. Each method of activation has proved to be effective, but the best method was with iron sulfate, sodium hydroxide and potassium permanganate.

1. Introduction

The development of civilization results increasing environmental pollution, including surface water and groundwater. Anthropogenic pollution which are getting into the natural waters are: surface active substances phenols, alcohols, components of pesticides and fertilizers, petroleum compounds, aromatic hydrocarbons, chlorinated organic compounds and heavy metals. Among the sources of contamination of natural waters, the most important are: household sewage, surface runoff from agricultural land, runoff from roads and rain.

Natural waters are characterized by variable chemical composition, which is determined by a number of factors, as geological conditions (construction of the substrate and topography, the course of the erosion of the substrate), weathering processes of physical, chemical and biological agents in the water environment, contamination of other components of the environment and the use the same waters and catchment. One of the most important from the standpoint of environmental pollution are anthropogenic heavy metals. Frequently emitted to the environment are: lead, cadmium, copper, iron, nickel, zinc, mercury and arsenic. Number of metal circulating in the trophic chain is increasing, and they are toxic to all organisms [6].

Heavy metals and radioactive elements are present in natural surface waters and groundwater. The form in which they appear in the waters is a function of their

concentration, dissolved oxygen, pH, carbonate hardness and the presence of organic substances. The natural source of metal is mainly hydrolytic phenomena mineral dissolution and leaching of sedimentary rocks. The dominant source of heavy metals in natural waters are, however, anthropogenic pollution, especially industrial wastewater. The largest share are here: metallurgical industry, mining, electrical and chemical industries.

Removal of metals from industrial wastewater is an important way to prevent contamination the whole environment with heavy metals. This is due to the fact that the environment followed by migration of water to the soil, and further uptake by plants, accumulation in the tissues and finally introducing the trophic chain.

Drinking water is the main source of life on Earth. All of us should share responsibility to ensure a healthy, secure and sustainable water supply for our communities, economy and environment, because our quality of life depends mainly on it.

Fresh water is one of our most vital resources, and when our water is polluted it is not only devastating to the environment, but also to human health. Most water comes from rivers, lakes and other surface water sources. Before it is delivered to our homes it is treated to remove chemicals, particulates (e.g., soot and silt) and bacteria. This clean, potable water is then used for cooking, drinking, cleaning, bathing, watering our lawns and so forth [14].

The quality of drinking water is linked to the quality of our lives. By supporting measures that improve our water and wastewater treatment systems, we can all contribute ensuring clean, safe water for ourselves and our communities.

Filtration is commonly the mechanical or physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium through which only the fluid can pass. The fluid that passes through is called the filtrate [16]. Water filters are used for mechanical filtration for a couple of very important reasons. This technology helps remove harmful contaminants, but retains beneficial minerals including calcium and potassium, and other.

1.1. Characteristic of the main types of filters

Filtration is the process of separating suspended solid matter from a liquid, by causing the latter to pass through the pores of some substance, called a filter. The liquid which has passed through the filter is called the filtrate. The filter may be paper, cloth, cotton-wool, asbestos, slag- or glass-wool, unglazed earthenware, sand, zeolite or other porous material [17]. For filtration of drinking water we use many types of filters. The following

can be mention: mechanical filters, reverse osmosis filters, filters with activated carbon, softening filters, iron removers, manganese removers and other.

Mechanical filters. Mechanical filters physically traps particles of debris in water. It is the first stage of the filtration process, and should always be placed so that water coming from the tank hits this media first. Keep in mind that most mechanical media also promote the colonization of beneficial bacteria [13].

Figure 1.1. Mechanical filters

Source: http://www.kreodom.pl

Reverse osmosis filters. Reverse osmosis (RO) is the most economical method of removing 90% to 99% of all contaminants. The pore structure of RO membranes is much tighter than UF membranes. RO membranes are capable of rejecting practically all particles, bacteria and organics >300 D molecular weight. In fact, reverse osmosis technology is used by most leading water bottling plants. Osmotic pressure drives water through the membrane; the water dilutes the more concentrated solution; and the end result is an equilibrium [12].

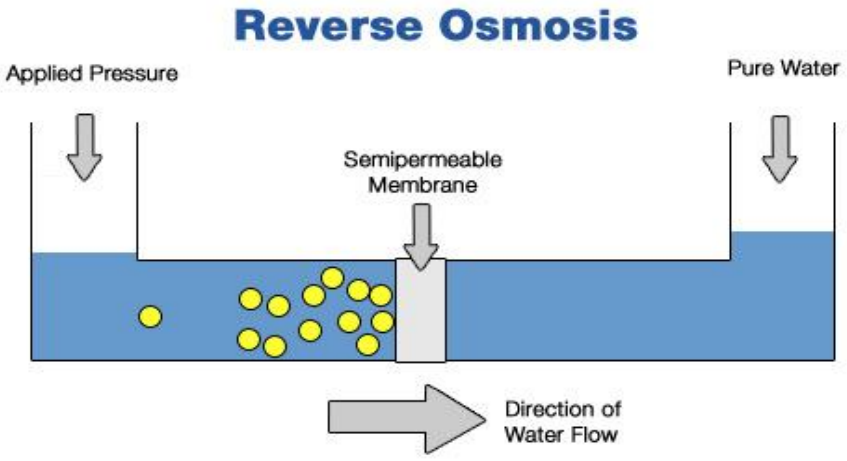

Figure 1.2. Reverse osmosis

Source: http://bande.co.za/what-is-reverse-osmosis/

Filters with activated carbon. Filters of this type are also called activated coal, because they are produced from carbon process. This type of filters has got low-volume pores that increase the surface area available for adsorption or chemical reactions [11]. Activated carbon is used in metal extraction, water, gold and gas purification, decaffeination, air filters, medicine and sewage treatment. Activated carbon is produced from carbonaceous sources materials by means of the following processes: physical reactivation and chemical activation.

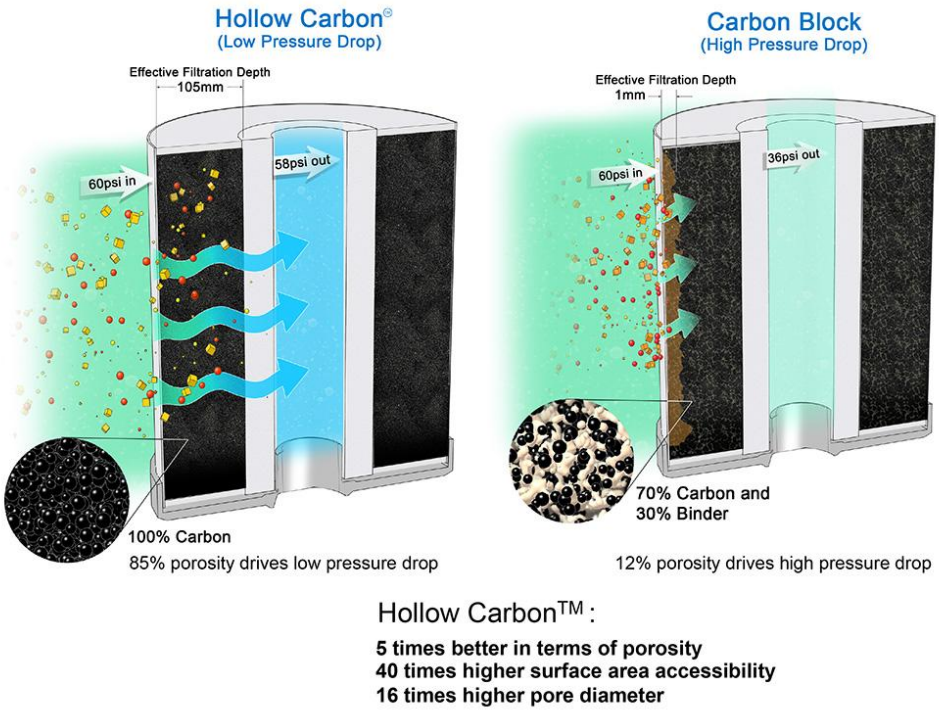

Figure 1.3. Filters with activated carbon

Source: http://www.selectoinc.com/our-technology/hollow-carbon-technology/

Softening filters. Water Softener Cartridges are used for removal of minerals through an ion exchange process, to prevent hard water scaling and staining, and also to prolong the life of RO membranes if used for pretreatment to an RO System [12]. This type of filters reduce the hardness of water, which is the reason for the formation of calcium deposits in pipes and plumbing on the mechanisms of dishwashers. Water softening consists of changing calcium and magnesium ions, which cause hardness in water, for into sodium ions.

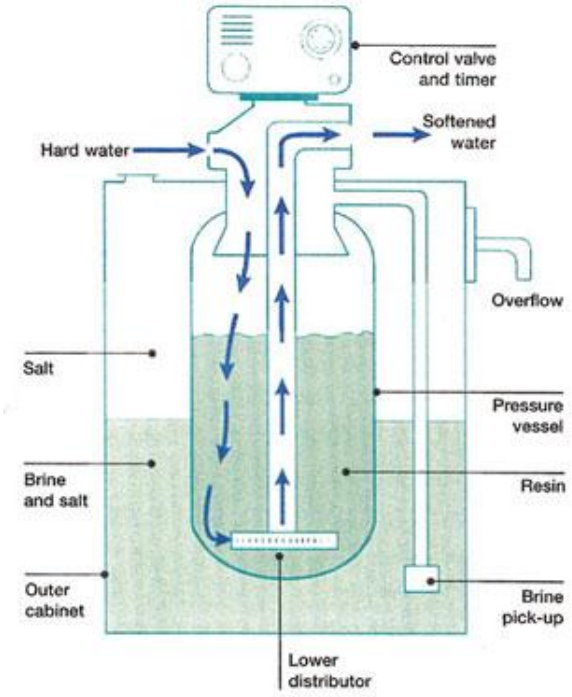

Figure 1.4. Softening filter

Source: http://woodchurchscience.edublogs.org/category/9w/

Iron removers. Corrosive water will pick up iron from pipes. We can find very small amounts of iron in water due to a large amount of iron in the soil. When water containing colourless, dissolved iron is allowed to stand in a cooking container or comes in contact with a sink or bathtub, the iron combines with oxygen from the air to form reddish-brown particles (commonly called rust). Manganese forms brownish-black particles. These impurities can give a metallic taste to water or to food. Over time, iron deposits can build up in pressure tanks, water heaters, and pipelines, reducing the quantity and pressure of the water supply [10]. Types of iron: Ferrous (Fe^{2+}), Ferric (Fe^{3+}), bacterial iron.

$$4\ Fe_2 + 3\ O_2 \rightarrow 2\ Fe_2O_3$$

$$Fe_2O_3 + 3\ H_2O \rightarrow 2Fe(OH)_3$$

Manganese removers. As in iron, the origin of manganese, in water, is both natural (dissolution of the reduced form Mn2+) and industrial (mining, the iron and steel industry, etc.). The same principle Is applied for its removal from water. Manganese does not endanger human health, nor the environment but it is unpleasant. In fact, the water gets a black colour and a metallic taste [17]. Small amounts of manganese and iron can changed the aesthetics and taste of drinking water.

$$Mn^{2+} \xrightarrow[\text{oxidation}]{} Mn^{4+} \xrightarrow[\text{precipitation}]{} MnO_2$$

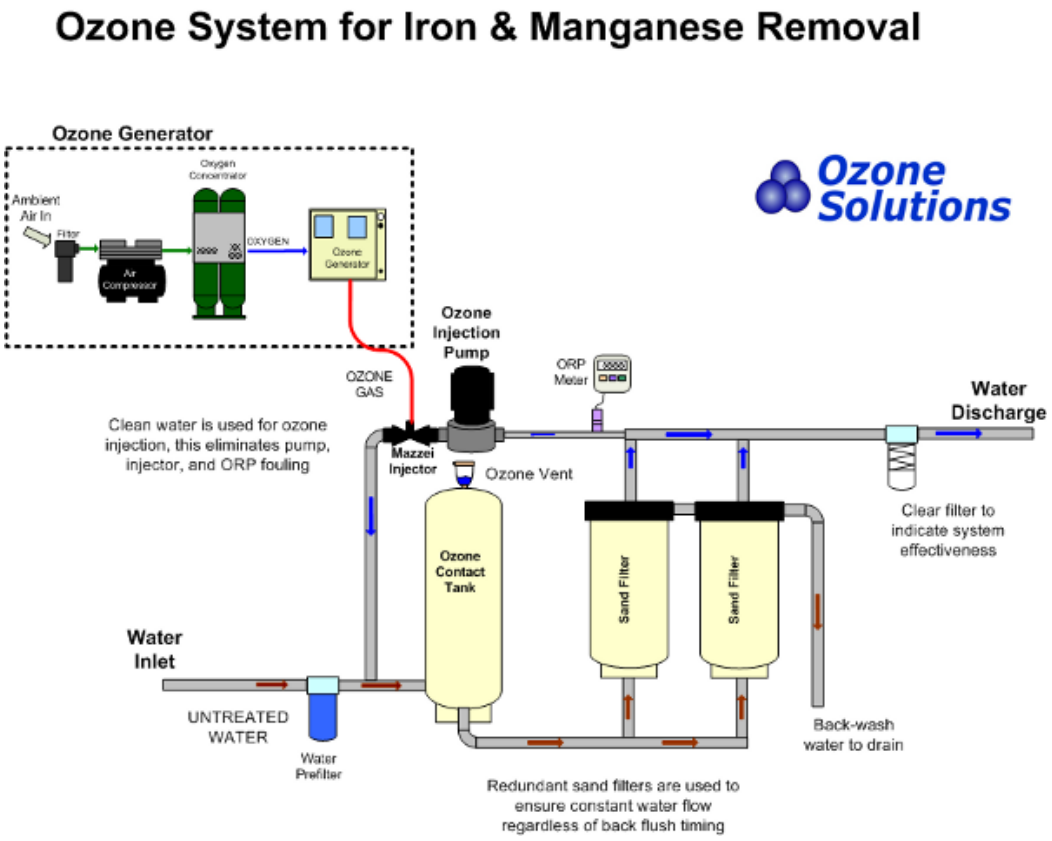

Figure 1.5. Iron and manganese remover

Source: http://www.ozonesolutions.com/info/ iron-and-manganese-removal-with-ozone

1.1. Description of zeolite and aluminosilicates

1.1.1.1. Characteristics of aluminosilicates

At first, we should to know, what aluminosilicates are. Alumina, Al_2O_3, and silica, SiO_2, are two most abundant minerals of the earth crust. The class of earth's minerals containing aluminium oxide and silicon oxide is called aluminium silicates [26]. This types of minerals are composed of aluminium, oxygen and silicon. They are the main component of clay minerals.

Aluminosilicates is a term used to describe fibrous materials made of aluminium oxide and silicon dioxide, (they are also called aluminosilicates fibres). These are glassy solid solutions rather than chemical compounds. The compositions are often described in terms of % weight of alumina, Al_2O_3 and silica, SiO_2. Temperature resistance increases as the % alumina increases. These fibrous materials can be encountered as loose wool, blanket, felt, paper or boards [27].

Among the main of aluminosilicates we have zeolites, kaolin, andalusite, dysten, silimanit and anorthite, and synthetic polymeric silicates, such as geopolymers. Aluminosilicates are obtained as a result of ion exchange Si^{4+} to Al^{3+} in the silicates. However,

it requires neutralization of the electric charge of the monovalent metal ion [8]. Aluminosilicates are formed by simultaneous precipitation of the aluminium and silicon oxides from salt solutions or by impregnating a gel with a solution of one component with solution of the second component, and while stirring the silica gel and aluminium hydroxide. They are belong to the hydrophilic adsorbents [4].

The representatives of aluminosilicates are feldspars, ultramarine and zeolites. The latter will be described separately. Feldspars have a loose structure and are airing under the influence of atmospheric factors (soil forming process). These include orthoclase $K(AlSi_3O_8)$ and albite $Na(AlSi_3O_8)$.

Ultramarines has got a three-dimensional skeleton structure containing the sulfur. They have numerous canals and open spaces similar to zeolites, but unlike them have no water molecules. This includes ultramarine $Na_8(Al_6Si_6O_{24})S_2$ and nozean $Na_8(Al_6Si_6O_{24})SO_4$.

Synthetic silicates are obtained by reacting the silica with aluminium hydroxide at a suitable pH and temperature of the process. This allows for preparation of aluminosilicates of different chemical properties of their surfaces.

Adsorption of metal cations such as zinc (II) from the wastewater can be carried out on the amorphous and crystalline aluminosilicates (Blazer, Zeolex23). This process occurs mainly through ion exchange. For zinc removing may also be used natural aluminosilicates (Ca-bentonite). Aluminosilicates are also used for the adsorption of mercury ions, chromium (VI), phenol, aniline, nitroethane and certain pesticides [8].

1.1.2. Characteristics of zeolite

The name 'zeolite' is said to have its origin in two Greek words zeo and lithos which mean 'to boil' and 'a stone'. The phenomenon of melting and boiling at the same time is a novel property. The name 'zeolite' was first used by the Swedish mineralogist Cronstedt to describe stilbite, the first recognized mineral zeolite, which was discovered in 1756. Over 100 years later, the reversible desorption/adsorption of water in this mineral was recognized [26].

Zeolites are referred to as hydrated aluminosilicate. They have porous structures, which are naturally occurring materials. Zeolite is a main class of hydrated aluminium silicates found in volcanic rocks. These minerals are very interesting for geologists, because they consist of a large cage, which looks like big structures which open channelways.

Figure 1.6. Types of tested zeolites. From left: fraction 4,0 – 8,0 mm and second one is fraction 0,5 - 1,0 mm

Source: www.subiopolska.com

Zeolites are highly absorbent porous minerals, composed largely of silica and aluminum. They are useful for their ability to capture and hold a variety of undesirable materials, just like a sponge absorbs water. However, not all zeolite is created equal [13.] Mineralogically, there are about 40 known types of natural zeolites (hydrated silicates) of which the most common is clinoptilolite. We can use this type of zeolite for example in aquarium or in filter for water.

Figure 1.7. Clinoptilolite

Source: www.roslinywodne.com

Storage capacity of water (so-called zeolitic water) in the tubules of the crystal structure is a characteristic of zeolites. Between polar water molecules, zeolite skeleton

are binding dipole. This water can be removed by heating, and then absorbed or replaced by other substances. Under conditions of too rapid water loss occurs in a structure damage irreversible. Desorption of zeolitic water is a function of time and temperature.

In the World we have got about forty-five natural types of zeolites, among which the most occurring and used are:

• clinoptilolite $NA_6[(AlO_2)_6(SiO_2)_{30}]\cdot 24H_2O$,

• chabazite $Ca_2[(AIO_2)_4(SiO_2)_8]\cdot 13H_2O$,

• mordenite $Na_8[(AIO_2)_8(SiO_2)_{40}]\cdot 24H_2O$.

They form in a number of relatively low temperature geologic environments.

Gas pockets in basalt and other volcanic rocks may contain dramatic crystal groups of zeolites. They are coming to us from the western United States and from Mexico. Natural zeolites can be formed also in desert lakes sediments, which pH is alkaline. we can observed the same process in alkaline soils in deserts, and in marine sediments. Zeolites occur in low-temperature metamorphic rocks in geologically young regions of mountain building, such as South Island, New Zealand.

Without natural types of zeolites, we can meet another one - synthetic zeolites. They have a wider range of properties and larger cavities than their natural counterparts. First production was in 1950s. Now, more than 100 different types of zeolite have been made on complicated chemical processes. The production of zeolites, which are synthetic is 12,000 tons.

Zeolites are manufactured in a number of ways; one important technique involves mixing sodium, aluminium, and silica chemicals with steam to create a gel. Another technique uses kaolin clay that has been heated in a furnace until it begins to melt, then chilled and ground to powder. This powder is mixed with sodium salts and water, aged, and heated [28].

The diversity of the selection of the ion exchange capacity is not only different types of zeolites, but also depends on the form of the zeolite, the solution pH, the composition (single or multicomponent).

It should be noted that the phenomenon of removal of metals is also influenced by the zeolite pH of the solution. On the one hand it determines the form of the metal in an aqueous medium, on the other hand affects the structure of the zeolite. Effect of pH on the structure of the zeolite is associated with dissociation of the functional groups at higher pH values. Natural zeolites contain significant amounts of calcium carbonate and bicarbonate,

and sodium. They affect the pH inside the zeolite and cause it can be higher than the pH of the solution.

From the reaction solution also depends on the dominant form occurrence of metal. It affects the ability of removing it by zeolites. Hence, for the reaction selectivity of the zeolite with respect to the solution of the metal will depend on the form of the parent metal and the complexes formed by zeolites. In addition, the kinetics of removal of the metal will depend on the size of the formed complexes [5].

1.1.2.1. The structures of zeolite

The atomic structures of zeolites are based on three-dimensional frameworks of silica and alumina tetrahedra, that is, silicon or aluminium ions surrounded by four oxygen ions in a tetrahedral configuration [28].

From figure on the next page (Fig. 1.7.) we can see, that oxygen is bonded to two adjacent silicon or aluminium ions, which are linking together. Clusters of tetrahedral form are further linked to build up the entire framework. In each of the different zeolites the polyhedral units may be sheetlike, equidimensional or chainlike.

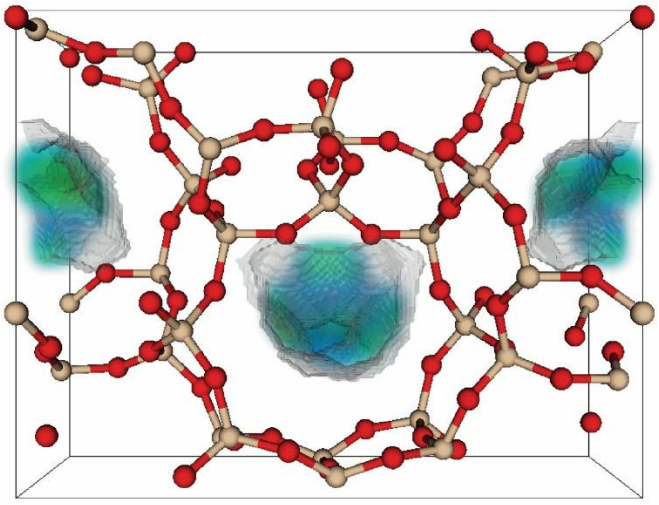

Figure 1.8. Zeolite atomic structure

Source: http://newenergyandfuel.com/http:/newenergyandfuel/com/2012/05/31/catching-co2-more-cheaply/figure_3b_1200dpi/

The aluminosilicates framework of a zeolite has a negative charge, which is balanced by the cations housed in the cage like cavities. Zeolites have much more open, less dense structures than other silicates; between 20 and 50 percent of the volume of a zeolite structure is voids. Silicates such as zeolites that have three-dimensional frameworks of tetrahedral are termed tectosilicates. Besides the zeolites, other tectosilicates include quartz and feldspars [28].

1.1.2.2. Application of zeolites in filtration and industry

Zeolites are distinguished by a number of unique characteristics of the physic - chemical properties. Mention may be a high adsorption capacity, the capacity of a molecular sieve, selectivity, ion-exchange capacity, resistance to acids and high temperatures. There's a lot of active sites. As a result, they provide an attractive material for use in processes using sorption and ion exchange. In addition, it is possible to modify the active sites. It is estimated that the use of natural zeolites in the world is approximately 3.6 megatons annually. The main producers are Cuba, Germany, Japan and South Korea in recent times as Australia, Indonesia, New Zealand. The predominant use of natural zeolites area is agriculture [1].

Natural zeolites are used in various areas of human activity for a long time. They began to be used much earlier than known and understood their property. Understanding the processes of synthesis of zeolites in natural conditions, has created the opportunity to take action on a laboratory scale. Initially employed for this purpose volcanic ash. Later, attempts were made to use fly ash from coal combustion processes [2].

Development of methods of the synthesis of zeolites resulted in large interest in the possibilities of their application in industry or environmental protection. However, it did not result in reduction in interest natural materials. Today, both types of zeolites, such as natural and synthetic fibers are equally widely used in the processes of physical, physic - chemical and biochemical [7].

Application in industry:

Zeolites, also referred to as molecular sieves, are widely used in various fields. Mainly used in the chemical industry. Other popular areas of use is a microelectronics, optics (production of materials with luminescent properties and nonlinear optical properties), medicine, environmental protection and agriculture, construction (as an addition to Portland clinkerand the manufacture of cement).

Zeolites are natural materials referred to as "clean" and safe. For this reason, they are used in detergents and cleaning powders, toothpastes fluoride, drugs and supporting filters digestion water purification in swimming pools.

Due to the ability to absorb the gases and air drying and purification of natural zeolites are used for ventilation in the cab and masks. They enable the absorption of odours and moisture from the air removing formaldehyde, chloroform and ammonia [3].

The application of zeolites are coming from their special properties:

- they can hold large molecules and break it into smaller pieces (catalytic cracking).
- they can selectively absorb ions that fit the cavities in their structures (molecular sieves);
- they can interact with water to absorb or release ions (ion exchange) [28].

Uses of zeolites:

- as water softeners, to remove calcium ions in reaction with soap to form scum,
- to clean radioactive wastes from the nuclear power plant,
- for absorbing sulphur dioxide from waste gases, which are a major cause of acid rain,
- to help purify the gases from power plants that burn high-sulfur coal,
- as molecular sieves are using to remove water and nitrogen impurities from natural gas,
- to help break down large organic molecules found in petroleum into the smaller molecules that make up gasoline, a process called catalytic cracking,

hydrogenating vegetable oils and in many other industrial processes involving organic compounds.

1.2. Removing of iron and manganese

Iron

Iron is generally removed from groundwater by the process of aeration or chemical oxidation followed by rapid sand filtration. Different mechanisms (physicochemical and biological) may contribute to iron removal in filters but the dominant mechanism depends on the physical and chemical characteristics of the water and process conditions applied. Nowadays, there are increasing numbers of publications on methods of biological iron removal which are reported to be much more efficient and cost effective than conventional physicochemical iron removal [15].

Although it is one of the essential elements of life in our body, in tap water is an undesirable component. Even small amounts of iron> 1 mgFe/dm^3, can cause dirty, brown water stains on sanitary gear. Iron in quantities a few grams can destroy underwear in the washing machine and change radically unpleasant taste of water and metal. Meanwhile, the water from the recognition of the iron content reaches up to 30 and more mgFe/dm^3 limit value - 0.2 mgFe/dm^3).

In the deep sea iron occurs mainly in the form of divalent Fe^{2+} , as ferrous bicarbonate $Fe(HCO_3)_2$ or ferrous sulphate $FeSO_4$. Both compounds are very unstable and readily undergo hydrolysis process, and upon contact with the atmosphere, as a result of dissolving oxygen in water, are oxidized to trivalent iron in the water to form ferric hydroxide $Fe(OH)_3$ in accordance with the reaction (for example the acid carbonate):

$$Fe(HCO_3)_2 + H_2O \rightarrow Fe(OH)_2 + H_2CO_3 \rightarrow Fe(OH)_2 + H_2O + CO_2$$

$$2\ Fe(OH)_2 + O + H_2O \rightarrow 2Fe(OH)_3\downarrow$$

Ferric hydroxide $Fe(OH)_3$ is a compound sparingly soluble in water, isolated as a dark red, and the fluff can be fairly easy to filter out sand deposits. The process of flocculation of the water gives a specific color. If we draw a bucket from a well and after a few days the water turns yellow, it is precisely the effect of the precipitation of iron compounds [20].

In the water surface (rare in the underground), iron is also present in the form of colloidal particles suspended FeS, $Fe(OH)_3$ or as an organic (humus). All of these forms are not subject to the process of hydrolysis and are difficult to remove from the water, giving it a natural color from pale straw – colored, the color of tea.

Manganese

It is much less common element in the water. The concentration of manganese can range from 0 – 10 mgMn/dm^3 (limit value - 0.05 mgMn/dm^3), where in it sporadic in surface water, more often in the deep sea, where it is present in the form of soluble salts of bivalent and forms chlorides, sulfates) [9]. The oxidized form of manganese dioxide MnO_2 as it becomes insoluble in water to form a black sludge. Manganese, like the element iron is undesirable in water intake on underwear and sanitary gear leaves brown spots, is also a breeding ground for bacteria [24].

Removal of iron and manganese

Both elements are not harmful to human health, therefore, in the category of water pollution are treated as nuisance compounds, which can cause:

- Change in the taste and odor of the water,
- Pollution and sanitary facilities oxide deposits,
- Increased risk of development of anaerobic bacteria, ferruginous

(eg Crenothrix, Leptothrix, Gallionelia, etc.) is largely responsible for the corrosion system,

- In water treatment processes - blocking ion exchange capacity of ion exchangers and fouling of membranes osmosis reverse osmosis systems.

Removal of iron and manganese requires a precise determination of the degree of contamination of water above mentioned elements and their own

nature [23].

In the case of underground water with a relatively high clarity, bacterial, in the presence of carbonates and sulfates is widely used ferrous aeration of water, and then filtering it. Aeration is carried out by raining the water slow filter in large installations) or by supplying air to the aerators, with the use of water on a compressor or an injector domestic

installations). Settling and filtration of the precipitate occurs flocculant directly in the bed. The ferric hydroxide precipitate is fairly easy to remove, so that a filter bed can be used for ordinary sand.

With a submersible water containing iron and manganese is advisable to raise the pH of the water. The oxidation of manganese is easier to carry out the reaction the alkaline water (pH should be above 9.5)., significantly shortens the time of oxidation of manganese to the water insoluble MnO_2 [21].

Good results for manganese removal catalyst bed in which seeds are coated with manganese oxide. Such media have a high sorption (collects on its surface, manganese ions, and other metals) may also be oxidized to ions Mn^{2+} Mn^{3+} form. Trivalent manganese is, however, susceptible to further oxidation of the oxygen dissolved in water to form Mn^{4+} and the formation of insoluble forms of MnO_2. Catalytic beds can be of natural or artificially created. When using catalytic deposits can be dispensed with pH adjustment. Catalytic beds (not all) of the time are "exhausted" and require regeneration. It is used in the most common solution of potassium permanganate.

If the content of iron and manganese in the water is high, the process should be a two-stage treatment, wherein it must first be removed from the water and then iron manganese. Selection of the percentage of individual components is carried out based on the composition of water and filtration velocity [22].

Iron present in water in colloidal form or in combination with organic compounds is much more difficult to remove. The same aeration of water just is not enough, you are the processes of chemical precipitation, or adding special substances into the water (coagulant), for connecting to small suspended particles into larger clusters of colloids capable of settling (sedimentation). The most commonly used coagulant is milk of lime or aluminum sulfate. Both methods require expensive equipment and are used on an industrial scale. In terms of family house water containing colloidal iron can try treatment on catalytic filters. The basic condition for such a treatment regimen is a lack of organic compounds in water.

5. Methodology

5.1. Covering of zeolites by potassium premanganate.

The research were based on the activation of zeolite mass by using potassium premanganate. This research were lasting about twelve weeks. This entire process was started

by weighing the two types of zeolite. Up to nine dishes (it was nine jars) with equal capacity 0.9 l were weighed one kilogram of zeolite. To four of these dishes were weighed one each kilogram of zeolite with fraction 0.5 - 1.0 mm and up to the next four - zeolite with fraction 4.0 - 8.0 mm. (Fig. 1.6) In total was prepared eight of the samples for testing.

Figure 2.1. Potassium premanganate

Source: https://www.flickr.com

After weighing the composite for testing, there was prepared manganese solution on this way: t was measured 10 g of potassium permanganate and added to the beaker. After that tap water was added to the beaker until the reagent is dissolved. The entire mixture was poured into a jar with zeolite. The water level in the jar should exceed about 1 cm above the zeolite. The entire procedure was repeated for the remaining 9 jars with two kinds of zeolites.

After this action all of prepared probes were successively left to stand for three-, five-, seven-and nine weeks in such a way that in each of analyzed week of studies was an probe with each of the two types of analyzed zeolite. This process is shown at the picture below (Fig. 2.2.)

Figure 2.2. Eight probes of Zeolites with manganese solution

Source: Own source

After three weeks, for second, third and fourth of analysed probes, there was added another dose of potassium permanganate. The aim was to strengthen of the saturation of zeolites by analysed reagent.

FIRST PROBE AFTER THREE WEEKS:

After three weeks first two of samples was stained to a purple colour in the following manner, as is shown in the figure below (Fig. 2.3.)

Figure 2.3. On the left: Zeolite with fraction 4.0 - 8.0 mm after three weeks. On the right: Zeolite with fraction 0.5 - 1.0 mm after three weeks

Source: Own source

SECOND PROBE AFTER FIFE WEEKS:

After fife weeks second two of samples was stained to a purple colour in the following manner, as is shown in the figure below (Fig. 2.4.)

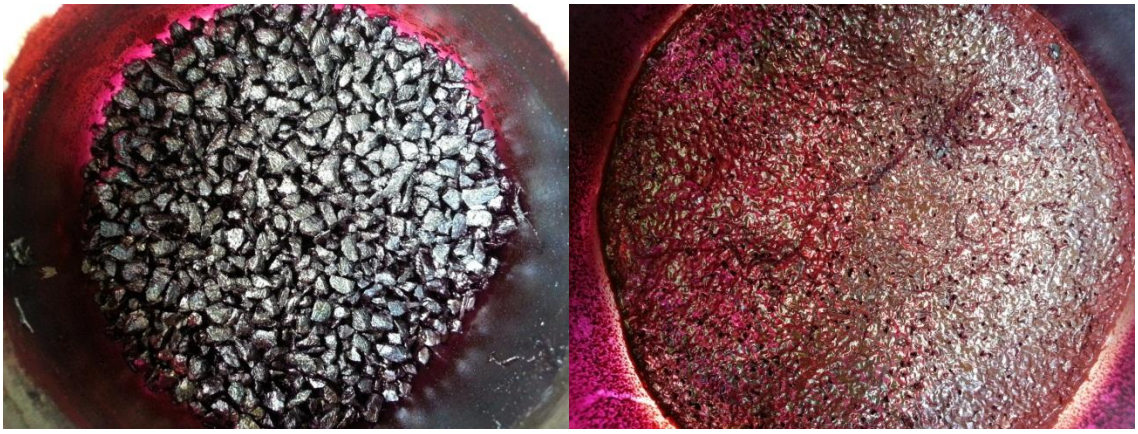

Figure 2.4. On the left: Zeolite with fraction 4.0 – 8.0 mm after fife weeks. On the right: Zeolite with fraction 0.5 – 1.0 mm after fife weeks

Source: Own source

THIRD PROBE AFTER SEVEN WEEKS:

After seven weeks second two of samples was stained to a purple colour in the following manner, as is shown in the figure below (Fig. 2.5.)

Figure 2.5. On the left: Zeolite with fraction 4.0 – 8.0 mm after seven weeks. On the right: Zeolite with fraction 0.5 – 1.0 mm after seven weeks

Source: Own source

FOURTH PROBE AFTER NINE WEEKS:

After nine weeks the last two of samples was stained to a purple colour in the following manner, as is shown in the figure below (Fig. 2.6.)

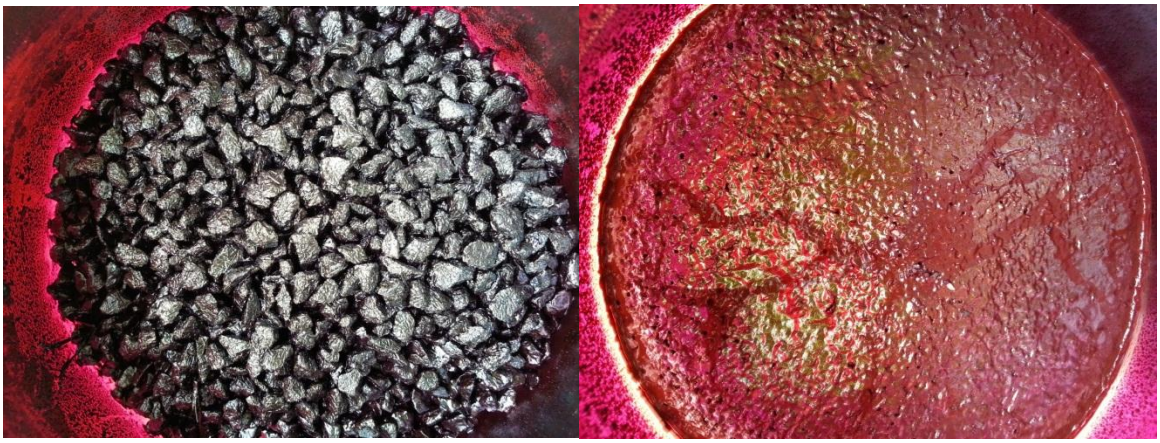

Figure 2.6. On the left: Zeolite with fraction 4.0 – 8.0 mm after nine weeks. On the right: Zeolite with fraction 0.5 – 1.0 mm after nine weeks

Source: Own source

The second step of analysis was drying of zeolite mass. Drying process is simple, but it is time consuming step. Whole process takes place in special dishes in sauna with water. This water was adjusted to a boil. Then small parts of zeolites were placed with a spoon in small dishes (Fig. 2.7.). For this purpose were used two saunas with water, which were contained in the laboratories. Then the vessel was set to twenty holes on saunas and allowed to dry.

Figure 2.7. Process of drying zeolite in sauna with water

Source: Own source

Figure 2.8. On the left: Zeolite with fraction 4.0 – 8.0 mm after drying. On the right: Zeolite with fraction 0.5 – 1.0 mm after drying

Source: Own source

2.2. Zeolite mass activation

After covering of zeolites by potassium permanganate and drying, our zeolite mass was activated. Process of activation was different for each one of zeolites. It was sodium hydroxide pure p.a., iron (II) sulfate (VI) (hydrate), ferric chloride, potassium permanganate and ammonium iron (II) sulfate hexahydrate pure p.a.

At first subjected to zeolite mass after three weeks. In beaker was disolved 40g of sodium hydroxide pure p.a. in 1 liter of distilled water. After that, for first two jars with two types of zeolite fraction was added solution with sodium hydroxide and allowed it for two hour. The water level in the jar should exceed about 1 cm above the zeolite. After this time was added 50g of potassium permanganate and it was allowed.

Figure 2.9. Sodium hydroxide pure p.a.

Source: Own source

Second step was activation of zeolite after fife weeks. At first was disolved 50g of iron (II) sulfate (VI) (hydrate) and 20g of manganese in 1 liter of distilled water. The solution thus

prepared was left for one hour. The water level in the jar should exceed about 1 cm above the zeolite. After this time was added 20 ml solution with sodium hydroxide, which was prepared like as in case of zeolite after three weeks and allowed it for next one hour. For the end for this two jars was added 10 g of potassium permanganate dissolved in slam amount of distilled water and dishes with zeolite mass was allowed.

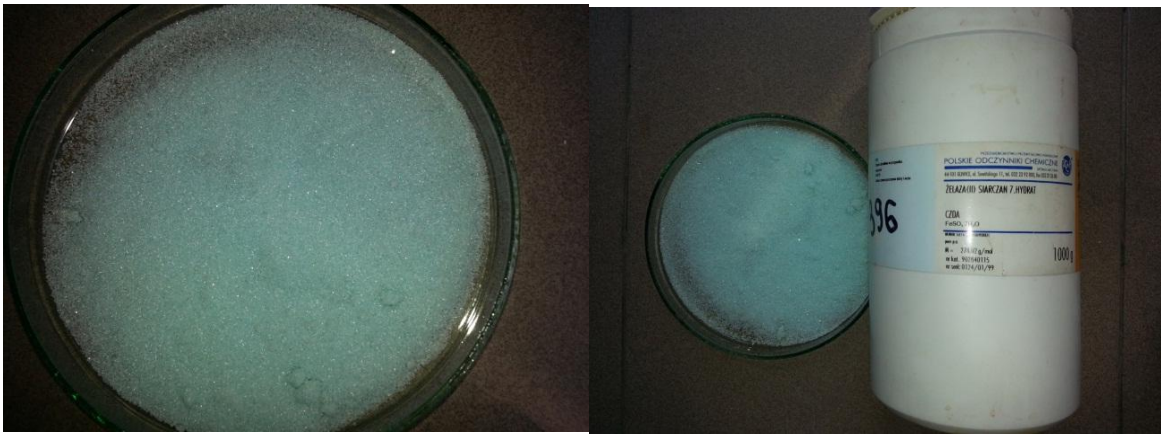

Figure 2.10. Iron (II) sulfate (VI) (hydrate)

Source: Own source

Third step was activation of zeolite after seven weeks. At first was disolved 50g of ammonium iron (II) sulfate hexahydrate pure p.a. in 1 liter of distilled water. The solution thus prepared was left for one hour. The water level in the jar should exceed about 1 cm above the zeolite. After this time was added 20 ml solution with sodium hydroxide, which was prepared like as in case of zeolite after three weeks and allowed it for next one hour. For the end for this two jars was added 10 g of potassium permanganate dissolved in slam amount of distilled water and dishes with zeolite mass was allowed.

Figure 2.11. Ammonium iron (II) sulfate hexahydrate pure p.a.

Source: Own source

The last step was activation of zeolite after nine weeks. At first was diluted 40ml of iron sulfate in 1 liter of distilled water. The solution thus prepared was left for one hour. The water level in the jar should exceed about 1 cm above the zeolite. After this time was added 20 ml solution with sodium hydroxide, which was prepared like as in case of zeolite after three weeks and allowed it for next one hour. For the end for this two jars was added 10 g of potassium permanganate dissolved in slam amount of distilled water and dishes with zeolite mass was allowed.

Figure 2.12. Iron sulfate

Source: Own source

On this way were subjected to activation eight jars with zeolite. They was left for another two weeks.

6. Results and discussion

On this way, activated zeolite after two weeks was subjected to a thorough wash. Rinsing started from the zeolite fraction 4.0 - 8.0 mm. On a fine sieve was placed small portions of zeolite after three weeks. Then sieve was placed above the sink, under a strong pressure, cold tap water. Descent water from zeolite was with strongly purple colour. It was caused by intrusion of potassium premanganate into the zeolite. The zeolite was washed in such a way that the sieve was rotated under a stream of water bubbled through it until the water contingents passing through the sieve until the water was completely clean. Then, purified in this way zeolite was passing from the sieve into a large beaker and applied to another portion of the colored zeolite for the sieve. Procedure was repeated with all portions of zeolite with fraction 4.0 - 8.0 mm. then all of the jars, in which the zeolite

was thoroughly washed on the walls from the remaining reagents and dried. Then zeolite was taking back to the pure jar.

In the same way was rising zeolite with fraction 0.5 - 1.0 mm. Zeolite after washing is shown in the pictures below (Fig. 3.1., 3.2.).

Figure 3.1. Activated zeolites after rising- view from the top

Source: Own source

Figure 3.2. Activated zeolites after rising – front view

Source: Own source

7. Conclusions

1. In results after activation of zeolite mass, we obtained a porous adsorbent with a high degree of activity.
2. Research were carried out to select the best method of activation of the zeolite for the removal of impurities.
3. Each one of activation methods has proved to be effective, but the best method was with iron sulfate, sodium hydroxide and potassium permanganate.
4. The porous structure of zeolite as the fill of filters can absorbed water pollution.
5. Activation of zeolite mass in a perfect way are suitable as the fill for filters supporting of removal of iron and manganese from water.

References

1. Eyde T.H.. Zeolites: Miting Engineering 57 (2005).
2. Faghihian H., Bowman R.S.: Adsorption of chromate by clinoptylolite exchanged with various metal cations, Water Research 39 (2005).
3. Ghobarkar H., Schäf O., Guth U.: Zeolites- from kitchen to space, Prog. Solid St. Chem. 27, (2005).
4. Nejmark I.J., Syntetyczne adsorbenty mineralne, Wyd. WNT, Warszawa (2005)
5. Ouki S.K., Kavannagh M.: Treatment of metals-contaminated wastewaters by use of natural zeolites, Water Science Technology 39 (2010).
6. Pawlaczyk-Szpilowa M.: Biologia i ekologia, Oficyna Wydawnicza Politechniki Wrocławskiej, Wrocław, (2012).
7. Rabo J. A.: Future opportunites in zeolite science and technology, Applied Catalysis A: General 229 (2002).
8. Sarbak Z., Adsorpcja i adsorbenty. Teoria i zastosowanie, Wyd. Nauk. UAM, Poznań (2005)
9. Article on ACS Publications, Ferrous Iron Removal Promotes Microbial Reduction of Crystalline Iron(III) Oxides, retrieved in 2005 from http://pubs.acs.org/doi/abs/10.1021/es9809859.
10. Article on Aquapura Water Products, Iron Remover, retrieved in 2014 from http://www.aquapurawater.ca/iron-remover-ezp-17.html.
11. Article on CPL carbon link, Properties of Activated Carbon, retrieved on 2nd of May 2008 from http://www.activated-carbon.com/.

12. Article on Free drinking water, Different Water Filtration Methods Explained, retrieved in 2014 from http://www.freedrinkingwater.com/ water-education/quality-water-filtration-method-page3.htm.
13. Article on Freshwater Aquariums, Using Zeolite in Aquariums, retrieved in 2014 from http://freshaquarium.about.com/od/filters/a/Using-Zeolite-In-Aquariums.htm.
14. Article on Grace Communications Foundation, The Importance of Clean Water, retrieved in New York in 2014 from http://www.gracelinks. org/2382/the-importance-of-clean-water.
15. Article on IWA online, Biological iron removal from groundwater: a review, retrieved in 2005 from http://www.iwaponline.com/jws/054/ jws0540239.htm
16. Article on Lenntech, Water treatment solution: Filtration, retrieved on the 15th October 2013 from http://www.lenntech.com/chemistry /filtration.htm.
17. Article on Lenntech, Water treatment solution: Iron removal by physical – chemical ways, retrieved in 15th October 2013 from http://www. lenntech.com/processes/iron-manganese/iron/iron-removal-physical-chemical-way.htm#ixzz32Ce5HB47.
18. Article on Lenntech, Water treatment solution: Manganese removal by physical – chemical ways, retrieved in 15th October 2013 from http://www.lenntech.com/processes /iron-manganese/manganese/manganese-removal-physical-chemical-way.htm.
19. Article on New Energy, New Energy And Fuel, retrieved in 30th May 2012 from http://newenergyandfuel.com/http:/newenergyandfuel/com /2012/05/31/ catching-co2-more-cheaply/figure_3b_1200dpi/.
20. Article on Science Direct, Arsenic removal from groundwaters containing iron, ammonium, manganese and phosphate: A case study from a treatment unit in northern Greece, retrieved in 15th of April 2008, from http://www.sciencedirect.com/science/ article/pii/S0011916408000672.
21. Article on Science Direct, Removal of iron and manganese from groundwater by oxidation and microfiltration, retrieved in 20th of November 2000, from http://www.sciencedirect.com/science/article/ pii/S00119 16400000904.
22. Article on Science Direct, Study on the removal of iron and manganese in groundwater by granular activated carbon, retrieved in 1st of November 2005, from http://www.sciencedirect.com/science/article/pii/S001191640 5004509.
23. Article on Science Direct, The behaviour of dissolved iron, manganese and zinc in the Beaulieu Estuary, S. England, retrieved in 3rd of May 2010 from http://www.sciencedirect. com/science/article/pii/0302352476900669.

24. Article on Science Direct, The removal of manganese, iron and ammonium nitrogen on impregnated activated carbon, retrieved in 5th of February 2007 from http://www.science direct.com/science/article/pii/S0011916406014226.
25. Article on Water Anywhere, Softening Filter Cartridges, retrieved on 2014 from http://www.wateranywhere.com/index.php?cPath= 255_263_450.
26. Publication about Aluminium Silicates, retrieved on 2013 from http://www.science. uwaterloo.ca/~cchieh/cact/applychem/alsilicate.html.
27. Publication about Aluminosilicates, retrieved on 2012 from http://www.answers. com/topic/aluminosilicate-1.
28. Publication on Encyclopedia of chemistry, retrieved in 2007 from http://www.chemistryexplained.com/Va-Z/Zeolites.html
29. Publication on North Dacota State University, It's All In Your Water Iron and Manganese Removal - WQ103, retrieved 27.07.2013 from http://www.ag.ndsu.edu/publications/ landing-pages/environment-natural-resources/iron-and-manganese-removal-wq-1030.
30. Publication on Department on Science Engineering, Science Direct, Synthesis and characterization of activated carbo-aluminosilicate material from oil shale, Reyad Awwad Shawabkeh; Department of Chemical Engineering, Mutah University, Box 7, AL-Karak 61710, Jordan; received 10 April 2004; received in revised form 5 June 2004;accepted 22 July 2004 http://www.researchgate.net/publication/242479172 _Synthesis_and_characterization_ of_activated_carbo-luminosilicate_material_ from_oil_shale/file/e0b4952a4cfae6814c.pdf.

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Escherichia coli K-12 strain with reporter fusion of recA::gfpmut2 for screening of anticancer and antidiabetic pharmacist residues in water ecosystems

Key words: *cancer, recA, gfp, cytotoxicity, genotoxicity, 5-fluorouracil, metformin*

Abstract: The reporter strain of Escherichia coli K-12 recA::gfpmut2 containing a plasmid-borne transcriptional fusion between DNA-damage genotoxin-inducible recA promoter involved in the SOS bacteria stress regulon response and fast folding GFP, enhanced (Green Fluorescent Protein) variant reporter gene-gfpmut2, have been used. Gfp reporter gene bacterial biosensor strain allowed the detection of cytotoxic and genotoxic activity of known anticancer drug– 5-fluorouracil (5-FU) and antidiabetic drug – metformin in PBS buffer and sample of surface water. Obtained data showed that recA::gfpmut2 genetic system was sensitive for applied drugs and drugs mixture. RecA promoter was a good bioindicator for cytotoxic and genotoxic effect screening of 5-fluorouracil, metformin and the mixture of the both drugs in PBS buffer and surface water. In experiment the positive fluorescence reactivity of Escherichia coli K-12 recA::gfpmut2 was obtained for each tested chemicals. The maximum point of the gfp gene stimulation in E. coli K-12 strain was noticed for co-administration of 0,001 mg/ml of 5-FU and 0,3 mg/ml metformin. The inhibition of reactivity gfpmut2 genetic system in surface water for mixture of 5-FU (0,001 mg/ml) and metformin (0,3 mg/ml) treated samples and for prolonged incubation (up to 24 h) was obtained. The results showed that E. coli K-12 recA::gfp mut2 strain could be potentially useful for first-step screening of cytotoxic and genotoxic effect of anticancer and antidiabetic pharmacist residues in water. The validation of recA::gfpmut2 genetic system in E. coli K-12 demands more experimental analysis.

1. Introduction

Cancer is one of the most challenging diseases to cure and the second leading cause of death in developed countries. Over the past few decades, it continues to be a worldwide health problem in spite of the rising number of nanoscaled technologies. Chemotherapy is the major therapeutic approach for the treatment of localized and metastasized cancers. Over the last decade, the number of cytostatic drugs used in cancer therapy has considerably increased. From June 2000 to July 2001, the consumption of antineoplastics in Austria reached approximately 800 kg/year (Institute for Medical Statistics, personal Communications) [1-4]. There is growing concern about the presence of human pharmaceutical (HPs) in the wider aquatic environment. Anticancer drugs used in chemotherapy have high pharmacological potency and possess fetotoxic, genotoxic and teratogenic properties and can induce subtle genetic and cell cycle

changes in aquatic fauna and flora under chronic exposure. Due to the improvement in detection limits (from <10 ng/l in 1998 to 1 < ng/l in 2011) to quantify anticancer drugs with liquid chromatography tandem mass spectrometry (LC–MS/MS) some of these chemicals have been reported in hospital waste effluents, effluents in sewage treatment plants (STPs) and river water, in a growing number of studies. The concern over these substances is their occurrence in freshwater systems which are then abstracted as a potable water supply, hence presenting a risk of human exposure, as well as posing a wider risk to freshwater and estuarine habitats [5-20].

5-Fluorouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is an antimetabolite of the pyrimidine analogue type. This chemotherapy agent has been shown to be effective in the treatment of a broad variety of solid tumors including pancreas, ovary, liver, brain, breast and gastrointestinal cancers, alone or in combination chemotherapy regimes [21-23]. Although the antimetabolite anticancer agent, 5-fluorouracil (5-FU), is widely used to treat several types of malignant tumors, it frequently causes intestinal mucositis. The pathogenic mechanisms of 5-FU-induced intestinal mucositis are still unclear, but several pathogenic elements are considered to be involved, including direct toxicity, oxidative stress, apoptosis, hypo-proliferation, and abnormal inflammation. Particularly, apoptosis is a critical event in the occurrence of 5-FU-induced intestinal mucositis since many apoptotic cells are observed in intestinal crypts before serious mucosal destruction in mice and humans [24-26]. Fluorouracil (5FU) is a fundamental component of all chemotherapic combinations for palliative and adjuvant treatments of colorectal cancer. Its main mechanism of action consists of inhibition of thymidylate synthase (TS) through an active metabolite, fluorodeoxyuridine monophosphate (FdUMP), which forms an inactive ternary complex with TS and 5-10 methylenetetrahydrofolate (MTHF). Intracellular MTHF levels, which are essential for ternary complex stabilisation, are controlled by the enzyme methylenetetrahydrofolate reductase (MTHFR). Consequently, the tissue activities of TS and MTHFR are presumed to be major determinants of 5FU clinical response. The activity of both enzymes is under genetic control and several gene polymorphisms are known to influence their tissue expression [21-28].

Metformin is an oral medication that lowers blood glucose (sugar) by influencing the body's sensitivity to insulin and is used for treating type 2 diabetes. Accumulated evidence suggest that metformin might be a potential drug for the chemoprevention of colorectal cancer (CRC) in diabetic patients. The antineoplastic effects of metformin have been reported to be associated with activation of AMP-activated protein kinase (AMPK) signaling pathway, improvement of insulin resistance and hyperinsulinaemia. Most recently, another antineoplastic benefit of metformin was reported. It might inhibit the survival of cancer stem cells (CSCs),

tumor-initiating stem-like cells: (TISCs) in breast, and pancreatic cancers and glioblastoma in vitro [29-34]. The measurements showed an almost ubiquitous presence of metformin in the aquatic environment - in sewage and surface waters. Concentration levels of metformin depend on the sewage fraction in the water and in most rivers are in the range of several to 100 ng/l, i.e. in the same order of magnitude (or even higher) than for other relevant pharmaceutical residues [2-14].

The primary source of cytostatic compounds in the environment are excretions from patients under medical treatment. As a consequence, one is very likely to detect these substances in hospital sewer systems or public sewage treatment plants (the latter in the case of out-patient treatment). In the Vienna General Hospital, nearly 80% of cancer therapy is done in the out-patient treatment ward. Therefore, one may expect to individualise relevant amounts of cytostatic agents in the public waste water. Besides the parent cytostatic compounds, metabolites may also contribute to the cytotoxic properties of hospital effluents. For this reason, the occurrence of cytostatic agents in waste water and in the environment has become an important issue not only to the scientific community, but has also raised public concern. In addition, little is known about the environmental impact of cytotoxic substances [1-14].

Recent years have seen continued growth of the interdisciplinary field of biosensors due to an increased interest between basic and applied sciences researchers for development of novel electronic devices that can be utilized for a variety of applications including human health care and environmental monitoring. A biosensor is an integrated miniaturized analytical device that integrates biological sensing elements like enzymes, antibodies, nucleic acids, cells etc. with transducer equipped with an electronic amplifier. Biosensors offer interesting features such on-site, simultaneous detection of multiple pathogenic agents utilizing selectivity of the biomolecules and the processing power of modern nano-electronics. They are known to provide fast and accurate results arising due to interaction of an analyte with a given biomolecule and have been found to have applications in various fields including clinical diagnostics, environmental monitoring, bioprocess monitoring, food, water and agricultural product processing, etc. According to IUPAC, a biosensor is a self-contained integral device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological element. Bacterial reporter strains biosensor systems, known as bacterial biosensors display an important role in the rapid, first-step biological screening and detection of genotoxic - DNA damaging chemicals, drugs and potential drug candidates. Several genetic engineering approaches have been employed over the years in the construction of bacterial reporter strains that respond to the presence of genotoxic compounds. Many of these share the same basic

principle: the fusion of a gene promoter, known to be activated by the presence of genotoxic chemicals, to a gene or a group of genes the activity of which can be monitored quantitatively, preferably in real time. The gene promoter acts as the sensing element, which – upon activation – drives the transcription of the downstream reporter gene(s). Consequently, the gene promoter will dictate the response spectrum of the construct and, to some extent, its sensitivity [35-49].

GFP fluorescence-based bacterial biosensors (fusion of gfp-reporter gene under control of the SOS dependent recA promoter) can detect the genotoxic mode of action of certain chemicals and are simple to apply, sensitive and easy to measure - in the concentration of GFP and in a consequence the fluorescence signal. GFP reporter protein has been isolated from coelenterates, for example the Pacific jellyfish Aequorea Victoria. GFP is being used increasingly to construct whole-cell biosensors, because of its useful properties such as: high stability, minimal toxicity for life cells and the ability to generate the green fluorescence without addition of external cofactors. Additionally it is possible non-invasive detection of gfp expression with application of simple in use equipment, for instance UV lamp, fluorescence microscope or spectrofluorymeter. The chromophore is responsible for GFP light and is produced posttranslationally in the presence of oxygen from serine-tyrosine and glicyne. Wild type GFP absorbs blue light at 395 nm and emits green light at 509 nm. To increase a rate of chromophore maturation, stability and to obtain the emission of stronger light signal several mutants of GFP were developed [41,43, 46-47].

The SOS regulon with recA promoter, is one of the most thoroughly studied genotoxic stress regulons for bacteria. The recA promoter transcription is induced upon DNA damage and induction of the SOS response is initiated by RecA protein activation to mediate the LexA repressor protein cleavage. With the cleavage of LexA, the promoters that it was bound to and repressing are then expressed that results in the induction of the SOS regulon, so each downstream gene product participates in the repair of the damaged DNA. The application of recA promoter for creation of effective genotoxicity bacteria biosensors is connected with broad involvement of RecA protein in several DNA repair pathways, including the repair of daughter-strand gaps and double-strand breaks, as well as in an error prone damage tolerance mechanisms called SOS mutagenesis [39, 41, 43, 46-47, 48].

The reporter genes determine the nature of the generated signal (bioluminescence, fluorescence, etc.) and thus also the instrumentation required for its acquisition. The host cell, the third major component in the construction of a genotoxicity reporter strain, is selected for ease of genetic manipulation, for its relevance, and – most importantly – for its effects on

detection sensitivity and threshold. In such living cell systems, bacteria are especially attractive due to their rapid growth rate, low cost, and easy handling [36-37].

The highly stable green fluorescent protein, GFP, of the jellyfish Aequorea Victoria was the first fluorescent protein the gene of which was utilized as a molecular reporter. The GFP protein has a high quantum yield and can be expressed in both prokaryotic and eukaryotic systems with no need of a substrate or cofactor. To increase the sensitivity of assays based on the GFP reporter system, several green fluorescent protein mutants were constructed [36-37, 41-43].

Due to HPs (especially anticancer drugs) highly potent mechanism of action (they directly or indirectly act with structure and function of DNA) these specific groups of drugs are conceived to be hazardous to living organisms and human health. There is a need to target HPs with environmental significance, quantify them, and assess their cytotoxic and genotoxic risk to living organisms [1-10]. So, the present study analyzed the potency of E. coli K-12 recA::gfp microbial biosensor strain for cytotoxic and genotoxic effect screening of two commonly prescribed HPs: 5-fluorouracil- anticancer drug and metformin – antidiabetic drug.

2. Materials and methods

2. 1. Chemicals

5-Fluorouracil and metformin was commercially obtained from pharmacy. Before using, drugs were dissolved in PBS buffer (1,44 g Na_2HPO_4, 0,24 g KH_2PO_4, 0,2 g KCl, 8 g NaCl per 1000 ml of distilled water, pH=7) at determined experimental concentrations.

2. 2. Bacteria strain and plasmid

In the experiment stationary phase cells: Escherichia coli K-12 recA::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2, genetically modified were used. They contained a pUA66 plasmid-borne transcriptional fusion between DNA-damage inducible, oxidative stress recA promoter involved in the SOS regulon response and fast folding GFP variant reporter gene-gfpmut2. The genetic structure of pUA66 plasmid is described in work of Zaslaver et. al., 2004 [47]. In the present work more stable and fast folding mutant of gfp gene - gfpmut2 with excitation and emission wavelengths of 485 and 507 nm was used [47].

2. 3. Bacteria growth condition

Escherichia coli K-12 MG1655 strains: Escherichia coli K-12 recA::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2 were cultured overnight in LB agar medium (Merck, Germany) at 30C° supplemented with 100 μg/ml of kanamycin (Sigma-Aldrich, Germany). Colonies were carried to LB broth medium (10 g NaCl, 10 g tryptone and 5 g yeast extract per 1000 ml of distilled water) with 100 μg/ml of kanamycin and incubated overnight at 30C°. After that, cells were washed with PBS buffer.

2. 4. Monitoring of bacteria growth and concentration

At the start of experiment the initial bacteria cells density was standardized to OD (Optical Density) value by use of spectrophotometer (Perkin Elmer Enspire 2300) at wavelength of 600 nm. The concentration of bacteria cells per ml of PBS was assessed by series dilutions system and expressed as CFU/ml values.

The dynamic of growth of bacteria strains treated with 5-FU and metformin was monitored by use of standard spectrophotometer analysis of Optical Density values at wavelength of 600 nm.

The values of bacteria growth inhibition (GI) during the treatment with drugs at the start of bacteria incubation with drugs - time 0 and after 3 and 24 hours was calculated according to the formula:

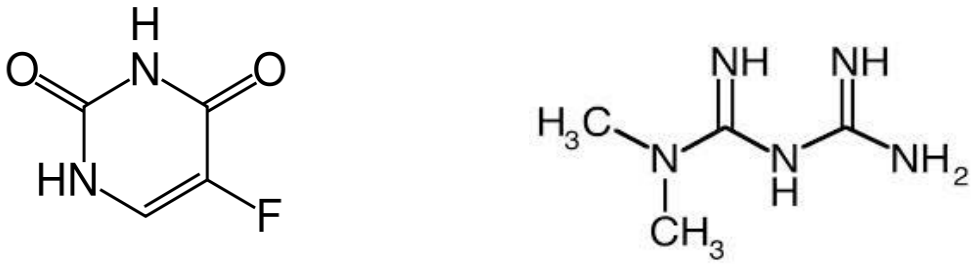
$$GI\ (\%)=OD_{CS}(\%) - D_{ODTS}(\%), \quad (1)$$

Where: $OD_{CS}(\%)$ – Optical Density of control sample, =100%,

$D_{ODTS}(\%)$ – the decrease in the value of Optical Density of bacteria samples treated with drugs.

2.5. Bacteria cells treatment with 5-fluorouracyl and metformin in PBS Buffet

1 ml of stationary phase bacteria cells (2×10^8 CFU/ml; OD=0,2) were suspended in 4 ml of PBS buffer and the following drugs were used for genotoxicity testing: 5-fluorouracyl (water solution of 50 mg/ml) (5-FU), metformin (water solution of 800 mg of metformini hydrochloridum) (M) and 5-FU+metformin (5-FU+M) in five different concentrations, for 5-FU: 0,0001; 0,001; 0,01; 0,1 and 1 mg/ml; for metformin: 0,3; 0,7 and 1 mg/ml and for 5-FU+metformin: (1) 0,0001+0,3; 0,001+0,3; 0,01+0,3; 0,1+0,3 and 1+0,3 mg/ml; (2) 0001+0,7; 0,001+0,7; 0,01+0,7; 0,1+0,7 and 1+0,7 mg/ml and (3) 0001+1; 0,001+1; 0,01+1; 0,1+1 and 1+1 mg/ml. Chemical structure of the both drugs are presented in Fig. 1.



5-Fluorouracil **Metformin**

Figure 1. Chemical structure of 5-fluorouracil and metformin

The concentration range of the drugs analysed in research were selected experimentally from the minimum level of recA::gfp construct sensitivity and according to the reviewed references recommendation, which indicated the concentrations observed in the environment [1-15]. The time of bacteria incubation with drugs (3h and 24 h) was estimated for monitoring of sensitivity of recA::gfp genetic construct for quickly (3 h) and later (24 h) response. The control sample - Escherichia coli K-12 recA::gfpmut2 strain in PBS buffer was not treated with drugs. For verification the correct activity of recA promoter, Escherichia coli K-12 strain containing pUA66 plasmid without promoter - Escherichia coli K-12 promoterless::gfpmut2 - was used as a control. Additionally, for assessment of genotoxic sentivity of recA::gfp construct, 4% acetone were used as negative control and 50 μM methylnitronitrosoguanidine (MNNG, known genotoxin) as positive control.

2. 6. Bacteria cells treatment with 5-FU and metformin in surface water

Surface water samples were collected in sterile flasks from Białka river. Samples were sterilized by filtration. 1 ml of stationary phase bacteria cells (2×10^8 CFU/ml; OD=0,2) were suspended in 4 ml of surface water and the following drugs were used for genotoxicity testing: 5-fluorouracil (5-FU), metformin (M) and 5-FU+metformin (5-FU+M), for 5-FU at concentration of: 0,001 mg/ml; for metformin: 0,3; 0,7 and 1 mg/ml and for 5-FU+M: (1) 0,001+0,3; (2) 0,001+0,7 and (3) 0,001+1 mg/ml. Drugs concentrations were selected for the highest stimulation of gfp gene expression in PBS buffer (for F_I= 9,31).

The conditions of bacteria incubations and the control protocols were the same as above.

2. 7. Analytical method for the intensity of gfp gene fluorescence (IF) analysis

After exposition of bacteria cultures to tested drugs, the strains were washed with PBS buffer and the intensity of fluorescence of gfp gene in the volume of 1 ml of bacteria cells suspension (1×10^4 CFU/ml) in PBS buffer was measured with spectrofluorometer (Perkin Elmer Enspire 2300). The measurements were done at excitation and emission wavelengths of 485 and 507 nm.

2. 8. Assessment of SFI values

The specific fluorescence intensity (SFI) value which is defined as the raw fluorescence intensity (IF) divided by the optical density (OD) measured at each time point at 600 nm was calculated according to the below formula for monitoring the dynamic of gfp expression after bacteria treatment with drugs:

$$SFI = \frac{IF}{OD} \tag{2}$$

where:

SFI – Specific Fluorescence Intensity,

IF – The raw fluorescence intensity of the strains at excitation and emission wavelengths of 485 and 507 nm,

OD – Optical Density at 600 nm of the strains.

2. 9. Detection of $S_{gfpexp.}$ value

For the increased SFI values with the level of gfp expression (5-FU and 5-FU+M) in comparison with the control sample the percent of stimulation of gfp ($S_{gfpexp.}$) were calculated, according to the formulas:

$$S_{gfpexp.}(\%) = I_{TS}(\%) - SFI_{CS}(\%) \tag{3}$$

where I_{TS} (%) – the increase for SFI values for tested drugs sample in comparison with the control sample, SFI_{CS} (%) – SFI for control sample, =100%.

2. 10. Assessment of F_I values

For each concentration of tested drugs the induction factors (F_I) were calculated. $F_I = (Fl_I/OD_0)/(Fl_0/OD_I)$, where Fl_I is the raw fluorescence of the culture treated with DNA - damaging compound; Fl_0 is the raw fluorescence of the control sample without genotoxin; OD_I

is the optical density at 600 nm of treated culture and OD_0 is the optical density of the control sample.

The SFI, $S_{gfpexp.}$ and $F_{I,}$ values express the potency of influence of the both drugs on the sensitivity of oxidative stress recA::gfp construct.

2.11. Classification of tested drugs as genotoxins

The F_I values were calculated for classification of tested drugs as genotoxins. According to references Ptitsyn et. al. (1997) [48] and Kostrzyńska et. al. (2002) [41] a chemical was identified as a genotoxin if its induction factor was 2 or more ($F_I \geq 2$).

2. 12. Statistical analysis

Statistics data obtained in this study are expressed as mean ± standard deviation (SD) for n = 6. The data were analyzed by use of standard statistical analyses, including one-way Student`s test for multiple comparisons to determine the significance between different groups. The values for $P<0.05$ were considered significant.

3. Results and discussion

In experiment the positive fluorescence reactivity of Escherichia coli K-12 recA::gfpmut2 was obtained for each tested chemicals.

Escherichia coli K-12 MG1655 recA::gfpmut2 strain treatment with 5-FU and metformin (5-FU+M) showed that simultaneous administration of the both drugs caused a significant increase ($P \leq 0,05$) in SFI, F_I and $S_{gfpexp.}$ values compared to non-treated cells (Table 1). Longer treatment with 5-FU and (5-FU+M) (up to 24 h) intensified SFI, F_I and S_{gfpexp} values. Bacteria cells incubation in PBS buffer under each concentration of metformin treatment caused a decrease in SFI values compared to control sample. A sustained decrease in SFI values was observed after 24 hours.

Figure 2 and 3 shows the dynamic of the SFI values for E. coli K-12 recA::gfp mut2 treated with 5-FU and 5-FU with combination with three concentrations of metformin (5-FU+M) after 3 and 24 hours incubation with drugs compared to control sample not-treated with drugs.

Bacteria treatment with 5-FU resulted in a progressive significant stimulation of SFI values for 1; 0,1; 0,01 mg/ml for 3 h and 24 h incubation compared to control sample and metformin treated cells. For 0,001 and 0,0001 mg/ml of 5-FU a significant increase for SFI

values was observed for 24 h. The maximum point for SFI value ($S_{gfpexp.}$= 831%) was possessed for 5-FU in concentration of 0,001 mg/ml and 24 h incubation time.

Bacteria cells co-administrated simultaneously with 5-FU and metformin exerted some influence on SFI. Parameters with the maximum point for SFI ($S_{gfpexp.}$ = 831%) were for 0,001 mg/ml of 5-FU and 0,3 mg/ml of M after 24 h incubation with both drugs. Metformin in 60% of applied concentrations significantly modulated 5-FU activity. It is seen, mainly at the 1; 0,1 and 0,01 mg/ml concentration of 5-FU and 0,3; 0,7 and 1 mg/ml of metformin, especially after 24 h incubation. In 40% of cases no significant differences in SFI between 5-FU and 5-FU+M were observed. The most frequently inhibition of SFI of 5-FU was detected after metformin administration at concentration of 0,7 and 1 mg/ml and for longer incubation (up to 24 h) (Fig. 2).

Simultaneous treatment of bacteria cells with 5-FU and metformin (at lowest concentration 0,3 mg/ml of metformin) compared to bacteria treated only with 5-FU significantly inhibited the SFI value of 5-FU at 1 mg/ml concentration and significantly stimulated at 5-FU concentration of 1; 0,001 and 0,0001 mg/ml. F_I values ≥ 2 were obtained for the all 5-FU concentration after 24 hours incubation. For co-administration of 5-FU+M for combination of 5-FU (0,001 mg/ml)+M (1 mg/ml) and 5-FU (0,0001 mg/ml)+M (1 mg/ml) the F_I values were below 2. $F_I \geq 2$ for metformin co-administrated with 5-FU in 75% of cases enhanced inhibition of SFI and decreased F_I values were observed as compared to the bacteria cells treated only with 5-FU.

Table. 1. SFI values for E. coli K-12 recA::gfp mut2 treated with 5-fluorouracil (5-FU), metformin (M) and combination of 5-fluorouracil and metformin (5-FU+M) in PBS buffer in three different metformin concentrations (0,3; 0,7; 1 mg/ml) in comparison with the control sample (bacteria strain in PBS buffer), T – time of bacteria strain incubation with drugs, F_I – induction factor values, $S_{gfpexp.}$ (%) – the percent of stimulation of gfp expression after treatment of bacteria cells with 5-FU and 5-FU+M in comparison with the control sample (100%)

5-FU (mg/ml)	**(M)** (mg/ml)	**T**	**Control sample** SFI±SD	**M** SFI±SD	**5-FU** SFI±SD	**F_I**	**$S_{gfp\ exp.}$ (%)**	**5-FU+M** SFI±SD	**F_I**	**$S_{gfp\ exp.}$ (%)**
1	0,3	3	18,54±1,77	17,02±1,54	39,33±3,90^{ab}	2,12	112	19,23±1,34^{**c}	- 4,07	- 307
		2			164,21±7,23^{ab}	5,06	406			
	0,7	4	32,45±3,56	33,12±3,34		2,12	112	132,23±4,25^{abc}	- 6,26	- 526
		3			39,33±3,90^{ab}	5,06	406			
	1	2	18,54±1,77	14,92±2,12^{a}	164,21±7,23^{ab}	2,12	112	30,0±3,35^{ab*}	- 6,04	- 504
		4				5,06	406	203,20±8,34^{abc}		
		3	32,45±3,56	31,73±3,16	39,33±3,90^{ab}					
		2		15,12±1,9228,13±3,55	164,21±7,23^{ab}			19,02±2,22^{**c}		
		4	18,54±1,77							
			32,45±3,56					196±5,35^{abc}		
0,1	0,3	3	18,54±1,77	17,02±1,54	22,32±1,45	- 9,92	- 892	19,36±2,33***	- 3,05	- 205
		2			322,0±8,91^{ab}					
	0,7	4	32,45±3,56	33,12±3,34	22,32±1,45^{*b}	- 9,92	- 892	99±3,65^{abc}		
		3		14,92±2,12^{a}	322,0±8,91^{ab}			15,40±1,83^{**c}	9,27	- 827
	1	2	18,54±1,77		22,32±1,45^{*b}	- 9,92	- 892	301±7,45^{ab*}	- 6,32	- 532
		4		31,73±3,16						
		3	32,45±3,56	15,12±1,92	322,0±8,91^{ab}			8,35±1,12^{abc}		
		2						205±4,55^{abc}		
		4	18,54±1,77	28,13±3,55						
			32,45±3,56							

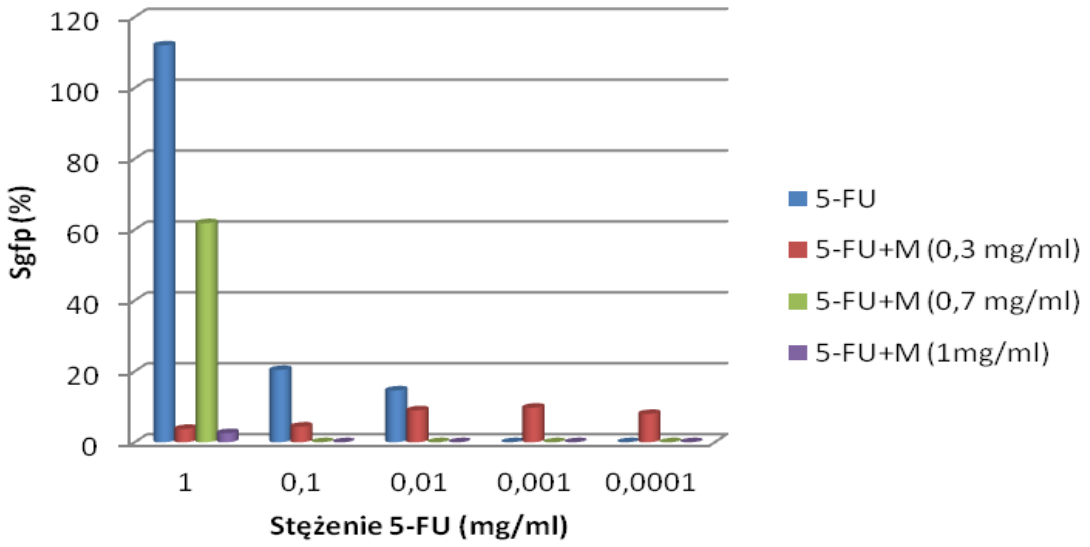
			32,45±3,56							
0,01	0,3 0,7 1	32 43 24 32 43 24	18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56	17,02±1,54 33,12±3,3 414,92±2,12[a] 31,73±3,16 15,12±1,9 228,13±3,55	21,25±2,45[**] 214,2±8,41[ab] 21,25±2,45[*b] 214,2±8,41[ab] 21,25±2,45[**] 214,2±8,41[ab]	-6,60 -6,60 -6,60	-560 -560 -560	20,21±2,20[***] 201±4,10[ab*] 15,22±2,34[**c] 207±6,30[ab*] 13,24±1,25[**c] 131±6,35[abc]	-6,20 -6,38 -4,04	-520 -538 -304
0,001	0,3 0,7 1	32 43 24 32 43 24	18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56	17,02±1,54 33,12±3,3 414,92±2,12[a] 31,73±3,1 615,12±1, 9228,13±3,55	18,16±2,33 116,2±4,16[ab] 18,16±2,33 116,2±4,16[ab] 18,16±2,33 116,2±4,16[ab]	-3,58 -3,58 -3,58	-258 -258 -258	20,35±2,15[***] 302±7,55[abc] 17,42±2,12[**] 101,33±7,10[a b*] 8,0±1,50[a*c] 50,33±5,30[*b c]	-9,31 -3,12 -	-831 -212 -
0,0001	0,3 0,7 1	32 43 24 32 43 24	18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56 18,54±1,77 32,45±3,56	17,02±1,54 33,12±3,3 414,92±2,12[a] 31,73±3,1 615,12±1, 9228,13±3,55	17,40±2, 12 114,30±6,33[ab] 17,40±2, 12 114,30±6,33[ab] 17,40±2, 12 114,30±6,33[ab]	-3,52 -3,52 -3,52	-252 -252 -252	20,02±2,55[***] 135±6,55[abc] 13,64±1,33[**] 103±5,60[abc] 12,11±1,66[**] 43±4,35[abc]	-4,16 -3,17 -	-316 -217 -

			18,54±1,77 32,45±3,56							

Source: Based on own data

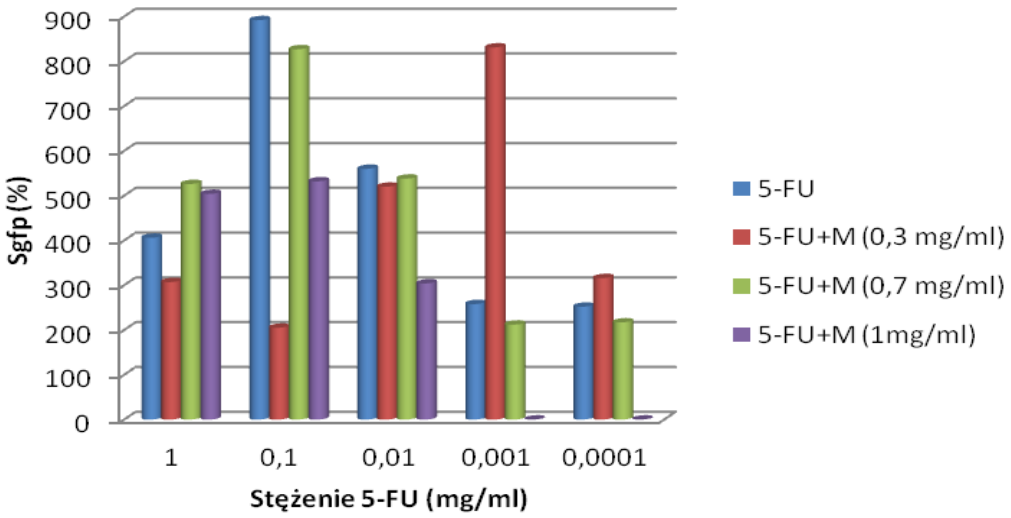
Mean values ± SD; n=6; a - significantly different from control ($p<0.05$); b - significantly different from metformin (M) group ($p<0.05$); c - significantly different from 5-fluorouracil (5-FU) group ($p<0.05$); * - no significantly different.

Fig. 2. The dynamic of the SFI values for E. coli K-12 recA::gfp mut2 treated with 5-FU and 5-FU with three concentrations of metformin (5-FU+M) after 3 hours incubation with drugs compared to control sample. SFI values is expressed as the percent of gfp gene fluorescence intensity (IF) stimulation of bacteria cells normalized by dividing by OD value. Mean values ± SD; n=6



Source: Based on own data

Fig. 3. The dynamic of the SFI values for E. coli K-12 recA::gfp mut2 treated with 5-FU and 5-FU with three concentrations of metformin (5-FU+M) after 24 hours incubation with drugs compared to control sample. SFI values is expressed as the percent of gfp gene fluorescence intensity (IF) stimulation of bacteria cells normalized by dividing by OD value. Mean values ± SD; n=6



Source: Based on own data

Bacteria cells incubated 24 h with the combination of 5-FU (at concentration of 0,001 and 0,0001 mg/ml) with 1 mg/ml of metformin resulted in a progressive decrease in F_I (below 2) and SFI values. For all applied concentrations of 5-FU there were no linear correlations between SFI values and 5-FU concentration. In the case of 5-FU+M (0,3 mg/ml), 5-FU+M (0,7 mg/ml) and 5-FU+M (1 mg/ml) the highest stimulation of SFI value was observed for 0,1 and 0,001 mg/ml of 5-FU and minimum for 0,1; 0,001 and 0,0001 mg/ml. Different reaction was noticed for 5-FU+M (1 mg/ml), where the strongest inhibition of SFI value was registered for 0,001 mg/ml of 5-FU and the highest stimulation of SFI for 0,1 mg/ml of 5-FU.

The monitoring of bacteria cultures growth (OD) (Fig. 4) at the start of bacteria treatment (time 0) and after 3 and 24 h incubation with drugs indicated significant increase in GI (growth inhibition) values for all tested concentration of metformin and 5-FU for 24 h treatment (Fig. 5). Addition of metformin to 5-FU and simultaneous action of the both drugs on bacteria cells significantly enhanced the growth inhibition values for 24 h incubation (Fig. 5).

There were no significant differences for shorter time (3h) of drugs influence on bacteria cells.

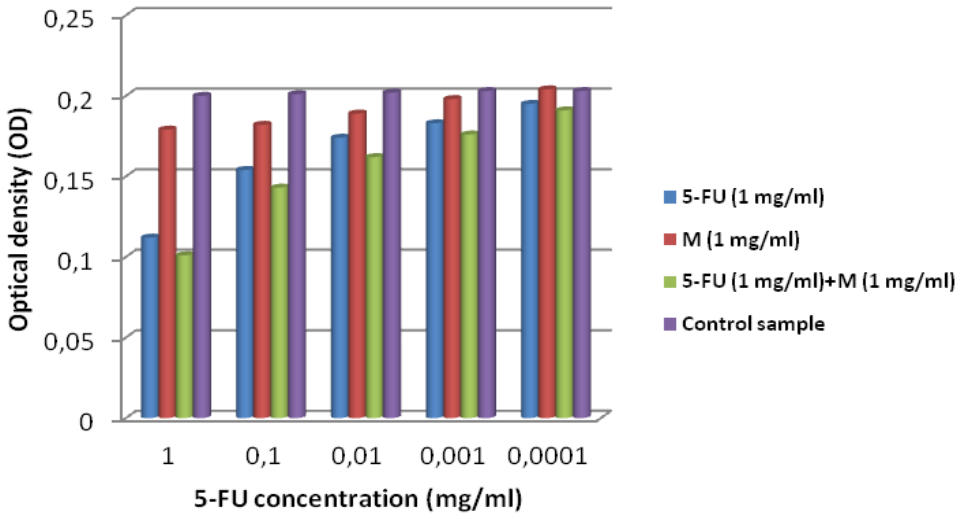
Prolonged treatment (up to 24 h) of bacteria cells with metformin at concentrations of 0,7 and 1 mg/ml significantly influenced the growth inhibition of bacteria. For 3 h of incubation there were no significant changes in OD values.

Bacteria incubation with PBS buffer (control sample) without any drugs addition resulted in no statistically differences in OD value from 0 to 24 hours continuous cultivation.

Treatment of gfp biosensor bacteria strain in surface water changed the sensitivity of recA::gfpmut2 genotoxic system and significantly decreased the stimulation of gfp expression and SFI value in comparison to incubation in PBS buffer (Tab. 2, Fig. 6). Prolonged treatment (up to 24 h) of bacteria cells with combination of 5-FU (0,001 mg/ml) and metformin (0,3 mg/ml) significantly influenced gfp expression with the maximum values for F_I=8,17 and 717% of S_{gfpexp} values comparable to control sample.

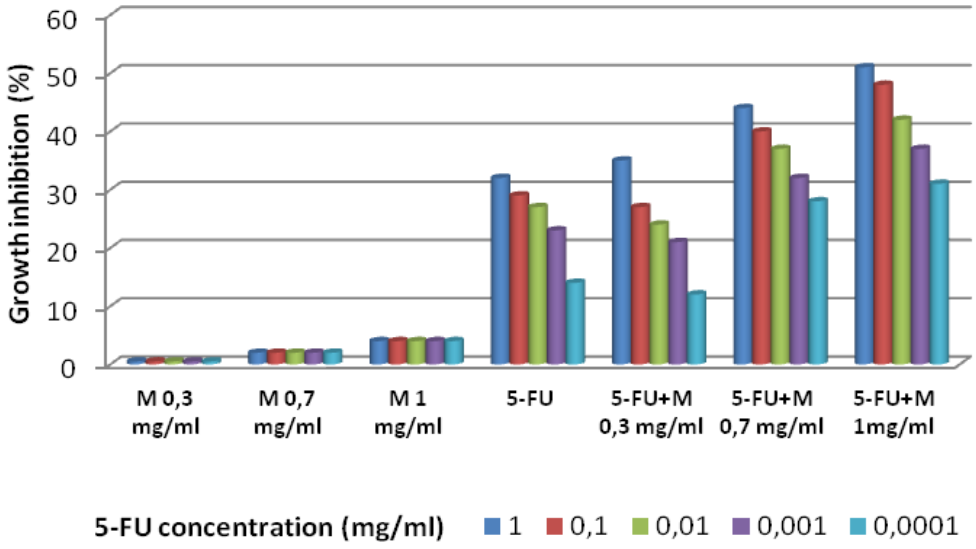
In this experiment, for assessment of genotoxic sensitivity of recA::gfp genetic biosensing system, 4% acetone was tested as negative control. For this chemical there was no increased in F_I values for 3 h and 24 h of incubation. Methylnitronitrosoguanidine (MNNG) – known genotoxin at concentration of 50 μM - was used as positive control. For this analyte F_I=8,4 for 24 h incubation time and F_I=2,8 for 3h (data not shown). These results proved stronger sensitivity of recA::gfp biosensing system for MNNG than for acetone stressor.

Figure 4. The dynamic of Optical Density (OD) value (600 nm) of E. coli K-12 recA::gfp cells after 24 hours of incubation with 5-FU (1 mg/ml), metformin (1 mg/ ml) and simultaneous incubation with 5-FU and metformin (5-FU (1 mg/ml)+M (1 mg/ml)) compared to control sample not-treated with drugs.
Mean values ± SD; n=6



Source: Based on own data

Figure 5. The comparison of growth inhibition (GI) values of E. coli K-12 recA::gfp cells after 24 hours of incubation with 5-FU, metformin and simultaneous incubation with 5-FU and metformin (5-FU+M) compared to control sample not-treated with drugs. Mean values ± SD; n=6



Source: Based on own data

Currently, two main groups of drugs are used: cytotoxic drugs and endocrine therapy drugs. Due to their intended function, the first ones could exert cytotoxic, genotoxic, mutagenic, carcinogenic or teratogenic effects on aquatic species [1-3]. Moreover, as they often act with structure and function of DNA, some authors consider that all eukaryote organisms might be susceptible to their toxicity. Various drugs are capable of damaging the DNA and triggering genotoxic and mutagenic effect in the living cells. If not repaired, or if produced in excessive amounts, damaged DNA can initiate a cascade of biological cellular, organic or individual effects, being in the consequence carcinogenesis [1-10]. Cytostatic anticancer drugs are an increasingly important issue in the environmental debate, mainly due to the lack of knowledge about the fate of these toxic substances. Over the last decades, 5-fluorouracil (5-FU) has been one of the most frequently used antineoplastic agents and may, therefore, be regarded as one of the pilot substances for environmental contamination. In 1997, the consumption of 5-FU in Austria amounted to 119 kg. During the last years, the consumption of drugs (including cytostatics) in Poland increased, too. For this reason, 5-FU may be regarded as one of the pilot cytostatics for the assessment of environmental contamination originating from hospital effluents [1].

Genotoxicity testing is used as a preclinical safety assessment tool to screen newly synthesized drug candidates based on the detection of potential carcinogenicity and heritable

mutations based on their responses to genotoxic actions. Genotoxicity of anticancer drugs is one of their most serious side effects due to the possibility of inducing secondary malignancies, so it is very important to develop new and improve current biological assays for anticancer drug genotoxic activity detection [1-5, 50] .

This study demonstrates that with the help of bacterial promoter elements, recA and in genetic fusion of gfpmut2 in E. coli K-12 strains biosensor, it is possible to predict and detect the potential mode of genotoxicity caused by anticancer drug – 5-fluorouracyl. This genotoxicity assay has the potential to be a useful tool in the analysis of unknown chemicals or novel drugs that are of health and environmental concerns. It was concluded that bacteria strains which were apply in this experiment could be potentially useful as a good alternative tool for the first-step screening of genotoxicity effect of anticancer drugs as a genotoxic biosensors.

5-FU was developed in a basic science laboratory, intensively studied biochemically, screened through animal tumors, and brought forth for clinical evaluation in human tumors. It became apparent that 5-FU was able to produce objective remissions in a variety of malignant tumors. It was expected that 5-FU would be damaging to any cells with a high replication rate and, consequently, would damage the mucosa of the small bowel along with the oral mucosa, hair follicles, nails, etc. It would not be unexpected that a denudation of small bowel mucosa might invoke both epithelial regeneration and increased DNA synthesis and accompanying neovascular proliferation [20-24].

Results of this study indicated that treatment of bacteria cells with 5-FU and mixture of 5-FU and metformin (5-FU+M) lead to above 9-fold stimulation (F_I=9,92 in the case of 5-FU and F_I=9,31 in the case of simultaneous coadministration of 5-FU and metformin) of bacteria genotoxin-sensitive recA promoter and gfp gene expression. The variable levels of efficiency of gfp expression are bioindicators of 5-FU genotoxic effect generation potency. Presented study with use of E. coli K-12 with genetic construct recA::gfp indicated 5-FU and metformin dose- and time-dependent ability on reaction with recA promoter.

Table. 2. SFI values for E. coli K-12 recA::gfp mut2 treated with 5-fluorouracil (5-FU) at concentration of 0,001 mg/ml, metformin (M) at concentration of 0,3; 0,7 and 1 mg/ml and combination of 5-fluorouracil (0,001 mg/ml) and metformin (5-FU+M) in surface water in three different metformin concentrations (0,3; 0,7; 1 mg/ml) in comparison with the control sample (bacteria strain in surface water), T – time of bacteria strain incubation with drugs, F_I – induction factor values, $S_{gfpexp.}$ (%) – the percent of stimulation of gfp expression after treatment of bacteria cells with 5-FU and 5-FU+M in comparison with the control sample (100%)

(M) (mg/ml)	T	Control sample SFI±SD	M SFI±SD	5-FU SFI±SD	F_I	$S_{gfpexp.}$ (%)	5-FU +M SFI±SD	F_I	$S_{gfpexp.}$ (%)
0,3 0,7 1	3 24 3 24 3 24	21,15±2,22 43,20±3,21 21,15±2,22 43,20±3,21 21,15±2,22 43,20±3,21	19,33±1,2*3,23±2,87[a] 18,13±2,1231,22±3,22[a] 17,24±2,45[a] 30,24±3,35[a]	25,20±2,85*[b] 170,5±7,15[ab] 25,20±2,85*[b] 170,5±7,15[ab] 25,20±2,85*[b] 170,5±7,15[ab]	-3,95 -3,95 -3,95	-295 -295 -295	22,85±2,95*** 353±8,33[abcA] 22,82±3252*** 113,24±8,33[abcA] 15,23±1,65[c] 60,23±7,35[abcA]	-8,17 -2,62 -	717 -162 -

Source: Based on own data

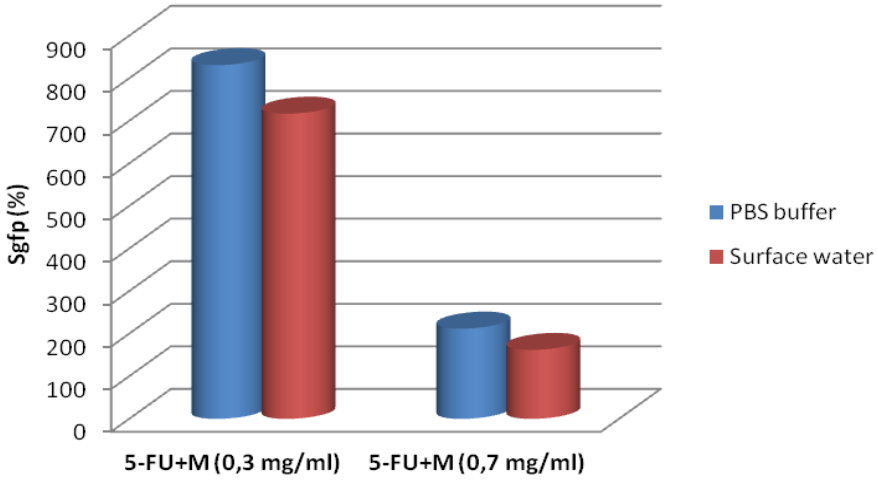
Mean values ± SD; n=6; a - significantly different from control ($p<0.05$); b - significantly different from metformin (M) group ($p<0.05$); c - significantly different from 5-FU group ($p<0.05$); A - significantly different from 5-FU+metformin (5-FU+M) group in PBS buffer ($p<0.05$); * - no significantly different.

Results obtained in the experiment are in agreement with studies of Kostrzyńska et al., 2002 [41]; Norman et al., 2005 [39]; Ptitsyn et al., 1997 [48] and some others, who presented data, that reporter gene systems (with gfp and lux reporters) were sensitive and useful for measurement of genotoxins, drugs and various chemicals in environmental studies [43-44].

Previous studies showed that recA promoter was induced by selected anticancer drugs [43]. An important group of anticancer agents belong to the family of antimetabolites. 5-Fluorouracil is the most relevant agent from this group and is one of the most frequently used antineoplastics in cancer therapy. Cytostatic agents act by either inhibiting cell growth or

directly killing cells. Because of these reaction mechanisms, many antineoplastic agents have cytotoxic, mutagenic and/or teratogenic effects [1].

Figure 6. The comparison of S_{gfp} (%) values for E. coli K-12 recA::gfp cells after 24 hours of incubation with 5-FU in concentration of 0,001 mg/ml co-administrated with metformin in PBS buffer and surface water. Mean values ± SD; n=6



Source: Based on own data

According to results obtained in this experiment metformin in simultaneous co-administration with 5-FU modulate the reactivity of recA-oxidative stress promoter in relation to 5-FU in E. coli K-12 recA::gfp mut2 living bacteria cells.

The mechanisms of metformin action on DNA molecules is still unknown. Results, from in vivo and in vitro studies so far undertaken, are controversial. While some reports indicated no genotoxic effects, others have assumed that metformin can produce oxidative stress due to DNA fragmentation [51-55]. The results of above experiment provided the conformation of the possible influence of metformin on the genes, similarly as Anedda et al., (2008) [33] and Amador et. al. 2012 [34]. In 100% of cases there were significant differences (comparable to the control sample) in the level of recA promoter sensitivity and gfp expression after bacteria treatment with the all applied concentrations of metformin and for short (3 h) and longer time of incubation (up to 24 h).

Kefas et. al. 2004 [53] studies indicated that activity of metformin is dose- and time-dependent. It was also confirmed by data obtained in our studies. Longer metformin exposure (up to 24 h) resulted in a progressive inhibition of 5-FU influence on recA promoter and gfp gene expression. F_I values ≥ 2 were obtained for 5-FU+M after 24 hours incubation, but not for 3 h. Only in one case of 3 h incubation of E. coli K-12 recA::gfp with 1 mg/ml of 5-FU triggered

the F_I value above 2 (F_I=2, 12). Generally, the strongest inhibition of gfp expression by 5-FU was noticed more frequently for adding of 0,1; 0,01; 0,001; 0,0001 mg/ml of metformin than for 1 mg/ml concentrations of antidiabetic drug. The highest concentrations of 5-FU (1 mg/ml) and metformin (1 mg/ml) stimulated SFI values. But 0,1 mg/ml of 5-FU with connection with 1 mg/ml of metformin inhibited SFI value. It suggests, the possible dose-dependent modulation of transcription of recA::gfp genetic construct by the higher concentration of the drugs.

According to some authors, the mechanisms of metformin effects on DNA molecules might possibly be mediated through its activation of AMPK, thereby increasing nitric oxide synthase [53]. Zou et al., (2004) [55] speculated that mitochondria-derived reactive-nitrogen-species mediate AMPK activation by way of metformin. Depending on the dose, nitric oxide is capable of inducing beneficial effects by playing a role in the gene regulation and signal transduction pathways possibly involved in defensive mechanisms against oxidative stress [53].

In the light of earlier reports and results of above experiment during metformin metabolism in living cells direct or indirect protective mechanisms against 5-FU induced oxidative stress may occur.

Our results showed 5-FU E. coli K-12 longer (up to 24 h) treatment significantly inhibited bacteria cells growth. Either metformin has cytotoxic effect by inhibition of bacteria cells growth for highest applied concentrations and 24 h treatment. Co-administration of metformin with 5-FU importantly, dose-dependently intensified 5-FU cytotoxic effect on living bacteria cells after 24 hours incubation. Our results are in agreement with earlier empirical studies of the other authors, who demonstrated that co-administration of metformin with chemotherapeutic agents intensified the inhibition of cancer cells proliferation and significantly improved cytostatic-induced cytotoxicity [29-34]. Janjetovic et. al. 2011 [52] have assumed that metformin can produce oxidative stress due to DNA fragmentation. DNA damage can initiate a cascade of cellular biological effects including cell death. The direct and indirect metformin influence on DNA could be the main mechanisms of its cyto- and genotoxicity.

We obtained the lighter reactivity of recA::gfpmut2 genetic system in surface water for drugs treated samples than in PBSbuffer. Probably it was a consequence of the inhibition of transcription by the presence of different chemicals in surface water which could influence gfp expression in bacteria strain. It is important, therefore, to check all river's water (specially in the hospital's surroundings) for the presence of drugs from these groups of chemicals,

The toxicity of 5-FU and metformin is well-known and established in experimental studies on bacteria, human cells and other organisms. In above experiment we applied E. coli K-12 bacteria cells as a model organism for genotoxic studies. Obtained data, are generally in

agreement with other results which were previously detected in in vivo and in vitro tests of higher organisms, including human cells.

4. Conclusions

1. The results of the presented study indicated that recA::gfpmut2 genetic system was sensitive for applied in experiment drugs and drugs mixture.
2. RecA promoter was a good bioindicator for cytotoxic and genotoxic effect screening of 5-FU, metformin and the mixture of the both drugs in PBS buffer and surface water.
3. The results indicated that E. coli K-12 recA::gfp mut2 strain could be potentially useful for first-step screening of cytotoxic and genotoxic effect of anticancer and antidiabetic pharmacist residues in water.
4. The validation of recA::gfpmut2 genetic system in E. coli K-12 demands more experimental analysis, which should be focused on the spectrofluorometry fluorescence screening of recA::gfpmut2 sensitivity after treatment of bacteria cells with anticancer drugs (or drugs candidate) from different groups of its biological mechanisms activity.

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References

1. Besse J.-P., Latour J. F. Garric J.: Anticancer drugs in surface waters.: What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs?, Environ. Intern., 39 (2012) 73–86.
2. Mahnik S. N., Rizovski B., Fuerhacker M., Mader R. M.: Determination of 5-fluorouracil in hospital effluents. Anal Bioanal Chem, 380 (2004) 31–35.
3. Zhang J., Chang V. W. C., Giannis A., Wang J. Y.: Removal of cytostatic drugs from aquatic environment: A review., Scien. Tot. Environ., 445–446 (2013) 281–298.
4. Kosjek T., Heath E.: Occurrence, fate and determination of cytostatic pharmaceuticals in the environment, Tr. AC. Trends Anal. Chem., 30 (2011) 1065–87.
5. Kümmerer K.: Pharmaceuticals in the environment, Annu. Rev. Environ. Resour., 35 (2010) 57–75.

6. Escher B. I., Baumgartner R., Koller M., Treyer K., Lienert J., McArdell C. S.: Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater, Water. Res., 45 (2011) 75–92.
7. Fent K., Weston A. A., Caminada D.: Ecotoxicology of human pharmaceuticals, Aquat. Toxicol., 76 (2006) 122–59.
8. Ferk F., Misik M., Grummt T., Majer B., Fuerhacker M., Buchmann C., et al.: Genotoxic effects of wastewater from an oncological ward, Mutat. Res. Genet. Toxicol. Environ. Mutagen., 672 (2009) 69–75.
9. Fick J., Soderstrom H., Lindberg R. H., Phan C., Tysklind M., Larsson D. G. J.: Contamination of surface, ground, and drinking water from pharmaceutical production, Environ. Toxicol. Chem., 28 (2009) 2522–7.
10. Hernando M. D., Mezcua M., Fernandez-Alba A. R., Barcelo D.: Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, Talanta 69 (2006) 334–42.
11. Johnson A. C., Jurgens M.D., Williams R. J., Kümmerer K., Kortenkamp A., Sumpter J.: Do cytotoxic chemotherapy drugs discharged into rivers pose a risk to the environment and human health?, An overview and UK case study, J Hydrol., 348 (2008) 167–75.
12. Khetan S. K., Collins T. J.: Human pharmaceuticals in the aquatic environment: a challenge to green chemistry, Chem. Rev., 107 (2007) 2319–64.
13. Kolpin D. W., Furlong E. T., Meyer M. T., Thurman E. M., Zaugg S. D., Barber L. B., et al.: Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance, Environ. Sci. Technol., 36 (2002) 1202.
14. Larsen T. A., Lienert J., Joss A., Siegrist H.: How to avoid pharmaceuticals in the aquatic environment, J Biotechnol., 113 (2004) 295–304.
15. Schulman L. J., Sargent E. V., Naumann B. D., Faria E. C., Dolan D. G., Wargo J. G.; A human health risk assessment of pharmaceuticals in the aquatic environment, Hum. Ecol. Risk. Assess., 8 (2002) 657–80.
16. Gęgotek A., Markowska A., Luczaj W., Bielawska A., Bielawski K, Ambrozewicz E., Skrzydlewska E, Effects of dinuclear berenil-platinum (II) complexes on fibroblast redox status, Adv. Med. Sci., 58 (2013) 63-72.
17. Fuks L., Samochocka K., Anulewicz-Ostrowska R., Kruszewski M., Priebe W., Lewandowski W.: Structure and biological activity of cationic[PtLCl(DMSO)]NO(3).DMSO complex containing a chelated diaminosugar:

methyl-3,4-diamino-2,3,4,6-tetradeoxy-alpha-L-lyxopyranoside, Eur. J Med. Chem., 38(7-8) (2003) 775-80.

18. Kruszewski M., Bożyk E., Oldak T., Samochocka K., Fuks L. ,Lewandowski W., Fokt I., Priebe W.: Differential toxic effect of cis-platinum(II) and palladium(II) chlorides complexed with methyl 3,4-diamine-2,3,4,6-tetradeoxy-alpha-L-lyxo-hexopyranoside in mouse lymphoma cell lines differing in DSB and NER repair ability, Teratog. Carcinog. Mutagen., 1 (2003) 1-11.
19. Samochocka K., Lewandowski W., Priebe W., Fuks, L.: Vibrational studies of Pt(II) and Pd(II) complexation by 3, 4-diamino lyxo-hexopyranoside, J Mol. Struct., 614 (2002) 203-209.
20. Samochocka K., Fokt I., Anulewicz-Ostrowska R., Przewloka, T., Mazurek A. P., Fuks L., Lewandowski W., Kozerski L., Bocian W., Bednarek E., Lewandowska H, J.: Sitkowski and W. Priebe, Platinum (II) and palladium (II) complexes with methyl 3, 4-diamino-2,3,4,6-tetradeoksy-α-L-lyxo-hexopyranoside, Dalton. Trans., 11 (2003) 2177-2183.
21. Capitain O., Boisdron-Celle M,. Poirier A. L., Abadie-Lacourtoisie S., Morel A.,Gamelin E.: The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. Pharmacogenomics J8 (2008) 256 – 267.
22. Ciaparrone M., Quirino M., Schinzari G., Zannoni G., Corsi D. C., Vecchio F. M., Cassano A., La Torre G., Barone C.: Predictive role of thymidylate synthase, dihydropyridine dehydrogenase and thymidine phosphorylase expression in colorectal cancer patients receiving adjuvant 5-fluorouracil.Oncology70 (2006) 366 – 377
23. Codacci-Pisanelli G., Pinedo H. M., Lankelma J., Van Groeningen C. J.,Van Kuilenburg A. B., Van Gennip A. H., Peters G. J.: Pharmacokinetics of bolus 5-fluorouracil: relationship between dose, plasma concentrations, area-under-the-curve and toxicity. J Chemother 17 (2005) 315 – 320 Cohen V, Panet-
24. Di Paolo A., Ibrahim T., Danesi R., Maltoni M., Vannozzi F., Flamini E., Zoli W., Amadori D., Del Tacca M. Relationship between plasma concentrations of 5-fluorouracil and 5-fluoro-5,6-dihydrouracil and toxicity of 5-fluorouracil infusion in cancer patients. Ther Drug Monit 24 92002) 588 – 593
25. Di Paolo A., Lencioni M., Amatori F., Di Donato S., Bocci G, Orlandini C., Lastella M., Federici F., Iannopollo M., Falcone A., Ricci S., Del Tacca M., Danesi R.: 5-fluorouracil pharmacokinetics predicts disease –free survival in patients administered adjuvant chemotherapy for colorectal cancer. Clin Cancer Res14 (2008) 2749– 2755

26. Dotor E., Cuatrecases M., Martines-Iniesta M., Navarro M., Vilardell F., Guino` E., Pareja L., Figueras A., Mollevı`D. G., Serrano T., de Oca J., Peinado M. A., Moreno V., Germa`J. R., Capella `G., Villanueva A.: Tumor thymidylate synthase 1494del6 genotype as a prognostic factor in colorectal cancer patients receiving fluorouracil-based adjuvant treatment.J Clin Oncol, 24 (2006) 1603– 1611
27. Gamelin E. C., Danquechin-Dorval E. M., Dumesnil Y. F., Maillart P. J., Goudier M. J., Burtin P. C., Delva R. G., Lortholary A. H., Gesta P. H., Larra F. G.: Relationship between 5-fluorouracil (5-FU) dose intensity and therapeutic response in patients with advanced colorectal cancer receivinginfusional therapy containing 5-FU. Cancer 77 (1996) 441 – 451
28. Gill S., Loprinzi C. L., Sargent D. J., Thome´ S. D., Alberts S. R., Haller D. G. ,Benedetti J., Francini G., Shepherd L. E., Francois Seitz J., Labianca R., Chen W., Cha S. S.: Heldebrant M. P., Goldberg R. M.. Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III Survival and toxicity determinants of adjuvant fluorouracil. British Journal of Cancer 100(10) (2004) 1549 – 1557.
29. Quinn B. J., Kitagawa H., Memmott R. M., Gills J. J., Dennis P. A.: Repositioning metformin for cancer prevention and treatment, Trends. Endocrinol. Metabol., 24 (9) (2013) 469-480.
30. Zhang Y., Guan M, Zheng Z., Zhang Q., Gao F., Xue Y.: Effects of Metformin on CD133+ Colorectal Cancer Cells in Diabetic Patients. PLOSone, 8 (2013) 2-8.
31. Rizos Ch. V., Elisaf M. S.: Metformin and cancer, Eur. J Pharm., 705 (2013) 96-108.
32. Scheurer M., Sacher F., Brauch H. J.: Occurrence of the antidiabetic drug metformin in sewage and surface waters in Germany, J Environ. Monit., 11 (9) (2009)1608-13.
33. Anedda A., Rial E., González-Barroso E. E.: Metformin induces oxidative stress in white adipocytes and raises uncoupling protein 2 levels, J Endocrinol., 199 (2008) 33–40.
34. Amador R R., Longo J. P. F., Lacava Z. G., Dorea J. G., Almeida Santos M DE F. M., Metformin (dimethyl-biquanide) induced DNA damage in mammalian cells, Gen. Mol. Biol., 35 (1) (2012) 153-158.
35. Hendriks G., Atallah M., Raamsman M., Morolli B., Van der Putten H., Jaadar H., Tijedens I., Esveldt-van Lange R., Mullenders L., Van de Water B., Vrieling H.: Sensitive DsRed fluorescence-based reporter cell systems for genotoxicity and oxidative strees assessment, Mutat. Res., 709-710 (2011) 49-59.
36. Biran A, Ben H., Yoav, S. Yagur-Kroll, R. Pedahzur, S. Buchinger, Y. Shacham-Diamand, G., Reifferscheid S.: Belkin, Microbial genotoxicity bioreporters based on sulA activation, Anal. Bioanal. Chem., 400 (9) (2011) 3013-24.

37. Biran A., Yagur-Kroll S., Pedahzur R., Buchinger S., Reifferscheid G., Ben Yoav H., Shacham-Diamand Y., Belkin S.: Bacterial genotoxicity bioreporters, Microb. Biotechnol., 3 (4) (2010) 412-27.
38. Reifferscheid G., Buchinger S.: Cell-based genotoxicity testing: genetically modified and genetically engineered bacteria in environmental genotoxicology, Adv. Biochem. Eng. Biotechnol., 118 (2010) 85-111.
39. Norman A., Hansen L. H., Sørensen S. J.: Construction of a ColD cda promoter-based SOS-Green fluorescent protein whole-cell biosensor with higher sensitivity toward genotoxic compounds than constructs based on recA, umuDC, or sulA promoters, Appl. Environ. Microbiol, 71 (5) (2005) 2338–2346.
40. Albano C. R., Lu A., Bentley W. E., Rao G.: High throughput studies of gene expression using green fluorescent protein-oxidative strees promoter probe constructs, J Biomolec. Screen., 1, 6, 6, (2001) 421-428.
41. Kostrzyńska M, Leung K. T., Lee H., Trevors J. T.: Green fluorescence protein based biosensor for detecting SOS-inducing activity of genotoxic compounds, J. Microbiol. Meth., 48 (2002) 43-51.
42. Park M., Tsai S. L., Chen W.: Microbial Biosensors: Engineered microorganisms as the sensing machinery, Sensors, 13 (2013) 5777-5795.
43. Cha H. J., Srivastava R., Vakharia V., Rao G., Bentley W.: Green fluorescent protein as a noninvasive strees probe in resting Escherichia coli cells, Appl. Environm. Microbiol., 65 (1998) 409-414.
44. Wei T., Zhang C., Xu X., Hanna M., Zhang X., Wang Y., Dai H., Xiao W.: Construction and evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals, Biosens Bioelectron., 44 (2013) 138–145.
45. Gu M. B., Mitchell R. J., Kim B. C.: Whole-cell-based biosensors for environmental biomonitoring and application, Adv. Biochem. Engin. Biotechnol., 87 (2004) 269-305.
46. Matejczyk M.: The Potency of application of microbial biosensors. (In Polish), Adv. Microbiol., 49 (4) (2010) 297-304.
47. Zaslaver A, Mayo A. E., Rosemberg R., Bashkin P., Sberro H., Tsalyuk M., Surette M., Alon U.: Just-in-time transcription program in metabolic pathways, Nat. Genet., 36 (5) (2004) 486 – 491.
48. Ptitsyn L. R., Horneck G., Komova G. O., Kozubek S., Krasavin E. A., Bonev M., Rettberg P.: A biosensor for environmental genotoxin screening based on an SOSlux assay in recombinant Escherichia coli cells, Appl. Environm. Microbiol., 63 (1997) 4377–4384.

49. Xu T., Close M. D., Sayler G. S., Ripp S.: Genetically modified whole-cell bioreporters for environmental assessment, Ecolog. Indic., 28 (2013) 125-141.
50. Kim H. J., Lee J. H., Kim S. J., Oh G. S., Moon H. D., Kwon K. B., Park C., Park B. H., Lee H. K., Chung S. Y., Park R., So H. S.: Roles of NADPH oxidases in cisplatin-induced reactive oxygen species generation and ototoxicity, J Neurosci., 17: 30 (11) (2010) 3933-46.
51. Attia S. M., Helal G. K., Alhaider A. A.: Assessment of genomic instability in normal and diabetic rats treated with metformin, Chem. Biol. Interact., 180 (2009) 296–304.
52. Janjetovic K., Harhaji-Trajkovic L., Misirkic-Marjanovic M., Vucicevic L., Stevanovic D., Zogovic N., Sumarac-Dumanovic M., Micic D., Trajkovic V.: In vitro and in vivo anti-melanoma action of metformin, Eur. J Pharmacol., 668 (2011) 373–382.
53. Kefas B. A., Cai Y., Kerckhofs K., Ling Z., Marten G., Heimberg H., Pipeleers D., Van de Casteele M.: Metformin-induced stimulation of AMP-activated protein kinase in beta-cells impairs their glucose responsiveness and can lead to apoptosis, Biochem. Pharmacol., 68 (2004) 409–416.
54. Za'tara, G. J., Bar-Tana, B., Kalderon, M., Suter, E., Morad, D., Samovski, D., Neumann P., Hertz R., AMPK activation by long chain fatty acyl analogs, Biochem. Pharmacol., 76 (2008) 1263–1275.
55. Zou M. H., Kirkpatrick S. S., Davis B. J., Nelson J. S., Wiles W. G., Schlattner U., Neumann D., Brownlee M., Freeman M. B., Goldman M. H.: Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species, J Biol. Chem., 279 (2004) 43940–43951.

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Modern control systems rainwater in built up areas

Key words: *stormwater management, urbanized watershed, runoff, hydrograph*

Abstract: The literature review of contemporary stormwater management practices at the urbanized watersheds is presented in the paper. It is fulfilled the classification of these methods depending their principle; there are analyzed the advantages and lacks of the best management practices. Problems of the stormwater sewerage system modeling are considered.

1. Introduction

Clean, fresh water is one of the most important natural resources, necessary for human development. Acute shortage of fresh water in some regions of the world and catastrophic floods in others, high pollution runoff and large hydraulic load on a system drainage, and the basins, causeactive search of effective ways to control rainwater, cleaning and management use of surface wastewater.

The importance of issues underlines the fact 39 UN declared 2005-2015 years International Decade for Action "Water for life "[1].

Significant changes in the hydrological balance of urban areas occur due to the constant increase in the area with waterproof coating (roofs, roads, sidewalks, etc.) and a corresponding decrease in natural filtering soils. Increasing the volume of rainwater in open waters, high pollution areas of cities and industry man-made pollution causing congestion existing sewer systems, reducing the effectiveness of their work, flooding in urban areas, worsening health status of water bodies where discharged rainwater [2, 3]. To address these issues should be performed comprehensive regulation of rainwater. The aim of this paper is to analyze the most common in the world of engineering practice management systems rainwater in built up areas.

2. Modern management techniques rainwater

Regulation of rainwater has become an important public problem in countries like USA, Canada, Germany, United Kingdom, Australia and others. In the western scientific and technical literature is firmly entrenched The term "control rain sewage." According to analysts,

one of the main features of the city water management is the situation faced by each individual city, even in the same country and in the same region is unique.

Theoretical and experimental investigation of the formation and regulating the flow of rainwater from urban areas involved Adams B., O. Akan, MI Alekseev, Babchenko IV, Belov NN, VA Bolshakov, Gorbachev PF, Guo J., James W., J. Dzopak, Dykarevskyy V.C., Kalitsun VI, Kitaev AL, Korinko IV, Kurganov AM, Pantelyat GS, R. Horton, W. Huber, T. Schuler, Y. Yaroshenko and others [4, 5, 6, 7, 8, 9].

In the design of new and reconstruction of existing systems Rainfall Wastewater is now important to move away from the principle of "as soon as possible collect surface wastewater, refer them to cleanse and drop body of water. Much more effective are measures of adjustment and use Rainfall runoff. In the United States for over 20 years acting regulatory requirements limit the growth of the maximum flow and surface runoff after building area.

Ukraine is in a zone of moderate continental climate, Range average annual precipitation layer in the state is relatively small: an average of 400 ... 500 mm of precipitation per year for southern, south-eastern regions and the Crimea to 800 ... 1000 mm Carpathian and Transcarpathia. However, existing territorial imbalances stocks freshwater different population density and concentration of industrial facilities already causing acute shortage of water in the eastern regions of the country, and western regions regularly suffer from floods [10].

It should be noted that for Ukraine of water supply is one of the lowest among European countries, whereas in hygroscopic gross national product is a first. Stock of local water resources in Ukraine is about 1,100 m^3 streamflow per person per year. For comparison, in Germany and Sweden - 2500 m^3, France - 3500 m^3 UK - 5000 m3, Belarus -5700 m^3, in the European part of Russia - 5900 m^3 [11].

In Ukraine practice on regulating, clearing and use of rainwater now received little attention. Majority big cities in Ukraine have overall or combined drainage system. Overall hydraulic system burden on sewers and buildings increases dramatically during the rainy season(10 times), which causes a temporary overflow drainage networks and overload sewage treatment plants (WWTP). Many small cities of Ukraine have rain drainage system partially or completely do not. Domestic and foreign research of the surface flow quality showed that in many ways it approaches contamination, and sometimes higher than the corresponding figures for household waste treatment [2, 3]. The crude runoff became a threat to the ecological status the settlement and for the state of water bodies. Often CBS offer to increase capacity to provide clean mix of domestic, industrial and surface waste water in clearing the rain. However, detailed analysis shows that the way in most cases is inefficient. Much better and cost advantageous is

to perform regulation of rainwater inlet cleaning, building and throughout the drainage basin. Management of rainfall flow allows more efficient use of water resources, improves operational performance drainage system and normalizes hydrological regime of the river, which is the discharge of treated wastewater.

Today scientists write about the most common operational practices in rain drainage as are : the establishment storage and regulating reservoirs rain wastewater (EDIS) eksfiltration building structures (pits, trenches, pools, etc.); advanced placement porous coating and green roofs, special ponds, artificial Mochara more. Table. 1 shows the classification of rainwater management methods, depending on how they work.

Some methods involve complex application of all three principles in the same building. Table. 2 shows main advantages and disadvantages of various management of rainwater.

Table 1. Classification rainwater management on the basis of work

Name structures	The principle of operation		
	accumulation	temporary detention	filtration
Rain tanks, tank; Storage tanks	+		
Regulatory tanks		+	
Ex Filtration Building			+
Green roofs		+	+
The increased roughness of the roof; performances and gravel backfill.		+	
Porous improved coverage			+
Open areas and lawns; vegetable strips; planted with grass ditches and pits		+	+
Bio Storage containers (open ponds)	+		+
Micro Landscape design; terraces	+	+	+

One of the most common means of regulating rainwater tanks is to use rainwater sewage (EDIS) types. With EDIS there is possible accumulation or runoff temporary detention, which

reduces the size of the reservoir, the performance of pumping stations and treatment plants, increases reliability and environmental safety of rain drainage.

Today, the global engineering practice developed and used a variety of designs multisection EDIS: from serial, parallel connection of cameras, multi [3, 4, 5]. EDIS provide water accumulation, take up little space of land on reservoir and can be used for other purposes, which is very important in urban environment. To adjust the volume costs output of EDIS used so-called output devices, such as type Hydroslide [12]. A promising solution is to reservoirs, wells, for example, type Stormceptor [13], whereby the adjustment costs and pre-treatment most contaminated portions of the first runoff.

Table 2. Comparison of different methods of managing rainwater

The name of the method or structure	Advantages	Disadvantages
Rain tanks, tanks, storage tanks	The accumulation and savings water; take up little space; ground tank can be used for other purposes.	The high cost of installation and operation; relatively small capacity.
Regulatory tanks	Detention runoff, reduce the maximum flow; Previous cleaning runoff.	The high cost of construction and operation.
Ex Filtration Building	Reduction of the flow volume and maximum flow; replenishment of groundwater; cleaning runoff.	Driving filtration medium in time; threat of groundwater contamination.
Green roofs	Reducing the flow volume and maximum flow; runoff retention time; aesthetic appearance; air purification; thermoregulation; fire aspect.	Increased load on constructions; possible contamination of the system and the roof leaks; the problem of freezing in winter.

The increased roughness of the roof; performances and gravel backfill.	Detention flow.	Additional cost of the device and increasing construction activity.
Porous improved coverage.	Reducing the volume of runoff; replenishment of groundwater; cleaning runoff.	High construction and operating costs; Pile possible, soil compaction; threat of groundwater contamination; swelling coverage when it freezes.
Open areas and lawns; vegetable strips; planted with grass ditches and holes.	Detention flow; a slight decrease in the volume of runoff; aesthetic appearance; cleaning runoff.	The need for large land areas; security; large operating expenses
Bio Storage containers (open ponds)	Adjustable flow with large areas; aesthetic appearance; versatility; cleaning runoff; potential increase in the value of land.	Require large areas land; contamination ponds and eutrophication; place breeding of insects and pests; safety problems operation.
Mikrolandshaft not modeling; terraces	Detention and decrease volume flow; aesthetic appearance; reduction pollution; water saving.	The high cost of design, construction and operation

Widespread in the world rainwater management techniques gained filters. Their work is exfiltration of runoff into the soil using exfiltration pits, trenches, grassy lowlands vegetation filter strips of perforated pipes [3, 5]. A promising method is advanced placement permeable porous asphalt surface type and porous pavers.

The last two decades actively studied and widely implemented environmentally friendly is method of regulating rainwater systems using green roofs. This method consists of placing on the roofs of buildings green space which primary function is to reduce the amount of rainwater flowing from the roof during heavy rain. Water partly retained in the soil substrate of green coating is absorbed by plants and returned to the atmosphere through evaporation. Studies

show that green roofs delay of 40 to 80% of the annual volume of rainwater and reduce the maximum flow rate of up to ten times and retard its occurrence over time [14]. Additional important benefits of green roofs is to improve the aesthetics of the urban area, air purification, thermal regulation of indoor environment (cooling in summer and warming in winter).

Managing rainwater large cities involve complex of networks and facilities. Therefore, the modeling of rain drainage systems associated with the solution of complex unsteady hydrodynamic, mass transfer and heat transfer problems are compounded by the stochastic nature of rainfall and the huge number of particular factors that affect the processes of formation of rainwater, organized its removal, cleaning and use.

Analysis of the literature shows that by all around the world there is no single universally accepted scientific approach to hydraulic calculation of networks and structures of rain drainage. Existing theoretical methods are based on substantial simplifications and partially or completely take into account a number of factors that affect the underlying of hydraulic processes. Different countries use a large number of purely empirical methods that usually does not theoretically grounded and have a narrow range of applications. Greater theoretical principles and establishing patterns that characterize the current storm sewage in the basin drain, in open and closed drainage networks and sanitation facilities rainy considering .Rain calculated parameters, configuration, altitude and hydraulic circuit characteristics of the basin runoff and actual configuration drainage trays, ducts and pipes and structural features of buildings to arrest, regulation or clean rainwater is relevant scientific problem, which is essential for improving efficiency and reliability of drainage systems.

First of all, in the design of stormwater drainage structures important is the ratio of the volume of runoff relative to the estimated amount of rain. Comparison of volume calculation by the method adopted in Ukraine and often used by foreign techniques (in particular, the method SCS USDA United States [15] shows a significant (20% or more) difference in the values of runoff coefficient that takes into account the loss of water from rain estimated the initial detention, infiltration and evaporation [16].

In the simulation of inflow hydrographs rain wastewater should be fully taken into account with geographic, survey and other features facility design. To determine the estimated costs of rainwater surface concentration and time there are used a large number of methods, mostly - empirical methods based on data. The experimental study certain specific pools drain and valid only for these pools. The existing methods of hydrographs construction do not considere configuration of basin runoff in the plan, altitude scheme of drain pool (all drain pool are accepted as flat surface of constant at all points in the direction angle calculation section).

Relevant are also improved methods of hydraulic calculation adjusting volume of EDIS and other capacitive structures. One of the problems in their design is the lack of domestic practice methods of calculation of EDIS multisection. In the operating procedure of single-section EDIS made many simplifications and approximations: does not overlook configuration of basin runoff in the plan, is not considered a number of design parameters of EDIS, which have a significant impact on the value of the adjusting volume coefficient (in particular, the rate of change of pressure, dimensionless diameter outlet pipeline).

3. Conclusions

1. Work considers the classification of modern management rainwater systems from built-up areas.
2. In this paper were described the advantages and disadvantages of the most common methods in practice.
3. There is given the task of modeling complex hydraulic processes in networks and wastewater facilities rainy considering several new structural parameters.

References

1. Water, a shared responsibility. The United Nations World Water Development Report 2. // UNESCO-WWAP 2006. − UNESCO, Berghahn Books. − 2006. − 601 p.
2. Alekseev MI Organization diversion of surface (rain and thawed) runoff from urbanized areas: Proc. allowance. / MI Alekseev, A. Hill. - M .: Publishing House of the DIA: Civil Engineering, 2000. - 352 p.
3. Mays L.W. Stormwater collection systems design handbook / LW Mays. - McGraw-Hill Professional, 2001. - 1008 p.
4. Dziopak J. Analiza teoretyczna i modelowanie wielokomorowych zbiorników kanalizacyjnych. / J. Dziopak. - Kraków: Politechnika Krakowska im. Tadeusza Kościuszki. Monografia 125, 1992.- 214 s.
5. Debo T.N. Municipal storm water management / T.N. Debo, A.J. Reese. - Lewis Publishers. - 1995 - 1154 p.
6. Schueler T.R. A Current assessment of urban best management practices. / T.R. Schueler. - Metropolitan Washington Council of Governments. - 1992.

7. Babchenko I.V. Rozpodilchi Cameri new type systems vodovidvedennya / Author. Dis ... Cand. tehn. Sciences: 05.23.04. Harkivsky Hold. tehn. Univ budivnitstva that arhitekturi. - H .: 2004. - 17 p.
8. Kitaєv AL Akumulyuvannya, clearing that vikoristannya poverhnevo-zlivovogo drain s teritoriy Promyslova of companies / Cand. Dis ... Cand. tehn. Sciences: 05.23.04. Harkivsky Hold. tehn. Univ budivnitstva that arhitekturi. - H .: 1997. - 18 p.
9. Y. Yaroshenko Warehouse, vlastivosti, tehnologiya konditsionuvannya that vikoristannya poverhnevo-zlivovogo i thawed stokiv / Stosovno mist that Promyslova of companies / Cand. Dis ... Cand. tehn. Sciences: 05.23.04. Harkivsky Hold. tehn. Univ budivnitstva that arhitekturi. - H .: 2000. - 19 p.
10. Budz OP Maksimalni vitrati Vod vesnyanoï poveni on richkah Pivnichno-Zahidnogo regionu Ukraine: Dis ... Cand. tehn. Sciences: 05.23.16 / Rivnensky Hold. tehnichny Univ. - Rivne, 1999. - 142 c.
11. Natsionalna dopovid about yakist pitnoï Vod is the camp Pitney vodopostachannya in 2007 rotsi // Ministerstvo s supply zhitlovo and communal Gospodarstwa Ukraine. - K.,2008. - 563 p.
12. Steinhardt Hydroslide. Discharge regulators for all ranges of application / Steinhardt GmbH, Taunusstein, 2004.
13. The stormceptor system for stormwater quality improvement. Owner'sManual. - Stormceptor, 2000.- 14 p.
14. Green roofs for stormwater runoff control / [Berghage RD, Beattie D., Jarrett A.R. and oth.] // EPA / 600 / R-09/026. - Cincinnati, OH: National Risk Management Research Laboratory. U.S. EPA, 2009. - 81 p.
15. Urban Hydrology for Small Watersheds // United States Department of Agriculture. TR 55, 210-VI-TR-55, Second Ed., June 1986. - 164 p.
16. VM Beetle Metodi rozrahunku ob'єmu doschovogo drain / VM beetle,I.I. Matlay // News Nat. y-tu "Lviv politehnika" "Teploenergetika.Inzheneriya dovkillya. Avtomatizatsiya. "- Lviv: NU "Lviv politehnika." -2010. - № 677. - S. 32-38.

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Good practice – better protection of the environment

Keywords: *Principles of Good Practice, local pollution, plant protection means, water protection*

Abstract: The paper discusses the legal provisions on the use of plant protection products, the principles of Good Practice and describes the threats, which are entailed by the use of plant protection products not in accordance with its rules. It is described how to prevent the contamination of the environment (both local and distributed), complying with the legal provisions talking about the way of storing plant protection products, preparation of plant protection products for the application, proceeding with the residues of the spray liquid after the treatment and proceeding during the cleaning of equipment for the application of plant protection products. It was found that making aware the users professionally applying the plant protection products about the threats which is brought by the use of chemical preparations necessary to prevent the formation of contamination of groundwater and surface water, and the formation of a threat for the health of humans and animals.

1. Introduction

The chemical plant protection both in Poland and in the European Union countries is subject to strict judicial scrutiny. Because of this there was an improvement in the protection and a reduction of availability of plant protection products (p.p.p.) for people, who are not trained to use them. The first legal act of the European Union strictly regulating the way of using the plant protection products was the Directive of the European Parliament and the Council 2009/128/WE of 21 October, 2009. The Directive regulates the framework for joint action for the sustainable use of pesticides. The purpose of this Directive is: achieving the sustainable use of p.p.p. by the EU member countries, reducing the risk, which entails the use of chemical preparations on the human health and environment, implementing the rules of the integrated plant protection by the professional p.p.p. users (i.e., taking into account the non-chemical methods during plant protection in order to minimise the threats connected with the use of plant protection products for the health of people, animals and the natural environment) [1]. The aim of the PE directive is also the non-legislative action, included in the National Action Plan to reduce the risk connected with the use of plant protection products for the years of 2013-2017. The tasks of the National Action Plan include:

- promoting good practices of the safe use of plant protection products,
- promoting the general principles of the integrated plant protection,

- modification of the training system for professional users of plant protection products, people engaged in sales of these agents and advisers,
- modification of the testing system of the technical condition of the equipment for using plant protection products,
- raising public awareness regarding plant protection products,
- ensuring the protection of small-area crops,
- ensuring the effective supervision over trade and the use of plant protection products [2].

There are many legal acts concerning the protection of the environment and human health, referring to plant protection products, among others: the Act on water law, the Nature Conservation Act, the Act on plant protection products and the regulation of the Minister of Agriculture and Rural Development on the application of p.p.p. or on protection and occupational health and safety during the application and storage of plant protection products and mineral and organic-mineral fertilizers [3,4,5,6,7].

The implementation of new provisions entails the need to raise awareness of farmers to adapt to the new requirements. To this end the training is conducted, not only of the sprayer operators, but also advisors concerning the use and sale of p.p.p..

The purpose of this study is to present the risks, which are connected to the use of plant protection products without accordance with the principles of good practice.

2. Principles of Good Practice

The use of chemical plant protection products entails high risks for the environment and the human health – including in particular health of the operator performing the treatments of spraying crops. The operator is exposed to the harmful effects of p.p.m. from the very beginning of the preparation of the spray liquid, then during the performance of the treatment and later on while cleaning the sprayer and equipment used to perform the treatment.

Due to the fact of the lack of sufficient knowledge, which should be owned by the operator while making the spray liquid and performing the spraying procedure using p.p.m. and due to failure to comply with the legal requirements, which strictly define, among others, which measures have been admitted to trading in a given year and how to deal with remnants of the spray liquid – the European Crop Protection Association (ECPA) in 2005 has already initiated the demonstration-training project TOPPS (Training the Operators to prevent Pollution from Point Sources). The project aims to raise awareness among the operators performing the spraying operations, consulting services and teaching units. The project is implemented using the multilateral cooperation of many European countries, which engage the local experts and

partner organisations to develop and spread the Code of Good Practice of the Plant Protection Organisation (DPOOR). In Poland the institutions involved in the project include the Institute of Horticulture in Skierniewice and the Institute of Environmental Protection – National Research Institute [8].

The task of TOPPS is to reduce pollution from the contamination of local and spread sources (caused by p.p.m.), including the runoff from agricultural areas, creation of protective areas overgrown with flora or propagating and implementing the principles of the DPOOR Code.

1.3.Protection zones overgrown with flora

The areas overgrown with flora (Fig. 1) are to protect water reservoir and waterways from residues of plant protection products as a result of the runoff from farmlands and orchards while maintaining the best possible water management and are aimed at reducing the soil erosion. Protection zones should be formed along the waterways and water reservoirs while maintaining the right width (Fig.2). The distance of using watercourses of the given plant protection product should be read from the label (instruction) of the application of the given measure and it should be treated as a necessary width of the whole protective zone, in which we find the zone overgrown with vegetation. If on the label there is no information concerning the protective zone, then from the watercourses you should maintain at least 5m of the width (Fig. 2a) with natural courses and water reservoirs and at least 2m of the width of the zone overgrown with vegetation (Fig. 2b) by the drainage ditches and artificial water reservoirs (also in case of tanks, in which water appears only periodically during the year) [8].

Figure 1. Protection of water tanks and courses – protective zones overgrown with vegetation.

Source: http://www.topps-life.org/sites/default/files/European-crop_PL_FINAL.pdf

For the creation of straps of protective zones one should use perennial plants (mainly grass suitable for the local environmental conditions), which even while inhibiting the growth limit the runoff of waters. Vegetation should be kept thick at the height of at least 15cm and limit the transit of machinery and passages for animals in the protection zone. In these zones one should also not use the treatments with plant protection products and fertilizer. One should not allow the accumulation of soil sediments, and if they appear, they have to be moved to the cultivated field. To avoid the runoff from the adjacent crop one should eliminate the crossings and paths crossing the protection zones.

The formation of protective zones overgrown with vegetation is estimated to reduce about 50 to 70% the amount of plant protection products, which get to surface waters and to reduce the harmful process of excessive water use, and to reduce the soil erosion. Thanks to the protective zones the biodiversity in the given area is increased by the formation of new habitats for fauna and flora. The law does not yet regulate the issues of creation of protective zones overgrown with vegetation, but it is an important recommendation, which helps fulfil the environmental goals, which is discussed by the Water Directive or the Directive on the Sustainable Use of Pesticides.

About 35% pollution of surface water with plant protection products is caused by the runoff and soil erosion. Pollution can be eliminated from the environment partially using the principles of Good Practice. Prevention of soil erosion and maintaining purity of water serves not only the environmental protection, but also the support of effectiveness and the sustainable production on farms.[9]

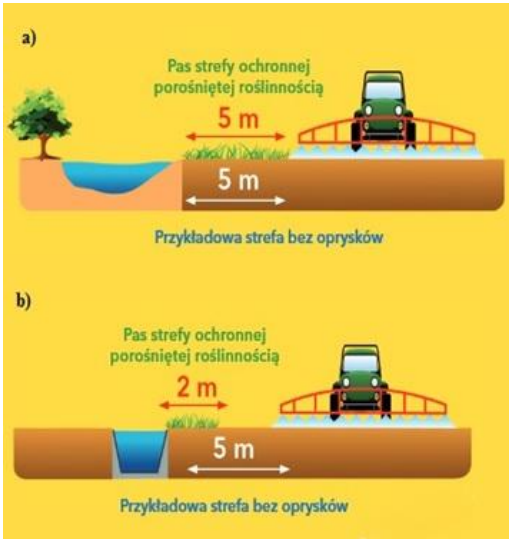

Figure 2. Determination of the protective zones covered with vegetation - a) by natural streams and reservoirs, b) by drainage ditches and artificial water reservoirs

Source: http://www.topps-life.org/sites/default/files/European-crop_PL_FINAL.pdf

1.4.Prevention of the environmental pollution

In order to prevent the local contamination the TOPPS program promotes training of sprayer operators, which are to make them aware of the goal and need to implement the principles of Good Practice talking about the rules of safe equipment cleaning after the performed treatment using plant protection products, the appropriate use of the spray liquid remnants or the right storage of plant protective measures.

The Regulation of the Minister of Agriculture and Rural Development on how to proceed with the proceeding while using and storing plant protection products based on art. 40 act. 3 of the Act of 8 March 2013 on plant protection products (Journal of Laws, item 455) [10] talks about the detail manner:

a) storing plant protection products,

b) preparation of plant protection products for the use,

c) dealing with spray liquid residues after the treatment using plant protection products,

d) procedure for cleaning equipment for the use of plant protection products.

Plant protection products should be stored in original, sealed containers at a temperature not less than 0°C and not more than 30°C, in a place not posing threat to the health of humans, animals and water purity – away from food and feed. Preparations should be located in the warehouse or closed cabinets, labelled and inaccessible to third parties [11]. In the place eliminating the risk of contamination of the soil or water as a result of leakage or seepage of harmful substances. Therefore, such warehouse should have the impermeable floor, from which the spilled or sprinkled preparation can be easily removed, lighting, ventilation, shelves of non-absorbent materials, drain into a separate container for p.p.m. residues. Measures stored in the warehouse should be sorted according to the destination and the degree of toxicity, powders should be over the liquids. In the warehouse there should be designated the place for measuring p.p.m. and storing the devices, with which the preparations are measured (weight, spatula, measuring jug). The equipment should also include tools and materials to eliminate the effects of spillage or scattering of preparations (brush, shovel, sawdust or other chemical absorbents) and plastic bags, containers for contaminated materials, health and safety manual, emergency phone numbers, fire extinguisher and the record of the warehouse p.p.m. state. To prevent the formation of p.p.m. residues, one should remember not to allow the measures to get expired, which we have in the warehouse, and to first use the ones, which are soon to be withdrawn from circulation. Do not store more measures than we need for essential uses. The disposed packages and useless measures are subject to the compulsory return to the point, where p.p.m. were

purchased. Under no circumstances you cannot burn them or bury them in the soil, because this contributes to the formation of local contamination of the environment [12].

Local contamination may also occur during the preparation of the spray liquid, this action according to the legal acts also should be performed in a safe distance (not less than 20m) from wells, reservoirs and waterways, areas susceptible to contamination and manholes. Both measuring of chemical preparations and filling the sprayer tank should be done when the user is equipped with the individual protection measures. It should be noted that during the preparation of the spray liquid solution one should not sill/spatter the preparations not damage the packaging. In order to avoid the local contamination empty packaging should be three times rinsed with clean water, and the washings must be poured to the tank with the spray liquid solution. Measuring preparations is done in a place sheltered from the wind, using separate tools and devices, which are stored together with p.p.m. You should carefully perform all actions in order not to allow the spilling or scattering of preparations, which cause the formation of local contamination. Spray liquid should be always prepared according to the strictly determined doses, given on the product label. Spray liquid is made directly before the use, and after this operation, the operator cannot leave it unattended. When filling the tank, one should pay attention not to allow the contamination of the sources of points of water collection (the supply hose should always be over the edge of the filling hole of the tank). When we have a sprayer with an extra water tank and preparation thinner – liquid is best prepared in a field or orchard (always in a different place, not to accumulate p.p.m. in one location, when they are spilled). If a small amount of the preparation is spilled on the biologically active ground, its action will be much faster neutralised, than in the case of spilling the preparation, e.g., in the household, on the sterile soil. However, if we make the liquid on a farm, this operation should be performed on the non-soaking ground, from which the spilled/scattered preparation can be easily collected or on the sodded areas, or positions of the BIOBED type [13].

Another problem is the excess of the spray liquid remaining in the tank and the residue resulting from the structure of the liquid system of the sprayer, which cannot be sprayed due to technical reasons. To avoid the excess of the unused liquid, it is worth paying attention during the selection of the sprayer to choose such a technically constructed tank and liquid system, which accumulates the smallest possible volume of liquid remaining after the properly performed treatment. Surplus of the spray liquid remaining after performing the treatment can be limited to the minimum thanks to the performance of the careful calibration of the sprayer before the treatment and the accurate maintenance of the designated parameters of the sprayer's operation during the performance of spraying. The amount of the remaining liquid in the tank

can be reduced by the operator also by turning the liquid stirrer off – when in the final phase of the treatment the pump starts sucking the air, and the pressure drops.

The significant surplus of the remaining preparation should be used within 24h, then you can leave them in the sprayer, if they do not cause, e.g., clogging of nozzles and filters. If the use of this liquid is not possible, then until it is developed or utilised, it is stored in a tank designed and labelled specifically for this purpose (sealed and protected from the access of third parties).

The residues of the spray liquid in the sprayer tanks should be disposed in a safe manner to prevent local contamination, i.e., the liquid remaining after the treatment in no case should be poured onto the ground, road or any other place preventing its collection or quick neutralisation by the biologically active surface, specially adapted for this purpose. Spilling such liquid causes the penetration of p.p.m. into the soil to lower layers, to the groundwater, which move the chemicals often in the unknown direction to remote areas (what causes the spread contamination). Remains of the spray liquid should be diluted several times (10x) and e.g. sprayed on a field, on which the procedure has been performed previously (not to exceed the maximum permissible dose of the preparation it is best done in a place, in which the spraying has been started – such a place should be planned before starting the procedure). The well diluted spray liquid can be also exploited by the use of the bio-remediation system (e.g. BIOBED (fig.3), VERTIBAC), thanks to such stations, active substances undergo the accelerated biodegradation. Biodegradation should take place in a place that prevents the release of active substances outside and in a way that is not causing new threats. Biodegradation time is not shorter than one year, but it depends on the type and content of p.p.m. in the residues [14, 15].

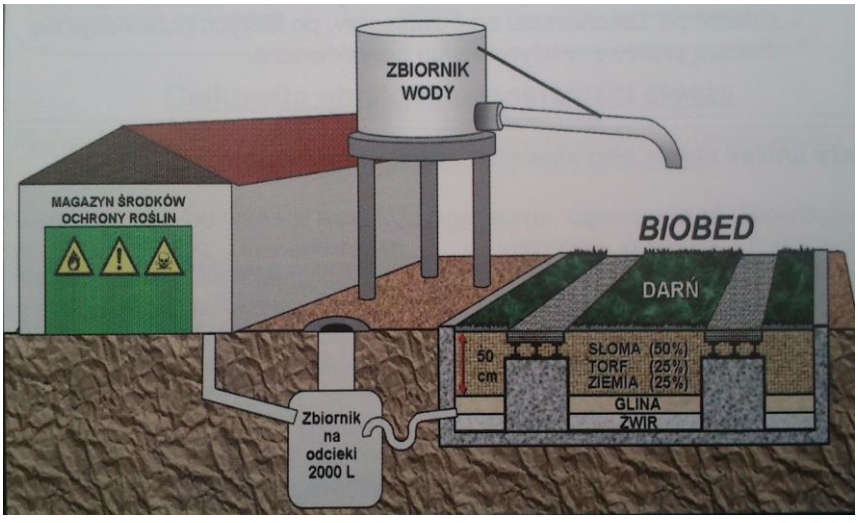

Figure 3. A BIOMED type of a station used for the biological neutralisation of p.p.m.

Source: Godyń, A., Doruchowski G.: Poradnik - mycie opryskiwaczy. Institute of Pomology and Floriculture, 9. Skierniewice 2009

Cleaning of a sprayer is divided into external and internal. External cleaning, if it is necessary, should be performed just after the completed procedure in a field or orchard, in which the spraying has been performed. Because of this the removal of p.p.m. is limited outside the place, where the procedure was performed. Safe and effective washing of chemical preparations prevents the premature wear of the equipment and the threat to both the environment and people operating at the spraying procedure. The operator while washing the equipment should obligatorily wear the protective clothing (like in the case of the preparation of the spray liquid). Each washing of the sprayer should take place in a different part of a field to prevent the excessive accumulation of p.p.m. in one place and the formation of local contamination. Currently, sprayers are equipped with a clear water tank (designed for washing the tank and rinsing the liquid installation) and a set for external cleaning (a hose with a brush or a lance/airless gun and a hose reel). By using a brush or a pressure washer for cleaning, we not only thoroughly remove the impurities but also create a relatively small volume of water contaminated by p.p.m. [16].

The most polluted elements of the sprayer are: a boom, construction elements around the nozzles, wheels, both of the tractor and the sprayer, and a fan (in the case of orchard sprayers). Washing is performed from top to bottom. While carrying of the washing in a field with a limited amount of clean water, first we should wash the most contaminated elements. To limit the external pollution of the sprayer, we should reduce the drift of the spray liquid while spraying, e.g., by limiting the operational speed of the machine, lowering the boom, using sprayers with larger droplets and performing the treatment in the appropriate weather conditions. To prevent the local environmental contamination, the external sediments of p.p.m. remaining on the sprayer should be removed very carefully – especially when the machine is not garaged and it stands for a long period of time outside. Rain rinses the residues of chemicals especially from places hard to wash by a sprayer, so they are accumulated in the base. That's why we should avoid the prolonged stay of the sprayer in one place in order not to accumulate the rinsed residues of p.p.m. [17, 18].

If the sprayer is not suitable for washing in a field (i.e. does not have the possibility of connecting the pressure washer to the clean water tank), the treatment should be performed in a place, where it will be possible to collect the contaminated water to the closed tank or its drainage to the bioremediation system (e.g. Phytobac). The alternative can also be the station of biobed, which is composed of the biologically active substrate containing microorganisms neutralising a small concentration of p.p.m. When washing the sprayer on the biomed station, we should use a small volume of water (50-100l), what allows the use of a pressure water,

which limits the amount of water at the same time with the effective cleaning. If we do not have the opportunity to wash the sprayer on a special stand, we do it on the biologically active surface, adapted to quickly absorb and neutralise the residues of plant protection products through biodegradation (e.g. turf area). Washing the sprayer should be performed at a distance of at least 20m from surface waters and other sensitive areas. You have to be careful in order not to allow the direct or indirect contamination of surface water and groundwater [19].

Internal cleaning consists of rinsing the sprayer's liquid installation and internal walls of the tank of the p.p.m. residues, which could have a negative influence on the plants in another procedure, state and functioning of the sprayer and the health and safety of people.

Washing the sprayer should be performed as often as it results from the actual need, because during this process is formed a large amount of contaminated water, which should be safely utilized. The residue of the spray liquid in a tank and liquid installation should be diluted, preferably 100 times, and spray on a field on a previously sprayed crop. Spraying the residues one should increase the speed in relation to the speed, at which the treatment was performed and reduce the pressure in the sprayer in order not to wash out the previously applied liquid or not to cause its overdose [17].

If it is not necessary, you should avoid washing the internal sprayer. However, this action is necessary, when:

- another procedure will be performed on another crop, for which the previously applied preparation is not registered,
- the preparation carries the risk of plant damaging (in another procedure),
- the equipment inspection will be performed at the Sprayer Control Station or a review, repair or equipment calibration,
- after the season ended,
- spray liquid residues can cause clogging of nozzles, filters or other technical problems. In such cases, washing and rinsing of the internal installation is conducted by using special cleaning preparations increasing its effectiveness, safe for people and neutral for the environment [16] .

Washing the sprayer is recommended before going on public roads, after performing the procedure, especially before the stopover "in the open", after ending the series of treatments before a longer stoppage.

The need to wash a sprayer is determined not only by the legal provisions included in the Act on plant protection products, but also provisions in the label included in the applied plant protection product.

The Regulation of the Minister of Agriculture and Rural Development on how to proceed with handling and storage of plant protection products based on art. 40 par. 3 of the Act of 8 March 2013 on plant protection products (Journal of Laws, item 455) also talks about:
2) requirements, which should be met by the places or objects, in which plant protection products are stored, taking into consideration minimal distances from specified places or objects, after consideration of which these measures can be stored;
3) the way of warning about the intention to conduct the treatment using plant protection products posing a particular threat for the health of people and animals or to the environment of people, which may be exposed to the contact with these measures or are the owners of farm animals, which can be exposed to the contact with these measures.
According to § 3 of the above-mentioned regulation: preparation of plant protection products for use by the preparation of the spray liquid should take place:
1) in a way minimizing the risk of contamination:
a) of surface and ground water in the meaning of the Water Law provisions,
b) of the land, including as the result of a leakage or seepage of plant protection products into the soil profile;
2) at a distance of not less than 20m from wells, water intake and reservoirs and water bodies – in the case of the preparation of spray liquid using plant protection products intended for professional users.

Each operator of the sprayer during the preparation of the spray liquid is obliged to strictly comply with the instruction recommendations placed on the label of the given p.p.m. The operator preparing the spray liquid should observe the principles of Good Practice and care of own safety complying with the safety rules and applying personal protection measures (Fig.4) – protective clothing (non-absorbent coveralls or pants and a sweatshirt with welts at the end of the sleeves, rubber gloves – matched to the size of the hand, reaching to the joints, hidden under the sleeve of the coveralls, rubber boots with legs tucked under the pants, face shield with a transparent shield or goggles or tight goggles to protect the eyes with a half-mask with AP2 filter).

Figure 4. Operator of a sprayer dressed according to the Principles of Good Practice

Source: Doruchowski G.: Bezpieczne i racjonalne stosowanie środków ochrony roślin w sadownictwie. Ed. Inllort Institute of Horticulture in Skierniewice, 5, 2013.

In § 4 of the above-mentioned regulation (Journal of Laws, item 455) there is described the procedure for the development of residues of the spray liquid after the treatment using plant protection products. Proceed in a way to reduce the contamination of surface and ground water within the meaning of the Water Law provisions and the land, while the residues of the spray liquid after the treatment using plant protection products intended for professional users should be:

1) used after the prior dilution on the surface, on which the procedure was performed, in a place, in which the plant protection product was used in a smaller amount, if it is possible, or

2) disposed using technical solutions providing the biological degradation of active substances of plant protection products, or

3) disposed in a way other than the one specified in point 2, if it complies with the rules on waste [10].

An important element of limiting the use o plant protection products, which is required by the law, is also the performance of the accurate calibration of the sprayer. What consists of a series of steps: determination of the operational speed, with which we will perform the

treatment, the selection of the right type and size of nozzles (for treatments with favourable weather conditions atomizing, while with bad – thick droplets reducing the drift of the liquid by the wind) and determination of the flow of the nozzles. The flow is regulated by the change of the operational pressure [20]. Accurate calibration is important, because too high doses of liquid lead to the preparation dripping from plants, and what follows is the reduction of the amount of the active substance on the protected surfaces. Not to allow the unnecessary dripping of the liquid from plants, the operator should turn off the sections of nozzles while manoeuvring on a field and during each stop or break.

For the performed procedure to be conducted in a safe way, it has to be performed in conditions favouring the operation of plant protection products (at the temperature to 25°C, air humidity 50-95%, wind speed up to 4 $m \times s^{-1}$, rainfall < than 0,1mm during the procedure and <0,2 mm from 3 to 6 hours after the performed treatment). Plant protection products must not be used in buffer zones designated for surface water, hives and other not agricultural areas to prevent the risk of contamination as a result of the spray liquid drift.

Drift of the spray liquid causes the formation of the scattered contamination and takes place as a result of air movements, which moves the small droplets of the spray liquid outside the spraying zone. There are different ways enabling the limitation of this phenomenon in agricultural crops. We can prevent the reduction of liquid drift by using the sprayer with the targeted assistance air stream, and during bad weather conditions we can use thick droplet nozzles (ejector-vortex or flat stream 90° or anti-drift). To liquids we can also add the adjuvant, which increase the viscosity of the droplets. Protection zones overgrown with vegetation (grass strips, hedges) growing around the fields also limit the drift. In reducing the water runoff from a field an important aspect is the proper cultivation of soils – both during ploughing in the contour cultivation and the forming of plants in the permanent cultivation should be performed across the slope. There can be formed filtration strips out of vegetation growing along the watercourses or filtration strips of vegetation on/or between the fields and across the slopes. Furrows or small dams placed inside the field located on a slope can also limit and slow down the flow of water. Limitation of the drift also allows the maintenance of the beam at a height not less than 50cm and maintenance of the operational speed under 8 $km \times h^{-1}$[21].

3. Summary

The consequence of the failure to comply with the legal provisions taking about the principles of Good Practice is the allowance of the local and diffused contamination of the environment, e.g., by polluting ground and surface water and posing a threat for the health of

people and animals. One of the easiest, fastest and most effective ways to limit the water pollution as a result of the runoff is the formation of zones covered by vegetation. Such zones are set up along all water bodies and watercourses adjacent to the area of field and orchard crops. The reason for exposure to the effects of p.p.m. of both the natural environment and people is the lack of awareness of the threats, which are posed by the use of chemical preparations and the knowledge how to reduce them effectively.

Rederences

1. Dyrektywa Parlamentu Europejskiego i Rady 2009/128/WE z dnia 21 października 2009 r. ustanawiająca ramy wspólnotowego działania na rzecz zrównoważonego stosowania pestycydów. Dz.Urz. 24.11.2009 L 309/71.
2. Krajowym Planie Działania na rzecz ograniczenia ryzyka związanego ze stosowaniem środków ochrony roślin na lata 2013-2017. Ministerstwo Rolnictwa i Rozwoju Wsi 2012.
3. Ustawa o prawie wodnym z dnia 11 lipca 2001 r., Dz.U. 2001 nr 115 poz. 1229
4. Ustawa o ochronie przyrody z dnia 16 kwietnia 2004 r., Dz.U. 2004 nr 92 poz. 880.
5. Ustawa o ochronie roślin z dnia 18 grudnia 2003 r., Dz.U. 2004.11. 94.
6. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 31 marca 2014 r. w sprawie warunków stosowania środków ochrony roślin, Dz.U. 2014 poz. 516
7. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 24 czerwca 2002 r. w sprawie bezpieczeństwa i higieny pracy przy stosowaniu i magazynowaniu środków ochrony roślin oraz nawozów mineralnych i organiczno-mineralnych, Dz.U. 2002 nr 99 poz. 896.
8. http://www.topps-life.org/sites/default/files/European-crop_PL_FINAL.pdf , dostęp 29.01.2015 r.
9. http://www.topps-life.org/pl/page/chron-swoja-wode, dostęp 29.01.2015 r.
10. Ustawa o środkach ochrony roślin z dnia 8 marca 2013 r., Dz.U. 2013 poz. 455.
11. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 22 maja 2013 r. w sprawie sposobu postępowania przy stosowaniu i przechowywaniu środków ochrony roślin, (Dz.U. 2013, poz. 625).
12. Doruchowski G., Materiały szkoleniowe - Aktualne problemy techniki ochrony roślin. Listopad 2014
13. Doruchowski G.: Bezpieczne i racjonalne stosowanie środków ochrony roślin w sadownictwie. Wyd. Inhort Instytut Ogrodnictwa w Skierniewicach, 13-15, 2013.
14. Godyń, A., Doruchowski G.: Poradnik - mycie opryskiwaczy. Instytut Sadownictwa i Kwiaciarstwa, 11-13. Skierniewice 2009.

15. http://www.rolnictwoodpowiedzialne.pl/pl/odpowiedzialnosc-biobed, dostęp 29.01.2015r.
16. Doruchowski G.: Bezpieczne i racjonalne stosowanie środków ochrony roślin w sadownictwie. Wyd. Inllort Instytut Ogrodnictwa w Skierniewicach, 20-21, 2013.
17. Godyń, A., Doruchowski G.: Poradnik - mycie opryskiwaczy. Instytut Sadownictwa i Kwiaciarstwa, 13-21. Skierniewice 2009.
18. Wachowiak M.: Myj opryskiwacz- unikniesz kłopotu. Nowoczesna Uprawa Nr 4/2008. http://www.ppr.pl/artykul-myj-opryskiwacz-unikniesz-klopotow-168260-dzial-4031.php, dostęp 29.01.2015 r.
19. Doruchowski G.: Użytkowanie Biobedu. Agrotechnika Nr12/2008, http://www.rolnictwoodpowiedzialne.pl/pl/uzytkowanie_biobedu, dostęp 19.01.2015 r.
20. Tadel E.: Kalibracja opryskiwacza. Agrotechnika 4/2014, 65-67.
21. Godyń A.: Bezpieczne opryskiwanie. Agrotechnika 2/2012, http://rolnictwoodpowiedzialne.pl/pl/bezpieczne_opryskiwanie_agrotechnika, dostęp 29.01.2015r.

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Biomass combustion and Co-combustion in energetic system: some operational problems

Key words: *biomass, co-combustion, operational problems, ash*

Abstract: As concerns regarding greenhouse gas emissions from coal-fired power plant increase, there is greater focus on the feasibility of using biomass as a supplement fuel in existing fossil-fuel-based power systems. Coal-fired power plants are used worldwide for electricity production and provide over 42% of global electricity supply. However, emissions of greenhouse gases (CO_2, CH_4, etc.) from coal combustion systems are a major environmental concern. Among these gases, CO_2 is considered the most critical in terms of its contribution to global warming and coal-fired power plants account for 28% of global CO_2 emissions.

In Poland, coal accounts for 55% of its primary energy supply and 92% of electricity generation, raising significant climate change and environmental challenges. As a European Union member state, Poland has to apply different renewable technologies and increase the share of green energy in its energy mix to achieve the '20-20-20' targets and decarbonize its power sector. With biomass acknowledged as the most promising and important renewable energy source, co-combustion is probably the least complicated and one of the most advantageous ways of utilizing biomass for replacement of fossil fuels for stationary energy conversion.

A dedicated biomass combustion power plant is another option when considering biomass as a renewable energy source in the generation industry, however it is important to review the advantages of co-firing regarding lower specific costs, higher plant's efficiency and emission reduction.

Although co-utilization of biomass is a proven technology, it leads to serious technological problems: depending on the source of biomass, the introduction of biofuels in a coal-fired boiler may cause operational problems in the boiler due to the higher content of alkali metals and chlorine compounds. These elements reduce the ash melting temperature, causing ash deposition problems, such as slagging, fouling and sintering, and corrosion.

The co-firing strategy has demonstrated reduced nitrogen and sulphur emissions and could also be used to reduce net carbon dioxide emission impact. But the increase of biomass conversion will result in an increased production of biomass ash. Thus, there is growing interest in biomass ash utilization. Alternative uses of biomass-coal ash as a value-added product will then be necessary in order to lower further economic and environmental problems regarding its disposal.

Different analysis have to be conducted to avoid hazards within the furnace due to ash deposition and corrosion. When proper choices of biomass, coal, boiler design, and boiler operation are made, traditional pollutants (SO_X, NO_X, etc.) and net greenhouse gas emissions decrease.

Biomass co-combustion is a promising technology, nevertheless, significant development work is required before large-scale implementation can be realized without significant change in the power cycle efficiency or emission control.

1. Introduction

Coal-fired power plants are used worldwide for electricity production and provide over 42% of global electricity supply (International Energy Agency, 2010). However, emissions of greenhouse gases (CO_2, CH_4, etc.) from coal combustion systems are a major environmental concern. Among these gases, CO_2 is considered the most critical in terms of its contribution to global warming and coal-fired power plants account for 28% of global CO_2 emissions (International Energy Agency, 2010) [1].

One means of mitigating these environmental impacts is increasing the fraction of renewable and sustainable energy in the national energy supply, which can make a real contribution to the Governments' renewable targets and obligations.

Co-firing biomass and coal in traditional coal-fired boilers represents one combination of renewable and fossil energy utilization that derives the greatest benefit from both types of fuels. Biomass is a renewable energy source and considered as a CO_2-neutral fuel since it releases no net CO_2 emissions if carefully managed. Although a "standalone" or dedicated biomass plant could be another option when considering biomass as a renewable energy source in the generation industry, it is often technically and economically advantageous to use biomass as a supplemental fuel in existing coal-fired power plants. Co-firing capitalises on the large investment and infrastructure associated with existing fossil-fuel-based power systems, while requiring only a relatively modest investment to include a fraction of biomass in the fuel. When proper choices of biomass, coal, boiler design, and boiler operation are made, traditional pollutants (SO_X, NO_X, etc.) and net greenhouse gas emissions decrease.

In Poland, coal accounts for 55% of its primary energy supply and 92% of electricity generation, raising significant climate change and environmental challenges. Although Poland's carbon intensity – energy-related CO_2 emissions per unit of GDP − has been falling sharply since 1991, continued heavy reliance on coal makes Poland a relatively carbon intensive economy, compared to the IEA Europe average [2].

As an EU member state, Poland participates in the European Emissions Trading Scheme (EU-ETS) and has to comply with the EU climate and energy package, which sets the so called"20-20-20" targets for the whole EU: reducing greenhouse gas (GHG) emissions by 20% (or even by 30% if other developed countries commit to comparable reductions under a new global climate change agreement), increasing renewables' share in energy supply to 20% and

improving energy efficiency by 20% by 2020. This translates into the following targets for Poland:

- limit GHG emissions in sectors not covered by the EU-ETS to 14% above the 2005 level;
- reduce energy consumption by 20% of projected 2020 levels;
- increase the share of renewable energy to 15% of gross final energy consumption.

In order to achieve these targets, every country has to apply different renewable technologies and increase the share of green energy in the energy-mix. The distribution of RES in Poland is shown on the Figure 1.1:

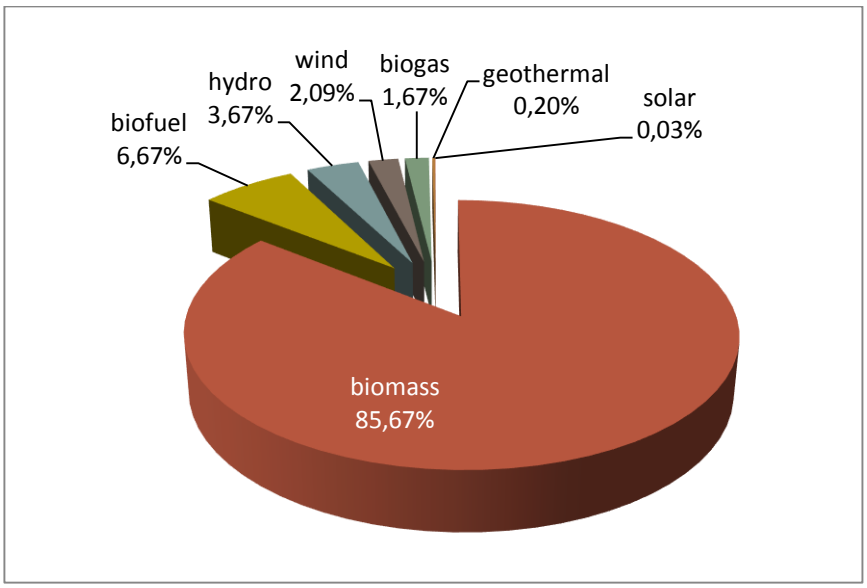

Figure 1.1 Energy production from renewable sources in Poland, 2010

Source: Is Using Biomass for Power Generation a Good Solution? The Polish Case. (Electrical Review). GUŁA A., WAJSS P., GORYL W., 2012 [3]

By 2010, biomass contributed with more than 85% of total energy production from RES in Poland. The most widely spread range of biomass usage in Poland is electricity generation produced in pulverized coal boilers in the existing power plants.

Co-combustion is probably the least complicated and one of the most advantageous ways of utilizing biomass for replacement of fossil fuels for stationary energy conversion. Although co-utilization of biomass is promising technology, it leads to serious technological problems. Depending on the source of biomass, the introduction of biofuels in a coal-fired boiler may cause operational problems in the boiler due to the higher content of alkali metals and chlorine compounds. These elements reduce the ash melting temperature, causing ash deposition problems, such as slagging, fouling, sintering, and corrosion.

The co-firing strategy has demonstrated reduced nitrogen and sulphur emissions and

could also be used to reduce net carbon dioxide emission impact, but the increase of biomass conversion will result in an increased production of biomass ash. Thus, there is growing interest in biomass ash utilization as its direct disposal will cause significant economic and environmental problems.

Biomass exploitation as secondary fuel in co-combustion processes is technically and economically feasible up to 20% m/m biomass/coal, where both bottom and fly ashes (from below the fire and removed from the flue gas, respectively) have successfully been applied without any major treatment [4]. However, still a large part of biomass ashes is disposed of in many countries. Quantity and quality are key factors in order to overcome the barriers for more widespread ash utilization. Yet not large amounts of biomass ashes are delivered in order to contemplate using it as a value-added product and quality fluctuations make it difficult for finding a single solution.

Additional research and development on ash utilization options is advice, being able to take measures to provide a constant ash quality. It is also needed to intensify collaboration between producer and end-user, raise awareness within markets and harmonize regulations and technical standards.

There has been remarkably rapid progress over the past last years in the development of the co-utilization of biomass materials in coal-fired boiler plants; also in Poland, where biomass co-firing is a promising technology for decarbonising its power sector. But further investigations and better understanding of the co-combustion process are necessary. There are still many problems and questions for engineers to face with to further develop this technology.

2. Combustion and Co-combustion technologies

Biomass combustion is generally used for heat production in small and medium scale units while co-firing in fossil-fired power stations enables the advantages of large size plants, which could not be applicable in the case of dedicated biomass combustion due to limited local biomass availability [5].

The most common combustion technologies are the following [4]:

Co-firing:

- Bubbling fluidized bed furnace: 5 up to 120 MW_{th}
- Circulating fluidized bed furnace: 25 up to 300MW_{th}
- Pulverized combustion furnace: 100 to 1000 MW_{th}.

Dedicated Biomass combustion plants:

- Grate furnace and underfeed stoker: 20 kW_{th} up to 100 MW_{th}

- Bubbling fluidized bed furnace: 5 up to 120 MW_{th}
- Circulating fluidized bed furnace: 1 up to 50 MW_{th}
- Pulverized combustion furnace: 1 to 350 MW_{th}
- Stove: 2 to 15 kW_{th} (household), 20 to 500 kW_{th} (utility buildings or industrial)
- Rotary kiln: 0.5 – 10 MW_{th}

The systems can be distinguished by the flow conditions within the furnace, hence describing fixed bed combustion, fluidized bed and entrained flow or dust combustion.

Under stoker furnaces are mainly used for wood chips and other similar fuels which exhibit relatively low ash content, while grate furnaces can be also used for fuels with higher ash and water content. Both bubbling fluidized bed furnace and circulating fluidized bed furnace are applied for large-scale applications. Biomass fuels used are generally wood or mixtures of wood and industrial wastes.

Co-firing technologies are usually implemented in existing coal-fired power plants. The most common type of co-firing facility is a large, coal-fired power plant, though related coal-burning facilities (cement kilns, coal-fired heating plants, industrial boilers, etc.) can be used. There are three technological approaches of co-firing biomass with coal in a power plant differing in terms of the boiler system design as well as the percentage of biomass to be co-fired. Biomass feedstock can be mixed with coal outside the boiler, or it can be added to the boiler separately. The approaches are: direct co-firing, indirect co-firing, and parallel co-firing.

(a) Co-combustion or direct co-firing.

Biomass is fed directly into the furnace after either being milled together with the base fuel (Fig. 2.1a) or being milled separately (Fig. 2.1b) [6]. The main application nowadays is direct co-firing in coal-fired power stations boiler, usually a pulverized coal (PC) boiler.

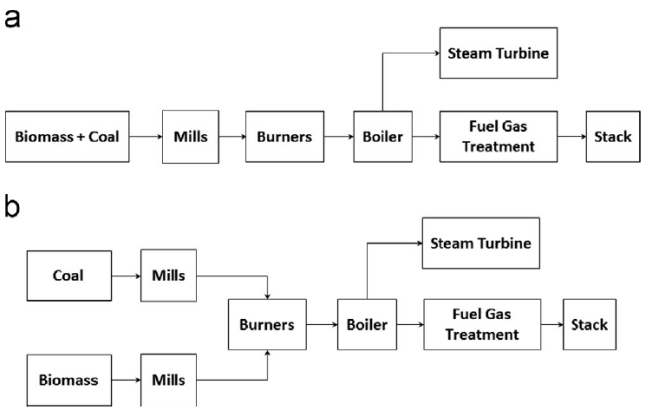

Figure 2.1 Direct biomass co-firing technologies: (a) Mixing biomass with coal. (b) Separate biomass feeding arrangement

Source: E. Agbor, X. Zhang, A. Kumar. A review of biomass co-firing in North America. Renewable and Sustainable Energy Reviews – Elsevier, 2014 [6]

It is a simple approach and the most common and least expensive method of co-firing biomass with coal in a boiler, although the co-firing rate is usually low, in the range of 3–5%. Further applications of co-combustion with coal are related to BFB, CFB, cyclone, and stoker boilers, which accept a much wider range of fuel size, composition, and moisture content than burners in pulverized coal boilers. The co-firing rate may rise up to 20% when cyclone boilers are used, but the best results are achieved with PC boilers.

(b) Indirect co-firing.

The biomass feedstock firstly undergoes a gasification process, and then the product gas is fed to a boiler furnace. Hence, a combination of gasification and combustion, as shown in figure 2.2. The gasification process of biomass produce a syngas which is rich in CO, CO_2, H_2, H_2O, N_2, CH_4, and also some light hydrocarbons. This syngas is then fired together with either natural gas or gasified coal in a dedicated gas burner.

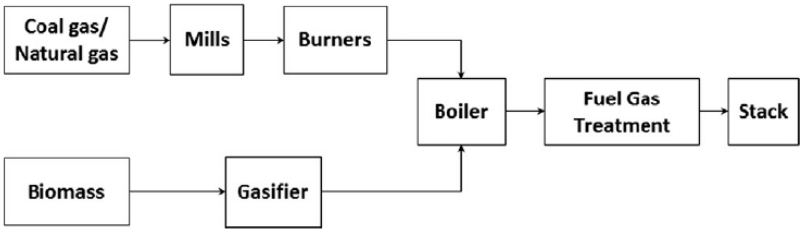

Figure 2.2 Indirect biomass co-firing technologies

Source: E. Agbor, X. Zhang, A. Kumar. A review of biomass co-firing in North America. Renewable and Sustainable Energy Reviews – Elsevier, 2014 [6]

The properties of the different kinds of biomass vary widely and have an impact on the heating value of the produced syngas, mostly related to the moisture content of the feedstock. High moisture content reduces the energy converted into syngas as more energy is consumed for drying and also leads to higher vapour content within the syngas, lowering therefore the percentage of combustible gases. The potential rate of biomass gas in the system is currently under investigations as higher percentages effects on combustion efficiency and emissions from pollutants are not yet determined.

On the other hand, gasification-based co-firing has lastly address several co-firing issues when compared with traditional co-firing technologies. It is fuel-flexible in terms of the base fuel and reduces boiler slagging by not feeding solid biomass.

Another form of indirect co-firing based on pyrolysis is currently under development, where a liquid bio-oil produce from biomass is co-fired with a base fuel such as natural gas in a power station [6].

(c) Parallel co-firing.

The biomass is burnt in a separate boiler for steam generation. The steam is used in a power plant together with the main fuel (Fig. 2.3). Parallel combustion enables a complete separation of the ashes and flue gases from both fuels and consequently, no disadvantages or limitations result from undesired alkali metals or contaminants in the ash. This technology offers lower operational risk and greater reliability but the installation of a completely separate external biomass-fired boiler represent the biggest drawback in compare with other technologies' investments costs.

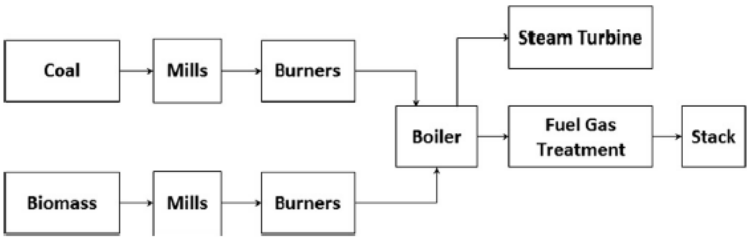

Figure 2.3 Parallel biomass co-firing technologies

Source: E. Agbor, X. Zhang, A. Kumar. A review of biomass co-firing in North America. Renewable and Sustainable Energy Reviews – Elsevier, 2014 [6]

3. Fuel

3.1 Fuel type

Biomass spans a heterogeneous set of organic matter, both by its origin as its nature. Within the definition, biomass for energy can include a wide range of materials. There are five basic categories of materials:

1) Virgin wood, from forestry, arboricultural activities or from wood processing;
2) Energy crops: high yield crops grown specifically for energy applications;
3) Agricultural residues: residues from agriculture harvesting or processing;
4) Food waste, from food and drink manufacture, preparation and processing, and post-consumer waste;
5) Industrial waste and co-products from manufacturing and industrial processes.

When co-firing biomass with another fuel, it is necessary to gain sufficient understanding of the properties of the fuels. Just as coal properties significantly differ across ranks, biomass varies dramatically from one type and category to another.

Virgin wood consists of wood and other products such as bark and sawdust which have had no chemical treatments or finishes applied, but will present different chemical and physical properties depending on the source it has been obtained (it may range in moisture content from dry to 60% or higher as freshly harvested, may have physical inclusions from the growing or

harvesting processes and there may also be chemical contaminants from the soil, water or air). In any case, woody biomass is considered as premium feedstock as it has generally low ash, sulphur and nitrogen content (which are all highly reactive and volatile, raising operational and technological challenges for co-firing activities).Virgin wood is suitable for a range of energy applications as heat/power generation. Both forest and agricultural wastes (straw, switch grass, corn stover, rice hulls, olive pits, etc.) have been successfully co-fired with coal in many installations in both North America and Europe.

Biowaste source (as the compilation of all different kinds of residues, by-products and organic wastes from agriculture, industry and households) are as well considered another possible source, although made of a wide variety of types, and therefore, the most appropriate energy conversion technologies and handling protocols vary from type to type.

Energy crops are grown specifically for use as fuel and offer high output per hectare with low inputs. In general, the principle purpose is to maximize the output of the desired harvest. Different organizations undertake research for different sites in order to identify the characteristics of potential energy crops, including biomass productivity under different conditions, susceptibility to disease or climatic events, etc. Policy on establishing energy crops is influenced by the land previously established with conventional forestry and the potential for harvesting fuel from this resource [7]. Although conventional forestry produces much lower levels of biomass output per hectare compared to many energy crops, the cost of producing each tonne of biomass in the forest are also significantly lower. Consequently there is little attraction in establishing energy crops on high quality agricultural land. Energy crops, conducted with the aim of producing transformable biomass into biofuels (instead of producing food, as has been the traditional agricultural activity) are already a reality in countries like Brazil and the United States that focus on production of sugar cane and corn, respectively, to obtain bioethanol.

3.2 Fuel properties

The kind of biofuel and its physical properties and chemical composition influence the whole process of biomass utilization (fuel supply, combustion system, solid and gaseous emissions). The most important characteristic are pointed below, with a description of their effects and a comparison to coal properties [8, 9].

- The volatile matter content of biomass is usually high. Typical values are in the range of 65 to 80% of the dry fuel compare to about 20 to 40% for coal. The volatile matter influences greatly the thermal decomposition behaviour, and it makes biomass a more

reactive fuel than coal.

- The moisture content of raw biomass can vary considerably. The moisture content is relatively low (15 to 30% of the raw fuel) for some energy crops harvested dry, such as cereals, miscanthus or perennial grasses, as well as for wood waste. On the other hand, the moisture content is much higher (40 to 60% of the raw fuel) for forestry biomass. In this last case, it can therefore be necessary to dry the biomass prior to combustion in order to avoid storage-durability or self-ignition problems.
- The ash content is another property that can vary widely. It can for example be very low (2 or 3%) for some energy crops such as miscanthus, or a bit higher (5 to 8%) for bark and some agricultural residues. It can also be as high as 15 to 20% for residues from rice agriculture, which makes it more difficult to use. Indeed, new problems then appear concerning dust emission, ash manipulation and disposal.
- The ash melting temperature is an important value to consider. For wood, there is no problem related to ash melting since the ash melting temperature is higher than 1100°C. However, for some energy crops, the high content of alkaline elements (K, Na) lower the ash melting temperature down to 750 or 800°C. Slagging and fouling problems can then be encountered.
- The gross calorific value of biomass varies from about 18 to 21 MJ/kg (dry basis). The lower values apply to herbaceous fuels while the higher ones are for fresh wood. In comparison, typical values for coal range from 28 to 35 MJ/kg. The net calorific value can be calculated from the gross calorific value, taking into account the moisture and hydrogen content of the fuel.
- The density is in most cases relatively low: 0,1 to 0,3. This increases the costs of fuel storage and transport. Moreover, the particle size distribution is for some biofuels inhomogeneous, which can make necessary additional pre-treatment.

Biomass pre-treatment technologies such as pelletization, briquetting and/or torrefaction could be effectively introduced to increase the heat value per volume of biomass, thus reducing the overall transportation cost. Pelletization is a process to physically densify fine biomass particles by applying pressure and heat. The result is a compact, low-moisture and low-eroding pellet, which can also repel water, improving logistics and storage options. The torrefaction process consists of heating biomass in the absence of oxygen, creating a charcoal-like substance with reduced moisture, small particle size, lower biological degradation and improved energy density. The resulting product after torrefaction can be also milled and

compressed into very dense pellets or briquettes. However, such technologies have extra costs and incorporating them in a large-scale biomass co-firing plant will certainly contribute to exceed the cost of operating an equivalent coal-fired plant. Nevertheless, this price difference could be overcome with a favourable CO_2 emission allowance price.

The chemical properties of biomass are also quite different from coal setting requirements for power plant operation. The carbon content is only about 46 to 51%, compare to at least 75% for coal. On the other hand, the sulphur content of biomass is also very low (less than 0,2%), which explains the low SO_X emissions. Alkali metals that are usually responsible for slagging and fouling are abundant in biomass fuel ashes and will be easily released in the gas phase during combustion. Also the chlorine content is to be examined carefully. It is usually low, except for some energy crops such as cereals, which can contain up to 1% of chlorine. It can have negative effect like corrosion and HCI emissions. This could be prevented by co-firing fuels containing sulphur and aluminium silicate peat or coal with chlorine bearing fuels [10].

4. Logistical and technical issues

Acquisition of biomass and transportation cost determine to a large extent the economic feasibility of co-firing. Biomass can be also used for biofuels and biomaterials production, involving market's prices competition. A biomass co-firing project requires a stable and cheap flow of biomass. Whether local availability of large quantities of cheap biomass exist the technology come to be very attractive, but insufficient local sources lead to the usage of high energy-density, pre-treated biomass pellets. In these cases, logistics and transportation play a very important role in the economic viability.

While estimates of total biomass resources vary significantly, a realistic assessment should only account for sustainable biomass - that is, resources that neither compete with food production nor involve changes in land uses, with can have adverse impacts on the climate and environment.

In order to reduce significantly the GHG emissions, more biomass should be consumed. However, high biomass share involve technical issues related to its unstable supply, as well as potential technological problems such as slagging, fouling and corrosion. Due to the current inability to manipulate logistical, technical and economic factors effectively, the present co-firing ratios rarely exceed 10% on a continuous basis, while according with IEA (IEA Bioenergy Task 32), co-firing more than 20% of biomass is technically feasible. Moreover, it is believed that biomass can potentially substitute even 50% of the coal. Table 4.1 features the range of co-firing ratios of common combustion technologies in biomass co-firing systems:

Table 4.1. Technical comparison of different boilers

Co-combustion system	Operation requirements	Co-firing percentage (% heat)	Technical features
Pulverized combustion	Fuel type: coal, sawdust and fine shavings; Particle size: < 10-20mm; Moisture content: < 20 wt%.	1-40%	Can decrease NO_X significantly. Limited by biomass particle size and moisture.
Fluidized bed combustion	Fuel type: various; Particle size: <80mm (BFB), <40mm (CFB); Temperature: < 900C.	CFB: 60-95.3%; BFB: 80%.	Most suitable system for co-firing. Soot formation is problematic, especially in CFB.
Packed Bed combustion	Fuel type: wide range; Particle size: < 30mm.	3-70%.	Not suitable for direct co-firing.
Cyclone combustion	Ash content: > 6%; Volatiles: > 15%; Except in a dried form, moisture content: > 20%.	10-15% by heat input or 20-30% by mass.	Minimal modifications are needed for feeding and mixing biomass and coal.

Source: Adapted from E. Agbor, X. Zhang, A. Kumar. A review of biomass co-firing in North America. Renewable and Sustainable Energy Reviews – Elsevier, 2014 [6]

Biomass co-firing may be affected by the following technical and logistical issues:

1. Fuel feedstock and its chemical and physical characteristics, which determine the whole combustion process and imply an impact on preparation, storage and handling properties.
2. Combustion technology, including boiler's capacity and performance, net efficiency and conversion losses.
3. Operational problems such deposition formation (slagging and fouling), corrosion and/or erosion and consequently changes in the life-time of equipment.
4. Flue gas cleaning operation and performance.
5. Ash disposal costs and/or revenue from ash applications.

5. Operational problems

5.1 Ash formation mechanisms

Solid fuels contain inorganic constituents that are responsible of ash formation during

the combustion process. The so called ‘ash-forming compounds’ vary greatly depending on the fuel’s nature and they may be present in the hydrocarbon structure or they may be found as external mineral particles or salts [11,12]. Generally older fuels contain more minerals while younger fuels contain a major fraction of species as organically bound and/or salts. The main compounds in wood fuels are usually alkali and alkaline earth metals attached to the carboxyl groups of carbohydrates. In the case of coal, typical minerals are silicates (e.g. clays, quartz), carbonates (e.g. calcite, siderite), sulphides (e.g. pyrite) and oxides. The physical and chemical transformations during thermal conversion of solid depend on several fuel characteristics:

- fuel mineral matter composition,
- ash levels in fuel,
- fixed carbon,
- volatile matter,
- mineralogy (particularly the levels of included or excluded mineral phases),
- char reactivity and char morphology,
- density and particle size.

Also the operating conditions are of major importance for the said transformations. Crucial parameters include the type of combustion system (air staging), temperature, pressure, heating rate and residence time, as they affect chemical equilibrium of numerous gaseous species as well as reactivity of gaseous, liquid and solid slag phase-bound minerals [13].

This inorganic residue after combustion travels as a suspension of fine particulate towards the stack, along with the flue gas and it can potentially create various problems such as near-burner slagging, boiler fouling, corrosion and erosion. It can also affect emissions in various ways. The two important physical transformations are fragmentation and vaporisation.

During combustion, a fraction of the ash-forming compounds in the fuel is volatilised and released to the gas phase. These primary particles are very small in size (5 to 10 nm), but they grow by coagulation, agglomeration and condensation during their way on the flue gas. This particles form the basis for the fine mode of the fly-ash, characterised by a particle size <1μm, and is emitted with the flue gas when the combustion plants are not well equipped with efficient dust precipitation technology.

The non-volatile ash compounds remaining in the char may be fragmented, resulting in residual ash particles, showing variety of composition, shapes and sizes. A fraction of the residual ash will be entrained with the flue gas and form the coarse part of fly ash (generally exceeding 5μm), while other fraction will stay on the grate and form bottom ash at the risk of causing slag formation due to a lowering of the melting point [12, 13].

Similarly, the inherent inorganic species may undergo several chemical reactions. Compared to coal, biomass fuels usually have higher concentrations of alkali/alkali metals (K, Na, Ca) and chlorine (Cl), which are highly active during combustion increasing the deposition rate on reactor walls or heat exchangers surfaces.

5.2 Ash deposition

Ash deposit formation represents a very important technical issue when co-firing ash-forming fuels, having an impact both in boiler design and operation. Thus, ash is one of the most studied characteristics of biomass, but unfortunately still with relatively poor understanding [14]. The reason for this problem is the complex character of the ash which originates both from natural and technogenic inorganic, together with organic and fluid matter during the combustion process [15].

Several problems related to ash and ash deposition can be defined as follows: slagging refers to the formation of fused or sintered deposits on heat-transfer surfaces and refractory in the furnace cavity subjected to radiant heat exchange; while the accumulation of ash deposits in the cooler convective section of the boiler are called fouling deposits and result from the behaviour of components as the gases cool down. Corrosion takes place when a component from the ash deposit or flue gas react with the metal tube wall. Also the tube surfaces in the high velocity sections of the convective part of the boiler are subjected to erosion due to the impact of particles, which is in turn exacerbated when fouling deposits represent a blockage. The slagging and fouling phenomena comprise a large number of variables that affect ash deposition process in various ways. A lot of research is conducted aiming to explain the mechanism of mineral matter transformation during burning, determine the influence of chemical composition of mineral matter and surrounding conditions on this mechanism in order to develop the necessary procedures to avoid hazards [16, 17].

6. Ash utilization

Generally, different ash fractions are generated by biomass combustion and co-combustion: bottom ash (collected from the combustion chamber), coarse fly ash (boiler fly ash, cyclone fly ash) and fine fly ash (from baghouse filters or electrostatic precipitators) [4]. The percentage of individual ash fractions is dependent on the combustion technology used. For example, in fixed bed furnaces bottom ash represents the main ash fraction (60-90%), while in fluidized bed furnaces is usually the fine fly ash the largest fraction of the total ash collected. The chemical composition of the ashes is also dependent on the combustion technology,

together with the type of ash fractioning, the kind of biomass fuel used and/or the combustion ratio when co-firing.

Ashes from the combustion of natural solid biomass generally contain valuable plant nutrients such K, P, Mg and Ca. The high contents of alkali metals, phosphorus and calcium make them unsuitable for the current established uses for coal's residues. But they are considered as viable source of nutrients for agricultural or forest land, thus reducing the use of artificial fertilizers. Nevertheless, biomass ash also contains substantial amounts of heavy metals (especially Cd and Zn) which have to be considered for the utilization strategy applied. There are significant differences regarding the heavy metal and nutrient content and the distribution among the ash fractions: fine fly ash usually display higher content of volatile heavy metals (Zn, Pb, Cd) together with semi-volatile nutrient K. On the other hand, non-volatile elements (Si, Ca, Mg) exhibit the highest concentrations in the bottom ash. A general characterization for the chemical composition of different biomass types would have key importance as main indicator for potential applications [14].

Coal ashes have been widely used for different applications, taking advantage on its physical and chemical properties for construction and building materials. It is the case of fly ash used as mineral admixtures in concrete, improving the performance and quality [18]. The properties of co-combustion residues differ from those of coal as they are directly connected with the individual blends components. But the same as the substitution of coal for biomass in coal-fired boilers brings about significant benefits, the utilization of co-firing ashes should be assessed as the same valid. Disposal of ash has caused significant economic and environmental problems, therefore different alternatives to use ashes as a value-added product have been investigated. Several properties of biomass ashes could become relevant and particularly attractive to a specific application. For instance, physical properties (particle size, density…), chemical properties (pozzolanic behaviour providing higher durability to concrete, amount of nutrients, macro elements, unburned carbon…). Although it would be necessary to assess as well the ecological properties as the heavy metals content and the leaching behaviour.

Several standards governing the utilization of co-firing ash residues (national standards, European norms and the analogous ASTM standards) define the significant parameters and characterization methods for ash utilization. The technical standard EN-450 for the use of coal fly ash for concrete was an important development in the regulation and is currently used in many European countries. Also the EN-13055 for the use of bottom ash applied in civil engineering. EN-450 is now under revision so residues from biomass and coal co-combustion (even at high ratios up to 50% m/m fuel input) will be also included. Thus, fly ash definition

will relate fine powder of mainly spherical glassy particles, derived from pulverised coal with or without co-firing materials [19].

Currently, co-firing up to 20 % (m/m) biomass/coal bottom ashes are used as concrete aggregate and the fly ashes that fulfil the requirements of the EN-450 are used as additive in cement or as concrete or asphalt filler. It is expected that the revision in EN-540 will seriously influence the European situation of fly ash utilisation. Legislation for ash utilization not (fully) cope with all utilization options that are technically possible due to lack of sufficient legal guidelines. In order to improve ash utilization, development is required in different areas as research, logistics, ash quality, collaboration, marketing, regulations and policy.

7. GHG Emissions and Environmental Impact

As concerns regarding greenhouse gas emissions from coal-fired power plant increase, there is greater focus on the feasibility of substituting biomass for coal in existing fossil-fuel-based power systems. Even though the share of biomass achievable greatly differs from the current co-firing ratio, it is necessary to exceed it on a continuous basis as a higher share of biomass directly implies lower GHG emissions. It has been estimated that co-firing biomass and coal at 20% could reduce CO_2 emissions from 45 million to 450 million ton/year by 2035 [20]. Biomass is considered a CO_2-neutral fuel as no net CO_2 emissions are release to the atmosphere if carefully managed, although whole life cycle of biomass should be taken into account when estimating the environmental consequences of co-combustion as harvesting, transportation and pre-treatment. In any case, a Life Cycle Assessment [20] shows that using biomass results in environmental benefits when comparing with coal-fired systems. Not only CO_2 emissions are reduced, but also greenhouse gasses and traditional pollutants, as SO_X and NO_X, due to lower sulphur and nitrogen content in biofuels. Despite the drawbacks of higher alkali content on ash deposition, this components may also have a positive effect on SO_X removal and higher amount of volatiles within biomass fuels make it possible to use it as a re-burn fuel, thus additional reduction of NO_X emissions could be achieved.

There has been remarkably rapid progress over the past last years in the development of the co-utilization of biomass materials in coal-fired boiler plants. Biomass co-firing is a promising technology for decarbonising the energy sector, but further investigations and better understanding of the co-combustion process are still necessary in order to provide energy at a reasonable cost and simultaneously protect the environment.

8. Conclusion

Co-combustion is probably the least complicated and one of the most advantageous ways

of utilizing biomass for replacement of fossil fuels for stationary energy conversion. Co-firing capitalises on the large investment and infrastructure associated with existing fossil-fuel-based power systems, while requiring only a relatively modest investment to include a fraction of biomass in the fuel. When proper choices of biomass, coal, boiler design, and boiler operation are made, traditional pollutants (SO_X, NO_X, etc.) and net greenhouse gas emissions decrease. Modern coal power plants can also achieve higher efficiencies than smaller stand-alone biomass plants, though the use of two different fuels increases the complexity of power generation. Although co-utilization of biomass is a promising technology, it leads to technological issues. Depending on the source of biomass, the introduction of biofuels in a coal-fired boiler may cause operational problems due to the higher content of alkali metals and chlorine compounds. These elements reduce the ash melting temperature, causing ash deposition problems, such as slagging and fouling, and corrosion.

Further research and development is needed for improving both boiler design and combustion technology. Understanding material properties and transformation during combustion will help to increase the share of biomass co-combustion and further mitigate hazardous emissions into the atmosphere. For EU countries such Poland, were GHG emissions from coal combustion systems are a major concern, the co-firing strategy will probably help to fulfil the 20-20-20 targets.

At present, a large co-firing potential exist [21] and even at small co-firing rate, the advantages of using biomass significantly outweigh the constraints associated with it. But before large scale implementation can be a reality, a long term strategy based on a strong policy framework is required.

References

1. Dahlquist E.: Technologies for Converting Biomass to Useful Energy: Combustion, Gasification, Pyrolysis, Torrefaction and Fermentation. CRC Press, Series: Sustainable Energy Developments 4, 2013.
2. International Energy Agency: Energy Policies of IEA Countries – Poland. IEA, 2011.
3. Guła A., Wajss P., Goryl W: Is Using Biomass for Power Generation a Good Solution? The Polish Case. Electrical Review, 2012.
4. R.J. van Eijk (KEMA): Options for increased utilization of ash from biomass combustion and co-firing. IEA Bioenergy - Task 32 Report, 2012.
5. Nussbaumer T.: Combustion and Co-combustion of Biomass: Fundamentals, Technologies, and Primary Measures for Emission Reduction. Energy & Fuels, 2003.

6. Agbor E., Zhang X., Kumar A.: A review of biomass co-firing in North America. Renewable and Sustainable Energy Reviews. Elsevier, 2014.
7. European Environmental Agency: How much bioenergy can Europe produce without harming the environment?. EEA ,2007.
8. Koppejan J. and Van Loo S.: The handbook of biomass combustion and co- firing, 2002.
9. Gonzalez Valdes, L.: The analysis of the mixture of the ash from biomass and coal. M.Sc. Thesis - Politechnika Wrocławska, 2014.
10. European Bioenergy Networks (EUBIONET). Biomass Co-firing - An effective way to reduce greenhouse gas emissions. EUBIONET Finland, 2003.
11. Bryers R.W.: Fireside slagging, fouling, and high-temperature corrosion of heat-transfer surface due to impurities in steam-raising fuels, 1996.
12. Shao Y., Wang J., Preto F. et. al.: Ash deposition in biomass combustion or co-firing for power/heat generation. Energies, 2012.
13. Solid Biofuels for Energy. A Lower Greenhouse Gas Alternative. Springer, 2011
14. Vassilev S. V., Baxter D., Andersen L. K., Vassileva C. G.: An overview of the composition and application of biomass ash. Part 1. Phase–mineral and chemical composition and classification. Fuel-Elsevier, 2012.
15. Vassilev S. V., Baxter D., Andersen L. K., Vassileva C. G.: An overview of the chemical composition of biomass. Fuel-Elsevier, 2009.
16. Stam A. F., Livingston W. R. et al.: Review of models and tools for Slagging and Fouling Prediction for Biomass Co-Combustion, 2009.
17. Lopez et al: Characterization of ash deposition. Heat Exchanger Fouling and Cleaning: Fundamentals and Applications. ECI digital archives Art. 38, 2003.
18. Xiao-Yong W.: Effect of fly ash on properties evolution of cement based materials. Construction and Building Materials 69 - Elsevier, 2014.
19. Grammelis P., Skodras G., Kakaras E.: Effects of biomass co-firing with coal on ash properties. Part I: Characterisation and PSD. Fuel - Elsevier, 2006.
20. Sahu S. G., Chakraborty N., Sarkar P.: Coal–biomass co-combustion: An overview. Renewable and Sustainable Energy Reviews - Elsevier, 2014.
21. IEA-ETSAP and IRENA: Biomass co-firing technology Brief. Technology Brief E21, 2013.

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The use of Chlorella vulgaris , Rapidly reduce the iron content in the effluent post-production

Key words: *Wastewaters, microalgae, Chlorella Vulgaris, Environment*

Abstract: The paper presents results of research on the use of microalgae Chlorella vulgaris to remove iron in the effluent post-production. Laboratory tests were used photo-bioreactor which enabled precisely controlled conditions of the experiment. The study was carried out for 14 days, controlling the loss of iron from the effluent. In addition, the parameters were controlled, nutrient content of selected conditioning kept breeding. In order to control the growth of biomass of microalgae assay was performed chlorophyll "a". It was founded during the research were obtained iron reduction of 29% with simultaneous increase in the biomass of microalgae referred to about 1364%. For the correctness of the culture was used conditioner F2 to provide the necessary nutrients for microalgae.

1. Introduction

The development of research based on the principles of sustainable development faces the difficulty of access to raw material research. In addition, there is emphasized the moral accent, as opposed to numerous communities conducting research related to the use of microalgae as a raw material for research in terms of perceiving them as a figment of scientists. However, as indicated by the authors obtained experimental results of these studies clearly are more perspective, and are becoming increasingly important in the scientific world. This is directly related to the research to take measures for the reduction of harmful human impact on the environment. All because the actions of the people must take into account that the environment is not good infinite, and thus should strive to implement and comply with the principles of sustainable development. The password created at the turn of the century is becoming increasingly important. This doctrine because changing approach to the environment. In view of this increasingly begins to approach environmental processes guided by the principles of sustainable development. The same concept is to integrate the objectives of environmental, social and economic policy Sustainable development means that the process of economic growth leads to increasing social cohesion and improving the quality of the environment. This is done through actions among which we highlight, among other things:

- Reduction the harmful effects of production and consumption on the environment;

- Active protection of natural resources;
- Reduction of anthropogenic impact on the biotic components of the environment.

It should be mentioned also that an important part of a balanced system of international law. Among the most important international legal documents that recognize the issue of sustainable development include: Agenda 21, the Convention.

Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters concerning.

Gradually, the following changes in the meaning of the ecosystem has its base in the development of scientific disciplines directly or indirectly related to environmental protection. The very concept of ecosystem is in fact very relativized to the level of expertise in the particular phenomenon is unusual. Sam increase our knowledge of the natural sciences has another important dimension namely a man realizes his responsibility and the weight that rests upon it for the smooth functioning of the environment. Ecology itself also not worked out a theory of self, and the results of her research, as well as biological and other sciences are generalized in the context of the theory of biological evolution. As a result of these changes has been the separation of the ecology of the environment. However Ecology situates himself as a basis for conducting actual research on environmental protection.

2. The meaning and use of microalgae in environmental protection

Microalgae also known as algae, it's easy autotrophic, aquatic organisms. This includes both eukaryotic and prokaryotic species, single and multicellular. The number of species is estimated at about 10 million, most of which unicellular algae to [1]. Microalgae in contrast to higher plants, multiply no matter what time of the year, and their biomass is homogeneous. In optimal culture conditions gives the possibility harvesting biomass even every few days. Microalgae as a component of plankton are salt water and fresh water (phytoplankton) and are covered with all kinds of surfaces (plants, rocks) to form a team of periphyton. Moreover, they can also occur on land, where inhabit moist places, are found kindle in snow and ice (where there are as snow algae).

Due to the extensive possibilities of using the biomass, which has specific properties of microalgae were the focus of learning. The overall scale algae origin are used, both inland and marine. Among the main biomass extracted from microalgae can distinguish the following areas of application:

- As food for fish;
- As a renewable material involved in the biological treatment of aquatic ecosystems;

-As material for the production of oxygen by carrying out photosynthetic processes;
- As a mineralizing soil material and thus enriching it in humus;
- As a source of protein for human food;
- As a food source with a high content of vitamins and endogenous to the body;
- As a material used in the textile industry;
- A material used in medical, pharmaceutical and food industries.

A very promising application of microalgae becomes possible to use them in a wastewater treatment technologies. This is of particular importance within the removal of nutrients. In addition, the possibility of disposal by microalgae hardly biodegradable substances, and the possibility of using microalgae as an alternative fuel generation III. In this case, as a result of a transformation of photosynthetic carbon dioxide and sunlight into rich in minerals and lipids biomass, which biomass becomes a rich source of triacylglycerols [3,4,5,6]. Without a doubt, help them in this simple biological structure, so that they are biodegradable faster than higher plants [7].

Same method of production of microalgae biomass is classified as open and closed methods. Regardless of the method used is a multi-step process, which is conditioned by many factors. Microalgae growth process requires the supply to the breeding of light, carbon dioxide, a suitable level of oxygenation of water, minerals and the use of the optimum temperature. Dietary requirements must be specified using the right formula microalgae biomass. Classical recognition to ensure proper food for microalgae is the use of optimized by Grobbelaar formula by applying the conversion factors between minerals (CO: H: NP), which is as follows: 0.48: 1.83: 0.11: 0.01 [8] .

The first method of biomass from microalgae culture is driving them through open joints. They provide a closed loop recirculation based in its design of the small volume of the channel. Microalgae mixing process is achieved by the use of variable turbine cycles. Additionally, thanks to their continuous operation prevents algae sedimentation process. The biomass is discharged through the turbine at the joint. The advantage of this method of cultivation of microalgae is low overall system cost, and high amount of biomass that is producing. Among the disadvantages to be considered a lack of ability to control the weather conditions which impact on the farming.

The second method by which the harvested biomass of microalgae cultivation is running in closed systems. For this purpose are used photobioreactors, which are closed systems constructed from materials transmitting light. It is generally accepted photobioreactors division into 3 types:

- Vertical-column;
- Cylindrical;
- Otherwise referred to as flat panels.

Critical parameter that determines the correct course of microalgae growth is light. For this reason are photobioreactors with the sunlight, and artificial light. The basic assumption of the impact of light on microalgae is the possibility of getting a single cell. For this reason, the device can be divided into jasna- zone located close to the light source and dark- zone located far from the irradiated surface. The mere presence of a dark area caused by the absorption of light by micro-organisms, and their self-shading. This phenomenon results in a photobioreactors zones:

- The outer layer of microalgae, which are exposed to too much light intensity, which can lead to photoinhibition;
- The middle layer of algae on the perfect lighting;
- The inner layer of the absence of light the microalgae where respiration processes proceed with high intensity [9,10].

Availability of carbon in contrast to compounds of nitrogen and phosphorus, rarely limits the growth of algae. Productivity, which takes place in the waters sweet meets another restriction to limited access to phosphates. The availability of silicon is, however, a limiting factor in the poor growth of diatoms in a oligotraphic lakes. Most other nutrients present in an aqueous medium in excess. Given that some microalgae are absolute autotrophic is many require an external source of vitamins, of which the most important are the thiamine, biotin, vitamin B_{12} and riboflavin and purine and pyrimidine compounds. the requirements of this kind referred to as auksotroii that reflects the richness of organic matter in the environment and the population is the result of the selection of microalgae non- synthetic all the ingredients needed to metabolic process could occur in the cells of microalgae. Most of microalgae tolerate a wide range of both pH and temperature. The growth rate of microalgae several times higher than the rate of growth of terrestrial and aquatic vascular plants. The study was used Chlorella vulgaris. And the main criterion for selection was oriented to the specific individual features of this species. This microalage characterized by high adaptability to changing environmental conditions and the culture. Suitable culture to stimulate the process have a direct effect on the possibility of obtaining various downstream processes. Chlorella vulgaris Is an organism cosmopolitan, occurring commonly in almost every aquatic and humid environment, both freshwater and saltwater like. Wide range of temperature tolerance does cause them even in the Arctic waters [11]. The very high potential for use of this species is to be seen particularly in

removing selected pollutants from water matrix, referred to as contaminants difficult.

1.2 Characteristics of the methods used to remove heavy metals from the water

The presence of heavy metals in waters, soils and the atmosphere depends on the chemicals, both natural and human economic activity. Metals enter the environment as a result of geochemical processes caused by the eruption of volcanoes, and as a result of weathering of rocks. Chemical form of metals in the environment depends on many factors, among them distinguish such as the properties of the metal and pH (the pH), redox potential, presence of other metals and ligands. The mere presence of metals because of their widespread prevalence in soils, water, bottom sediments and biota does not become a reason for the occurrence of contamination. The process contamination, because we can speak at a time when the metal is present in a large, relative to the level of the background concentration. This toxicity associated with their bioaccumulation in the food chain is one of the environmental and health problems of modern society. This is particularly serious in the case where metals are accumulating as a result of conducting industrial processes. When they arise because solutions containing different metal ions. Such solutions arise both in the metallurgical industry and in other industries as a side effect of the technological process used. Metals in the waste water can come from, leather industry, the paints, fertilizers, pesticides, textile, and electrochemical. In industrial plants, in which the waste water contains heavy metals, there should be a separate process systems for the screening. This is not only for safety reasons, but also economic reasons. It has been proven that the purification of metals smaller amounts of wastewater from individual departments is more cost-effective than the mixed sludge from sewage pooled [12,13,14]. Conventional techniques for removing heavy metals from water and wastewater include min. chemical precipitation, reverse osmosis, evaporation, and ion exchange. These methods have many advantages, but it is often found that they are very effective at low concentrations, and produce toxic sludges with a high cost of carrying out the process. Table 1 summarizes the advantages and disadvantages of the most commonly used methods for removal of metals from aqueous solutions.

Table 1. Selected methods for removal of metals from aqueous solutions and from wastewater

Method	Advantages	Disadvantages
Chemical precipitation and filtration	- simplicity; -costs	- For large concentrations - Ineffective
Electrochemical methods	- recovery of metal	- For large concentrations;

		- High cost
Reverse osmosis	- Pure water for re-use	- High pressure - Clogging of the membranes
Ion exchange	-The possibility of regeneration of resins -Effective method (possible recovery of metal)	- Sensitive to a suspension of ions form of metals -costs resins
Evaporation	- Pure water for re-use	- High energy consumption; - High cost - Pellet remains

Source: Sanak-Rydlewska S.: Eliminacja jonów ołowiu za pomocą sorbentów naturalnych. Międzynarodowa Konferencja nt.: "Zarządzania środowiskiem w aspekcie zrównoważonego rozwoju terenów uprzemysłowionych",Szczyrk 20–22.03.07, s. 115–255.

2. Materials and methods

2.1 The used of species microalgae in research

Chlorella vulgaris used in the research work was donated by Dr. Philip Pniewski, Head of the Collection Baltic algal cultures in Gdynia in the course of scientific cooperation. Inoculate was delivered to the Department of Water Environmental Science, Faculty of Food in a plastic tube with a capacity of 50 ml filled with a standard F-2 medium (pH 7.5) for freshwater microalgae. Then strain pedigree was transferred to the culture system based on the methodology used in the breeding closed algae photobioreactors on laboratory conditions. Cells were passaged into new containers with fresh medium 1 once a week, each time checking the sterility of the culture microscopically. Breeding was was carried out under constant conditions in photobioreactors at 26°C at light 64 μE / m^2 / s obtained by HPS lamps in the light / dark cycle of 12/12 hr. The test consisted of photobioreactors horizontal orientation, where the total volume was 10 dm3. The contents were mixed photobioreactors using a mechanical stirrer with a capacity of 5dm3 / h. This treatment technology also allowed to supply CO_2 to the solid culture medium. Losses due to the abstraction of water samples for testing were not replenished, so that the samples were not diluted by additional quantities of clean water. Were added to the culture at a concentration of iron ions 500μg / dm3, using standard Merck of Ferrum.

2.2 Research and hydrochemical determination

Test samples for th hydrochemical analysis were taken every 24 hours for 14 days of the experiment. Each photobioreactor collected raw sewage. The contents of the selected indicators were conducted in accordance with the methodology specified by the APHA (1995). Determination of iron phenanthroline general method involves the reduction of the iron contained in the water to bivalent iron by means of hydroxylamine and reduce by 1,10-phenanthroline. The increase in biomass is determined by the chlorophyll "a". In order to standardize and maintain repeatability were used glass filters GF-F class whatman company. Samples were analyzed in 3 replications. In parallel with the cultures containing iron ions were carried out control cultures without the addition of iron ions. In the experimental work model waste water was used, which consisted of water with a standard conditioner, and considered metal.

3. Results

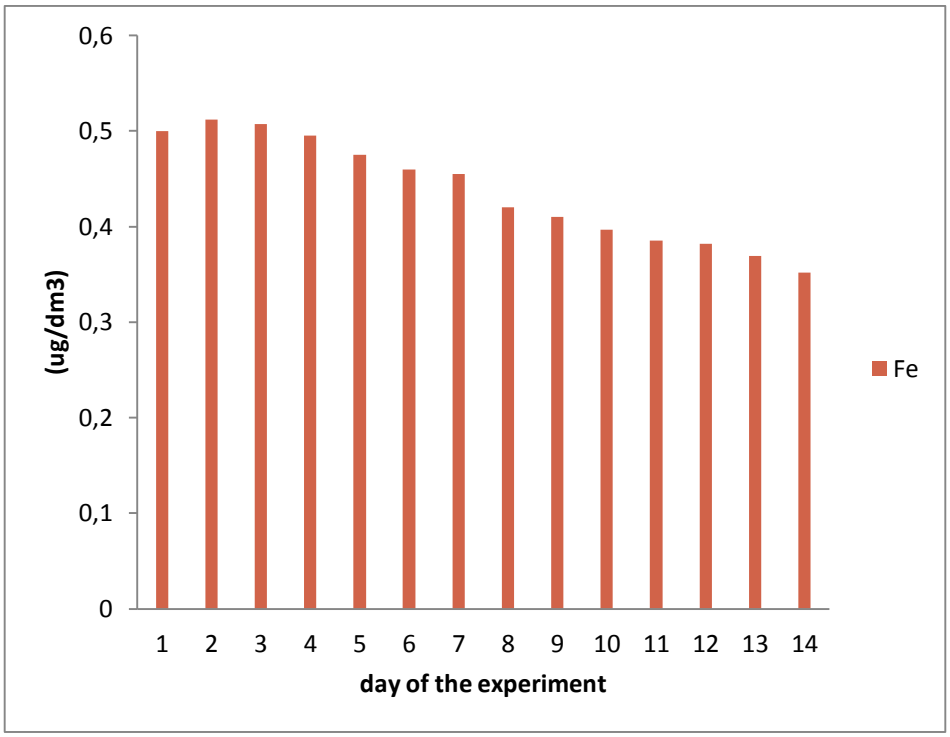

Figure 1 The values of iron ions during the experiment

Source: Own

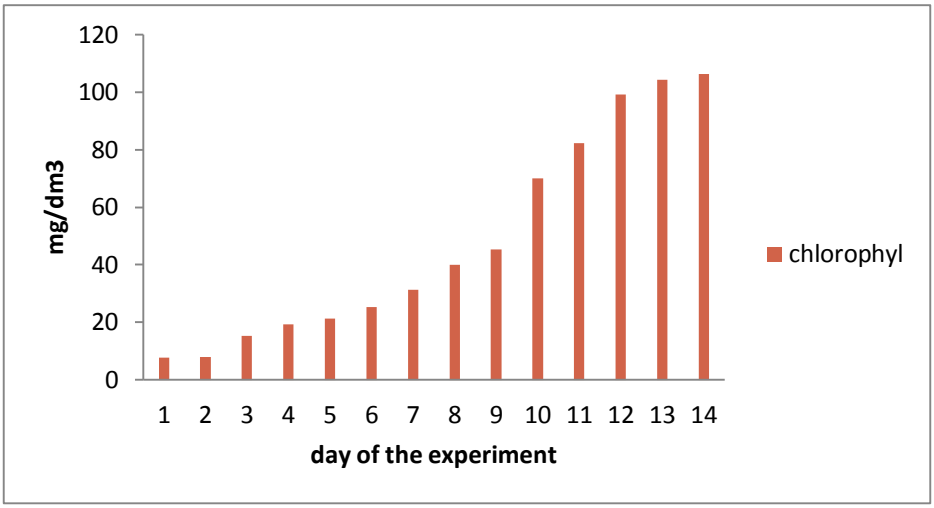

Figure 2 . Chlorophyll "a" in the course of the study

Source: Own

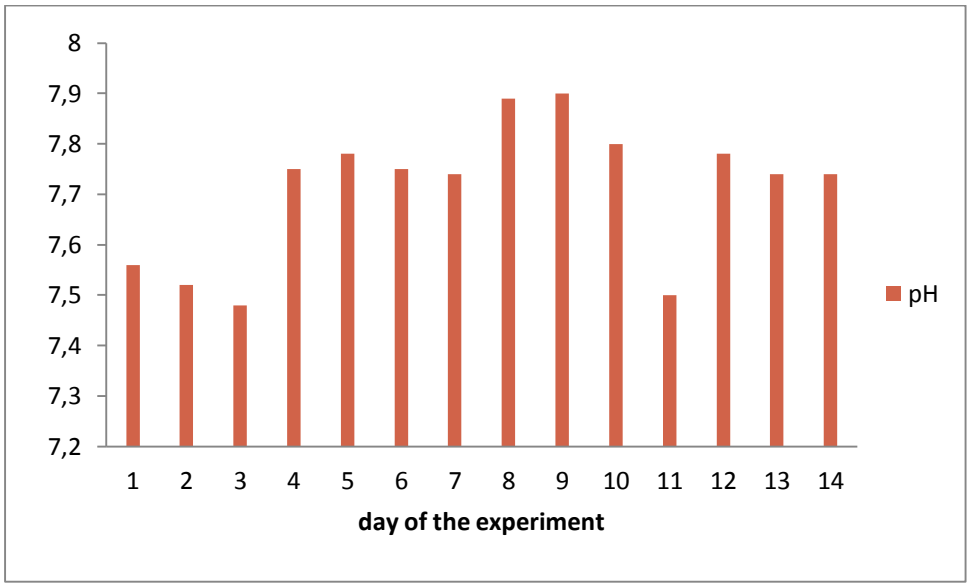

Figure 3 . The pH values during the test

Source: Own

Fig. 1 shows the resulting reduction of iron ions, which was achieved in the course of the study. The initial value was 500 ug / dm^3 on the first day of the experiment, and the last measurement was 352 ug / dm^3. With the decline of the iron ion content of microalage increased the biomass content, which was determined on the basis of measurements of chlorophyll "a", which is an indicator of the size of primary production. As indicated in Figure 3 pH fluctuated in optimal values, which are scheduled for freshwater microalgae.

Approaching critical analysis of the results should be borne in mind that metal toxicity is closely related to their bioavailability for microalgae. With this issue is related to the pH value of the environment, the presence of other ions and chelators. Acidic environment favors

the solubility of metal compounds, and thus increases the concentration of the fractions for microalgae of bioactive [15].

In the present study the compaction process has been applied on the surface of cells, which was defined as biosorption. This process, however, has not been fully understood or defined or. according to Shumate and Stranber biosorption is the result of ion exchange adsorption by exchanging metal ions from the ion spray occupying active sites of microorganisms; Surface metal precipitation as hydroxides, salts or insoluble complexes, and chemical reactions metabolites secreted extracellularly, and collecting and crystallization products formed within the guard cell. [16]. In contrast Tsezos considers that biosorption both extracellular and intracellular download metals by microorganisms associated with their metabolic activity and microbial activity independent of the compaction surface. Biosorption can be described as metal compounds retention within the cell casings, metal transport across cell membranes to allow their subsequent intracellular accumulation, adsorption or ion exchange adsorption of the natural [17,18]. The results of the study carried out by Ozer and others affirm that microalgae having the ability to carry out photosynthetic processes have a greater ability to remove chromium (VI) equal to 29.6 mg $*g^{-1}$. Research in the field of wastewater treatment technologies demonstrate the competitiveness of photobioreactors suspended in solution compared to conventional solutions using activated sludge or trickling [19].

In our studies we obtained a reduction of iron ions of approximately 30%, similar to Meier et al. The authors also show that the biosorption process can be an alternative to the classical methods of removing metal from aqueous matrices [20]. Other researchers have found that without a doubt, an alternative to using the biosorption process of dead organic matter may be natural biomass of microalage, particularly, the use of which is currently the subject of numerous research. It has been found that it is cheap, fast increasing biomass renewable raw material, present in large quantities in the seas and oceans, and ease of production in the laboratory. The use of these organisms in industrial wastewater treatment technologies is of particular importance to the need to seek new, environmentally beneficial and economically viable solutions. The observed trend of continuous tightening of standards defining acceptable concentrations of heavy metals in waste water also results in the - in spite of advanced conventional technology- purification methods are insufficient and too costly. This applies in particular waste water, in which the metal concentration does not exceed 100 mg / dm^3 [21].

An important component in the development of research related to the removal of metals from aqueous matrices is the choice of biological material that could potentially be used as

biosorbent metal ions from the metal ions. This material needs to be assessed, with a view to determining its capacity biosorption. The authors, who were occupied with the study of the process kinetics of biosorption of metal ions in waste water are usually based on experiments in which biosorbent is contacted with a solution of metal ions with a defined initial concentration. In the case of using algae as often biosorbentu contacting process is carried out in shakers , under isothermal conditions and constant intensity of shaking, in order to ensure maximum contact with the solution of immobilized microalage. The kinetics of the process is followed until there is equilibrium sorption system, manifested in August no change concentrations of metal ions in the solution. Where the use of biomass of living micro-organisms (the possible presence of mechanisms for bioaccumulation and biotransformations) when bound to a maximum quantity of metal ions from a solution, is sometimes observed natural phenomenon, partial desorption. Given the above, it is desirable to develop new technologies that are designed to characterize the kinetics of the process, ie. Speed metal sorption and desorption using a specific of biosorbent. For biosorption equilibrium is achieved, usually within a few minutes, which significantly differs from the sorption processes using activated carbon or ion exchangers, in which the time required to achieve equilibrium is measured in hours. Download stabilize state of iron ions in our study, we observed after the first phases of acclimatization, and metabolic processes, similar conclusions were formulated by Filipiuk and al. [22].

Although traces of heavy metals are essential as co-factors for many enzymatic activities in microalgae, as in most organisms, higher concentrations of heavy metals are toxic. Nonetheless, some microalgae can absorb large quantities of heavy metals from wastewater and store them in different cytoplasmatic structures without toxic consequences. They use trace amounts of essential metals for growth and metabolically ignore the non-essential heavy metals. Microalgae have an affinity for polyvalent metals, leading to their application as cleaning agents in water and wastewater containing dissolved metallic ions. For removing metals, the microalgae of choice were usually species of Chlorella vulgaris and Scenedesmus. For example, a strain of Chlorella can live in a cadmium and iron -rich suspension and remove up to 65% of this pollutant [23]. As with copper, iron does not produce a significant environmental hazard, despite its endless uses. Alginate immobilized and free cells of A. doliolum and C. vulgaris showed higher uptake rates of copper and iron, suggesting that immobilization offers some protection against metal toxicity. Compared with free cells, immobilized cells showed greater efficiency for removing iron, even after three cycles, although there was a gradually

decrease in efficiency in the second and third cycles [24]. Our results is more efficiency in a factor of time.

That same results is reported by Pankowski et. al. They reported that increasing the iron concentration from 6.25 nM (3.5×10^{-4}mg/l) to 300 nM (1.7×10^{-2} mg/l) increased the growth rate of Fragilariopsis cylindrus from 0.34 d^{-1} to 0.57 d^{-1} and that of Fragilariopsis curta from 0.14 d^{-1} to 0.28 d^{-1} [25].

4. Conclusions

This study focused on a specific problem of the removal of iron ions from the drain of ions form. A special feature is the presence of iron in the form of bound and conditioned by a number of elements of the environment. The results indicate the possibility of using microalgae rapidly reduce the substantial loads of iron from waste water. In the era of exploration technology solutions that do not adversely impact on the environment is an alternative to the proposed solution compared to the conventional methods used so far.

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References

1. Weiner J.: „Życie i ewolucja biosfery, Podręcznik Ekologii ogólnej. Wydanie drugie poprawione i unowocześnione, Wydawnictwo Naukowe PWN, Warszawa 2006.
2. Barsanti L,. Gualtieri P: „ Alage: Anatomy, Biochemistry, and Biotechnology", CRC Press, 2006.
3. Banerjee A., Sharma R., Chisti Y., Banerjee U.C., Botryococcus braunii: a renewable Source of hydrocarbons and other chemicals. Crit. Rev. Biotechnol. 2002 , 22, 245-279.
4. Melis A., Green alga hydrogen production: progress, challenges and prospects. Int. J. Hydrogen Energy, 27,2002 , 1217-1228.
5. Lorenz R.T., Cysewski G.R., 2003. Commercial potential for Haematococcus microalgae as a natural source of astaxantin. Trends Biotechnol.2003, 18, 160-167
6. Spolaore P., Joannis-Cassan C., Duran E., Isambert A., Commercial applications of microalgae. J. Biosci. Bioeng. 2006, 101, 87-96.
7. Kuligowski K., Tonderski A., Wójcik M: „Biogaz z alg- szanse i zagrożenia: Konferencja - Międzynarodowe Spotkanie Klastrów Ekoenergetycznych, Gdańsk 2010.

8. Molina Grima E., Acien Fernandez, Garcia Camacho F., Chisti Y., Photobioreactors: light regime, mass transfer, and scaleup. J. Biotechnol. 1999, 70: 231-247.
9. Frąc M., Jezierska – Tys S., Tys J, Algi – energia jutra (Biomasa, Biodiesel). Acta Agrophysica; 2009, 13(3), 627 – 638.
10. Zimny H. , Ekologia ogólna. Wydawnictwo Mikron. Warszawa, 2002
11. Nagashima H., Hayashi N, Ohtani S., Moose H., Effects of temperature on the photosynthesis of the Antarctic greem alga, Chlorella vulgaris. Proc. NIPR Wymp. Polar 1994, 1, Issue 1 304 pp.
12. Klimiuk E., Łebkowska M., Biotechnologie w ochronie środowiska, PWN, Warszawa 2003.
13. Fu. F., Qi W., Removal of heavy metal ions from wastewaters, A review.Journal of Environmental Management 2011,92, 407-418
14. Quintelas C., Rocha Z., Silva B., Fonseca B., Figueiredo H., Tavares T., Biosorptive performance of an Escherichia coli biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(III). Chemical Engineering Journal, 2009, 152, 110-115.
15. Megeay M,. Towards an understanding of the genetics of bacterial metal resistance, Trends Biotechnol, 1991, Vol 9, 17-24
16. Shumate II S.E., Stranberg G.W,. Accumulation of heavy metals mucrobial cells. M. Moo-Young, C.N. Robinson, J.A. Howell (Eds.), Pergamon Press, Oxford, 1985, 235-247.
17. Tsezos M., Adsorption by microbial biomass as a process form removal of ions from process or waste solutions. [w:] Immobilisation of ions by bio-sorption. H. Eccles, S. Hunt (Eds.), Ellis Horwood Ltd., Chichester, UK, 1986, 201-218
18. Klimiuk E., Łebkowska M. , Biotechnologie w ochronie środowiska, PWN, 2003, Warszawa.
19. Podedworna J., Żubrowska-sudoł M.,Dlaczego warto w technologii ścieków wykorzystywać bioreaktory ze złożem ruchomym? ,Gaz, Woda i Technika Sanitarna 9, 2011, 324–327.
20. Urbańska M., Kłosowski G., Algi jako materiał biosorpcyjny- usuwanie i odzysk metali ciężkich ze ścieków przemysłowych, Ochrona Środowiska i Zasobów Naturalnych, 51, 2012.
21. Aksu Z., Equilibrum and kinetic modelling of cadmium (II) biosorption by C. vulgaris in a batch system: effect of temperature. Sep. Purif. Technol. 21, 2001, 285-294
22. Filipiuk D., Fuks L., Majdan M., Biosorpcja jako metoda odzysku i usuwania metali ciężkich z wodnych ścieków przemysłowych. Przem. Chem. 2006, (85)6: 417-422.

23. Matsunaga, T., Takeyama, H., Nakao, T., Yamazawa, A., Screening of marine microalgae for bioremediation of cadmium polluted seawater. J. Biotechnol.1999, 70,33–38.
24. Rai, L.C., Mallick, N., 1992. Removal and assessment of toxicity of Cu and Fe to Anabaena doliolum and Chlorella vulgaris using free and immobilized cells. World J. Microb. Biotechnol. 1992, 8, 110–114
25. Pankowski A, McMinn A. Iron availability regulates growth, photosynthesis, and production of ferredoxin and flavodoxin in Antarctic sea ice diatoms. Aquatic Biology, 2009 4: 273-288.

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Escherichia coli gfp reporter gene microbial biosensors for genotoxicity screening of gentisic acid and sodium gentisate

Key words: *Genotoxicity, SOS-biosensors, GFP, Gentisic acid, Sodium gentisate*

Abstract: Genotoxic activity of gentisic acid and sodium gentisate with use of biosensors strains of Escherichia coli K-12 recA::gfpmut2, Escherichia coli K-12 ssb::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2, which contained a plasmid-borne transcriptional fusion between DNA-damage inducible recA and ssb promoters involved in the SOS regulon response and fast folding green fluorescent protein (GFP) variant reporter gene-gfpmut2 was studied. Many changes in the genotoxic activity of different concentration of studied compounds with application of recA::gfpmut2 and ssb::gfpmut2 have been observed. Gentisic acid and sodium gentisate caused increase in genotoxic effect for the both promoters of E. coli SOS regulon. Different concentration of tested compounds and duration of bacteria incubation influenced the genotoxic sensitivity of recA::gfp and ssb::gfp genetic biosensing constructs in E. coli K-12. Obtained results suggest that E. coli K-12 ssb::gfp mut2 biosensor strain is more sensitive for genotoxic effect of gentisic acid and sodium gentisate than E. coli K-12 recA::gfp mut2.

1. Introduction

A biosensor is an analytical device which integrates a biological recognition element with a physical transducer to generate a measurable signal proportional to the concentration of the analytes [1]. Nowadays, bacterial-based genotoxicity biosensors systems display an important role in the biological monitoring and detection of DNA damaging chemicals for environmental applications [2]. Biosensors have been created to provide even cheaper, faster and potentially more cost-effective alternatives and to accommodate high-throughput screening [3, 4]. Living organisms-based biosensors, as like bacterial biosensors can perform functional sensing and provide measurement, such as bioavailability, genotoxicity or general toxicity. Above, due to their specificity, fast response time, low cost, portability, ease to use and giving a continuous real time signal they are famous for dynamic development and represent of the advantages compared with traditional methods [5, 6, 7, 8, 9]. In such living cell systems, bacteria are especially attractive due to their rapid growth rate, low cost, and easy handling [10,

11]. Recently, bioluminescent biosensors with lux, luc or gfp genes have been developed to detect a variety of chemicals, genotoxic agents and factors, which are responsible for DNA damage, oxidative damage or cell growth inhibition [3, 12, 13].

The reporter protein is a key element to a promoter-based whole-cell biosensor. Many different reporter proteins have been investigated for the use in whole-cell biosensors. Fluorescence-based reporters, including GFP and its many spectral variants, have also been popular [14]. This protein has been isolated from coelenterates, for example the Pacific jellyfish Aequorea Victoria [8]. Whole-cell biosensors with GFP are being used increasingly, because of its useful properties such as: high stability, minimal toxicity for living cells and the ability to generate the green fluorescence without addition of external cofactors. Additionally it is possible non-invasive detection of gfp expression with application of simple in the use equipment, for instance UV lamp, fluorescence microscope or spectrofluorometer. The description of gfp and other reporter genes are broadly given elsewhere [12, 15, 16].

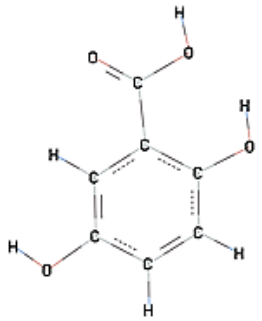
The SOS regulon with recA - most explored SOS-responsive, oxidative stress promoter for genotoxicity assessment is one of the most thoroughly studied stress regulons for bacteria [17]. The both promoters recA and ssb are involved in SOS response and belong to oxidative stress promoters in Escherichia coli [3, 9, 11, 18].

The recA promoter transcription is induced upon DNA damage and the induction of the SOS response is initiated by RecA protein activation to mediate the LexA repressor protein cleavage. The popularity of application of recA promoter for creation of effective genotoxicity bacteria biosensors is connected with broad involvement of RecA protein in several DNA repair pathways, including the repair of daughter-strand gaps and double-strand breaks, as well as in an error prone damage tolerance mechanisms called SOS mutagenesis [8, 17]. The second, very important DNA sequences, which are involved in bacterial SOS response are ssb that encodes SSB proteins (single-stranded DNA-binding proteins), which are among the most widespread cellular proteins. This being true of proteins of eukaryotic organisms as well as of eubacterial and archaeal proteins, they are also encoded by bacteriophage and adenovirus genomes. The main feature of this protein group is their high nonspecific affinity to single-stranded DNA (ssDNA). Just this peculiarity of SSB proteins and their participation in the main cellular processes maintaining genome integrity as well as the possibility of genetic information transfer define their role in the life cycle of all living cells [19]. They are essential proteins for replication of the chromosomes of E. coli and its DNA phages. In addition, SSB participates in recombination and repairing of E. coli DNA and in the concerted SOS response of this bacterium to DNA-damaging agents [4].

The mechanism of the induction of the SOS response regulon genes and its application in microbial biosensors were widely described by Gu et al. [8]. The examples of biosensors and an application of these devices are broadly reviewed by Xu et al. [20]. Park et al. [21] in excellent review paper described recent approaches based on protein/cellular engineering and synthetic biology as an emerging tool in the field of whole-cell biosensors.

Gentisic acid (2,5-dihydroxybenzoic acid, Fig. 1) belongs to polyphenols that occur in plants, fruits and vegatables. It was found in citrus fruits, grapes, artichoke, sesame, sandalwood, olive [22]

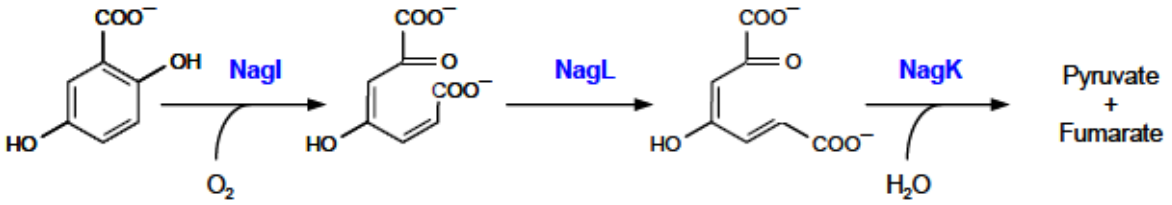
Figure 1. Chemical structure of gentisic acid



Source: Own research

For example gentisic acid was identified in extracts of Boreva orientalis [23], Smilax china [24], Decalepis hamiltoni [25] and Medicago truncatula roots [26] among other phenolic acids. It can accumulate in plants after non-necrotizing infection, for example in citrus exocortis viroid infected tomato plants, but not in these plants infected with the necrotizing pathogen Pseudomonas syringae [27]. Gentisic acid also acts as pathogen in activating defence genes in tomato [28]. Belles et al. [28] basing on radiolabeling studies showed that in healthy leaf tissues benzoic and salicylic acids are rapidly converted to gentisic acid. Moreover many derivatives of gentisic acid were found naturally, such as for example gentisic acid 5-O-β-D-glucoside was identified in fruits of Boreva orientalis [23] or 2-O-β-glucopyranoside in whole plant of Cotoneaster orbicularis [29]. Virus and viroid infection in tomato and cucumber are resulted in the accumulation of high levels of gentisic acid xyloside [27, 28]. Occurrence of derivatives of gentisic acid in M. truncatula roots suggests that they can also play a role in defence response system of this plant [26]. Phenolic acids are known as secondary plant metabolites and microbial degradation products [25, 30]. Gentisic acid is an active metabolite of degradation of various compounds for example: rutin, quercetin, salicylic acid and acetylsalicylic acid (Aspirin) [31]. It was identified and quantitated by Imbenotte et al. [32] as one of the three metabolites of acetylsalicylic acid. It is also a by-product of tyrosine and benzoate metabolism.

The metabolic pathway where Pseudomonas putida NCIMB 9869 after growth on 3,5-xylenol uses the gentisate for oxidizing m-cresol was described by Huang et al. [33]. Gentisate 1,2-dioxygenase is the enzyme that catalyzes the degradation of gentisic acid. The aerobic gentisate



pathway is shown in Fig. 2.

Figure 2. Pathway of aerobic gentisate degradation

Source: Own research based on Rabus et al. [34] and Wöhlbrand et al [35]

Initially, a gentisate 1,2-dioxygenase (NagI) forms maleylpyruvate, which is subsequently isomerized to fumarylpyruvate (NagL) and hydrolytically cleaved (NagK) to fumarate and pyruvate [34, 35].

Antimicrobial activity of phenolic acids and their derivatives against Gram-positive and Gram-negative bacteria, yeast, and fungi was measured by means of the minimum inhibitory concentration (MIC - the lowest concentration of chemical compound, which would inhibit the visible growth of the microorganism after the respective incubation) and described by Merkl et al. [36]. Gentisic acid in the concentration 5 mmol/l inhibited the growth of Escherichia coli DMF 7503, Bacillus cereus DMF 2001 and Listeria monocytogenes DMF 5776. In the case of Fusarium culmorum DMF 0103 and Saccharomyces cerevisiae DMF 1017 the higher concentration of gentisic acid was needed (10 mmol/l and 20 mmol/l, respectively).

Phenolic acids such as hydroxybenzoic acids are widely occurring species in wastewater generated in food-processing industries (especially olive oil production, wine distilleries). The effect of these compounds on aquatic organisms was investigated by Kamaya et. al. [30]. They studied an acute toxicity of benzoic acids to the crustacean Daphnia magna. The toxicity of benzoate derivatives was strongly influenced by the number of OH groups as well as the position of OH substituents. The toxic effects decreased in order 2,6- > 2,5- > 2,4- > 2,3- > 3,4- > 3,5-dihydroxybenzoic acids. All dihydroxy derivatives showed higher toxicity than non- and monohydroxylated benzoic acids. Gentisic acid showed the toxicity with 48 h EC_{50} of 59.5 mg/l. The pH is an important factor to assess the ionizable compounds, because the unionized form of a weak acid such as benzoic acid is generally thought to be more toxic than the ionized form. According to Wang and Lay [37] sodium 2-hydroxybenzoate was significantly less toxic than the corresponding acid. The acute and chronic effects of the main metabolites of

acetylsalicylic acid were assessed by Marques et al. [38] using standard (Daphnia magna) and autochthonous (Daphnia longispina) cladocerans. Gentisic acid was the most toxic among the three tested metabolites: salicylic, gentisic, and o-hydroxyhippuric acids.

The aim of the present study was to evaluate genotoxic activity of gentisic acid and sodium gentisate with use of biosensors reporter strains of Escherichia coli K-12 recA::gfpmut2, Escherichia coli K-12 ssb::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2, which contained a plasmid-borne transcriptional fusion between DNA-damage inducible recA and ssb promoters involved in the SOS regulon response and fast folding GFP variant reporter gene-gfpmut2. In presented research more stable and fast [39] folding mutant of gfp gene - gfpmut2 with excitation and emission wavelengths of 485 and 507 nm was used.

2. Materials and methods

2.1. Materials

2.1.1. Organisms

24 hours old Escherichia coli K-12 MG1655 strains: Escherichia coli K-12 recA::gfpmut2, Escherichia coli K-12 ssb::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2, genetically modified strains are obtained from Prof. Uri Alon, (Department of Molecular Cell Biology & Department of Physics of Complex Systems, Weizmann Institute of Science Rehovot, Israel). They contained a pUA66 plasmid-borne transcriptional fusion between DNA-damage inducible recA and ssb promoters involved in the SOS regulon response and fast folding GFP variant reporter gene-gfpmut2 [39]. These strains were cultured overnight in LB agar medium (Merck, Germany) at 30C° supplemented with 100 μg/ml of kanamycin (Sigma-Aldrich, Germany).

2.1.2. Chemicals (tested compounds)

Gentisic acid (2,5 - dihydroxybenzoic acid) and sodium gentisate of analytical grade were purchased from Sigma-Aldrich Company.

2.2. Methodology

During the whole experiment the 30C° as a temperature for strains incubation and for gentisic acid and sodium gentisate treatment was selected to prevent overgrowth and reduce background fluorescence. Additionally, it is known that lower temperatures are optimal for correct GFP folding [17]. Colonies were carried to LB broth medium (10 g NaCl, 10 g tryptone and 5 g yeast extract per 1000 ml of destilled water) with 100 μg/ml of kanamycin and incubated 24 hours at 30C°. After that, cells were washed with PBS buffer (1.44 g Na2HPO4, 0.24 g KH2PO4, 0.2 g KCl, 8 g NaCl per 1000 ml of destilled water) and the Optical Density (OD) of bacterial cultures was standardized with spectrophotometer to 0.2 at wavelength of 600 nm. Cells were resuspended in PBS buffer and were tested for their ability to detect sublethal levels of genotoxic activity of gentisic acid and sodium gentisate in five different concentrations: 0.0001; 0.001; 0.01; 0.1 and 1 mg/ml. Samples were incubated with chemicals for 3 and 24 hours at 30C°. The control samples of Escherichia coli K-12 recA::gfpmut2, Escherichia coli K-12 ssb::gfpmut2 and Escherichia coli K-12 promoterless::gfpmut2 strains, not treated with gentisic acid and sodium gentisate were conducted in the same condition. For verification the correct activity of recA and ssb promoter, Escherichia coli K-12 strain containing pUA66 plasmid without promoter - Escherichia coli K-12 promoterless::gfpmut2 was used as a negative control. After exposition of bacteria cultures to tested analytes strains were washed with PBS buffer and the intensity of fluorescence was measured.

2.3. Analytical method

The intensity of fluorescence was measured with spectrofluorometer (Perkin Elmer Enspire 2300). The measurements were done at excitation and emission wavelengths of 485 and 507 nm. The growth of bacteria strains was monitored with spectrophotometer (Perkin Elmer Enspire 2300) at 600 nm. The specific fluorescence intensity (SFI) which is defined as the raw fluorescence intensity (IF) divided by the optical density (OD) measured at each time point at 600 nm was calculated according to the formula:

$$SFI = \frac{IF}{OD} \tag{1}$$

where:
SFI – Specific Fluorescence Intensity,
IF – The raw fluorescence intensity of the strains at excitation and emission wavelengths of 485 and 507 nm,

OD – Optical Density at 600 nm of the strains.

For each concentration of chemical compounds the induction factor (FI) was calculated. FI = (FlI/OD0)/(Fl0/ODI), where FlI is the raw fluorescence of the culture treated with DNA - damaging compound; Fl0 is the raw fluorescence of the control sample without genotoxin; ODI is the optical density at 600 nm of treated culture and OD0 is the optical density of the control sample. A chemical was identified as a genotoxin if its induction factor was 2 or more, according to Ptitsyn et. al. [40] and Kostrzyńska et. al. [17].

2.4. Statistical analysis

Nine parallel measurements were performed for each concentration of gentisic acid and its sodium salt. The obtained results were analyzed by use of STATISTICA (ver. 10.0) software and the analysis of variance was performed with the Tukey's test**.** The values for $p \leq 0.01$ were considered significant.

3. Results and discussion

The values of SFI of gfp expression for 24 hours old E. coli K-12 recA::gfp mut2 after 3 and 24 hours incubation with gentisic acid are gathered in Table 1.

Table 1. The specific fluorescence intensity (SFI) of gfp expression for 24 hours old E. coli K-12 recA::gfp mut2 after 3 hours and 24 hours incubation with gentisic acid. Data points represent means±SD, n=9

Concentration of gentisic acid		SFI for 3 hours of incubation with chemical	% of stimulation of gfp expression*	Induction factor FI	SFI for 24 hours of incubation with chemical	% of stimulation of gfp expression*	Induction factor FI
[mg/ml]	[mol/dm3]						
Control sample		18.575 ±2.247	—	—	33.513 ±3.751	—	—
0.0001	6.49·10-7	26.125 ±1.378	41	1.431	40.393 ±6.216	21	1.227
0.001	6.49·10-6	26.922 ±2.986	45	1.453	37.630 ±2.927	12	1.378
0.01	6.49·10-5	30.589 ±6.441	65	1.622	59.271 ±10.402	77	1.752
0.1	6.49·10-4	34.960 ±6.328	88	1.867	68.887 ±26.403	106	1.985
1	6.49·10-3	26.680 ±4.215	44	1.429	68.578 ±26.042	105	1.970

Source: Own research; *- the highest levels of stimulation of gfp expression (above 30% changes in the values of gfp expression in comparison with the control)

The higher values of SFI were observed for 24 hours of incubation. The levels of stimulation of gfp expression in percentage are also presented. For 3 hours of incubation almost the same % of stimulation was observed for all concentrations, only for 0.01 and 0.1 mg/ml the significant increase was noticed. The highest genotoxic activity was observed for 0.1 mg/ml concentration. In the case of 24 hours incubation greater differences took place - gentisic acid in 0.0001 and 0.001 mg/ml concentrations stimulated gfp expression only insignificantly. The level of stimulation in higher concentrations considerably increased. For 24 hours of incubation of E. coli K-12 recA::gfp mut2 with gentisic acid almost the same % of stimulation was observed at concentration ≥ 0.1 mg/ml. Comparing % of stimulation between 3 hours and 24 hours of incubation the highest difference was noticed for 0.001 mg gentisic acid /ml. Percentage of stimulation of gfp expression obtained for 24 hour of incubation with gentisic acid was four times lower than that obtained for 3 hours incubation. For higher concentrations the increase was observed for longer incubation (about 2 times).

Data obtained for Escherichia coli K-12 ssb::gfp mut2 bacteria strain after 3 hours and 24 hours incubation with gentisic acid were gathered in Table 2.

Table 2. The specific fluorescence intensity (SFI) of gfp expression for 24 hours old E. coli K-12 ssb::gfp mut2 after 3 hours and 24 hours incubation with gentisic acid. Data points represent means±SD, n=9

Concentration of gentisic acid		SFI for 3 hours of incubation with chemical	% of stimulation of gfp expression *	Induction factor FI	SFI for 24 hours of incubation with	% of stimulation of gfp expression *	Induction factor FI
[mg/ml]	[mol/dm3]						
Control sample		31.260 ±3.951	—	—	22.631 ±4.321	—	—
0.0001	6.49·10-7	38.705 ±2.300	24	1.257	53.186 ±4.030	135	2.429
0.001	6.49·10-6	45.246 ±4.331	45	1.513	64.019 ±4.884	183	2.980
0.01	6.49·10-5	49.267 ±8.302	58	1.621	67.541 ±6.488	198	3.216
0.1	6.49·10-4	60.430 ±8.654	95	1.991	68.025 ±6.833	201	3.241
1	6.49·10-3	60.866 ±2.845	44	2.000	62.82 1±6.804	178	2.947

Source: Own research; *- the highest levels of stimulation of gfp expression (above 30% changes in the values of gfp expression in comparison with the control)

The increase of percentage of stimulation of gfp expression was noticed for 24 hours of incubation in comparison with 3 hours incubation for all concentrations of gentisic acid. The largest increase (in nearly six times) was observed for the lowest concentration of gentisic acid, while the lowest one (more than twice) was obtained for 0.1mg/ml of gentisic acid. In comparison with the recA promoter the stronger genotoxic reactivity (changes in the values of gfp expression in comparison with the control sample) was noticed for 24 hours of incubation.

The highest reactivity of two genetic systems, for both E. coli K-12 recA::gfp mut2 and for E. coli K-12 ssb::gfp mut2 was observed after 24 hours incubation with 0,1 mg/ml of gentisic acid. Higher changes in the level of gfp gene expression (above 30% of changes in gfp expression in comparison with the control sample) were noticed in the case of E. coli K-12 ssb::gfp genetic construct (Table 2). It suggests that E. coli K-12 ssb::gfp mut2 biosensor strain is more sensitive for genotoxic effect of gentisic acid than E. coli K-12 recA::gfp mut2. The values of FI calculated for data obtained after 24 h incubation indicated that gentisic acid is genotoxic compound.

Data concerning an influence of the second tested chemical - sodium gentisate- on gfp gene expression were shown in Tables 3 and 4, respectively for both recA and ssb promoters. Results for E. coli K-12 recA::gfp mut2 after 3 and 24 hours incubation like in previous case with gentisic acid have shown higher values of SFI after longer incubation period.

Table 3. The specific fluorescence intensity (SFI) of gfp expression for 24 hours old E. coli K-12 recA::gfp mut2 after 3 hours and 24 hours incubation with sodium gentisate. Data points represent means±SD, n=9

Concentration of sodium gentisate		**SFI for 3 hours of incubation with chemical**	**% of stimulation of gfp expression ***	**Induction factor F_I**	**SFI for 24 hours of incubation with chemical**	**% of stimulation of gfp expression ***	**Induction factor F_I**
[mg/ml]	[mol/dm^3]						
Control sample		14.647 ±0.733	—	—	31.906 ±1.592	—	—
0.0001	$6.49 \cdot 10^{-7}$	21.277 ±1.630	45	1.450	39.727 ±5.695	25	1.262
0.001	$6.49 \cdot 10^{-6}$	21.662 ±0.969	48	1.481	36.107 ±2.806	13	1.133
0.01	$6.49 \cdot 10^{-5}$	23.634 ±0.470	61	1.619	45.928 ±5.322	44	1.433
0.1	$6.49 \cdot 10^{-4}$	23.193 ±1.816	58	1585	44.745 ±3.066	40	1.401

1	$6.49 \cdot 10^{-3}$	23.316 ±1.024	59	1.600	42.400 ±6.479	33	1.319

Source: Own research; *- the highest levels of stimulation of gfp expression (above 30% changes in the values of gfp expression in comparison with the control)

Table 4. The specific fluorescence intensity (SFI) of gfp expression for 24 hours old E. coli K-12 ssb::gfp mut2 after 3 hours and 24 hours incubation with sodium gentisate. Data points represent means±SD, n=9

Concentration of sodium gentisate		SFI for 3 hours of incubation with chemical	% of stimulation of gfp expression *	Induction factor F_I	SFI for 24 hours of incubation with chemical	% of stimulation of gfp expression *	Induction factor F_I
[mg/ml]	[mol/dm^3]						
Control sample		27.250 ±3.279	—	—	22.124 ±5.134	—	—
0.0001	$6.49 \cdot 10^{-7}$	36.545 ±3.030	34	1.352	53.394 ±5.128	141	2.538
0.001	$6.49 \cdot 10^{-6}$	41.928 ±0.321	54	1.574	58.673 ±3.630	165	2.817
0.01	$6.49 \cdot 10^{-5}$	42.881 ±0.443	57	1.610	55.445 ±1.737	151	2.692
0.1	$6.49 \cdot 10^{-4}$	54.070 ±6.795	98	1.989	54.967 ±3.017	148	2.640
1	$6.49 \cdot 10^{-3}$	38.498 ±0.964	41	1.449	50.758 ±9.590	129	2.335

Source: Own research; *- the highest levels of stimulation of gfp expression (above 30% changes in the values of gfp expression in comparison with the control)

The higher increase in comparison with control sample was noticed for 3 hours incubation, although there was no significant difference between whole range of sodium gentisate concentration. Only after 24 hours incubation of E. coli K-12 recA::gfp mut2 differences were noticeable (see Table 3). In terms of gfp expression stimulation levels for 3 as well as 24 hours incubation were insignificantly differ, although the increase of % of stimulation was observed for concentration ≥0.01 mg/ml.. The highest values of gfp expression stimulation was observed respectively for 0,01 mg sodium genistate/ml. Comparing percentages of gfp expression stimulation for 3 and 24 hours E. coli K-12 recA::gfp mut2 incubation the greatest differences

took place in 0,001 mg/ml. Percentage of stimulation of gfp expression obtained for 24 hour of incubation with sodium gentisate at above mentioned concentration was four times lower than that obtained for 3 hours incubation. For other concentrations of sodium gentisate the increase of % of stimulation of gfp expression was observed for shorter time of bacteria incubation with chemical.

In the case of using E. coli K-12 ssb::gfp mut2 system to study of sodium gentisate genotoxicity the higher values of SFI were observed for 24 hours of incubation, also for this period of time the greatest difference between control sample and tested concentration was noticed. The levels of stimulation of gfp expression in percentage gathered in Table 4 indicate, that the greatest stimulation for 3 hours incubation was noticed for 0.1 mg/ml, while for 24 hours incubationfor 0.001 mg sodium gentisate/ml. The increase of percentage of stimulation of gfp expression was observed for 24 hours of incubation in comparison with 3 hours incubation for all concentrations of sodium gentisate. The largest increase in nearly four times was observed for the lowest concentration of sodium gentisate (0.0001 mg/ml). The values of FI calculated for data obtained after 24 h incubation indicated that sodium gentisate is genotoxin compound.

Obtained data for Escherichia coli K-12 ssb::gfp mut2 (Table 4) in the case of 24 h incubation indicate an increase in its genotoxic activity in comparison with the recA promoter (Table 3), for the all concentration of sodium gentisate. For 3 hours incubation with chemical, received results do not explicit.

It suggests that E. coli K-12 ssb::gfp mut2 biosensor strain is more sensitive for genotoxic effect of sodium gentisate in comparison with E. coli K-12 recA::gfp mut2.

In Figures 3 and 5 dependences of the induction factor FI from dose of chemical compounds of E. coli K-12 recA::gfp mut2 (Fig. 3) and E. coli K-12 ssb::gfp mut2 (Fig. 5) are presented.

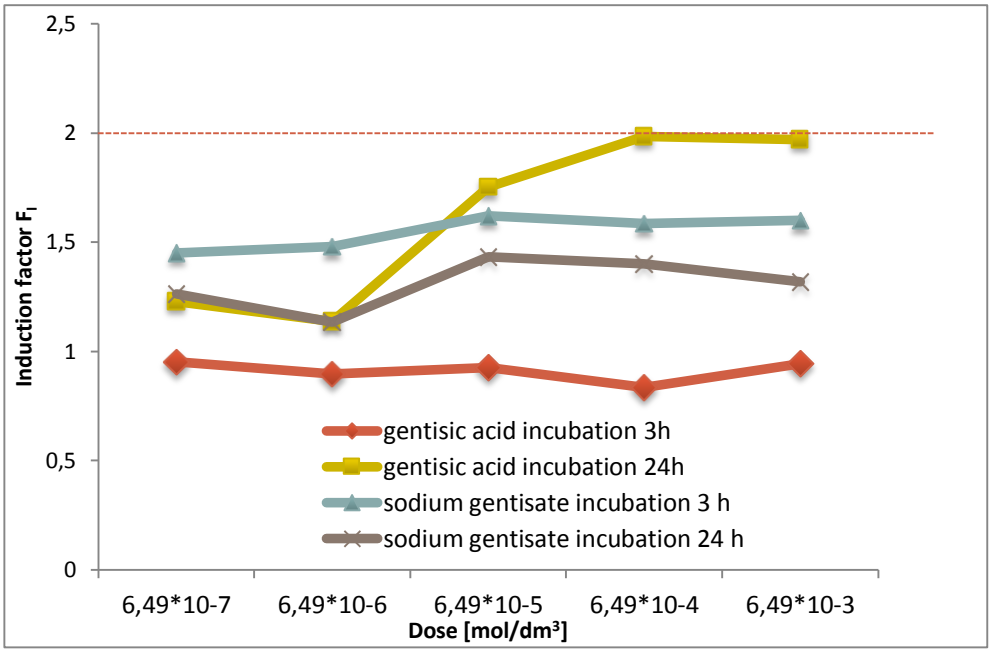


Figure 3. Induction of recA::gfp mut2 by gentisic acid and its sodium salt after 3 hours and 24 hours incubation

Source: Own research

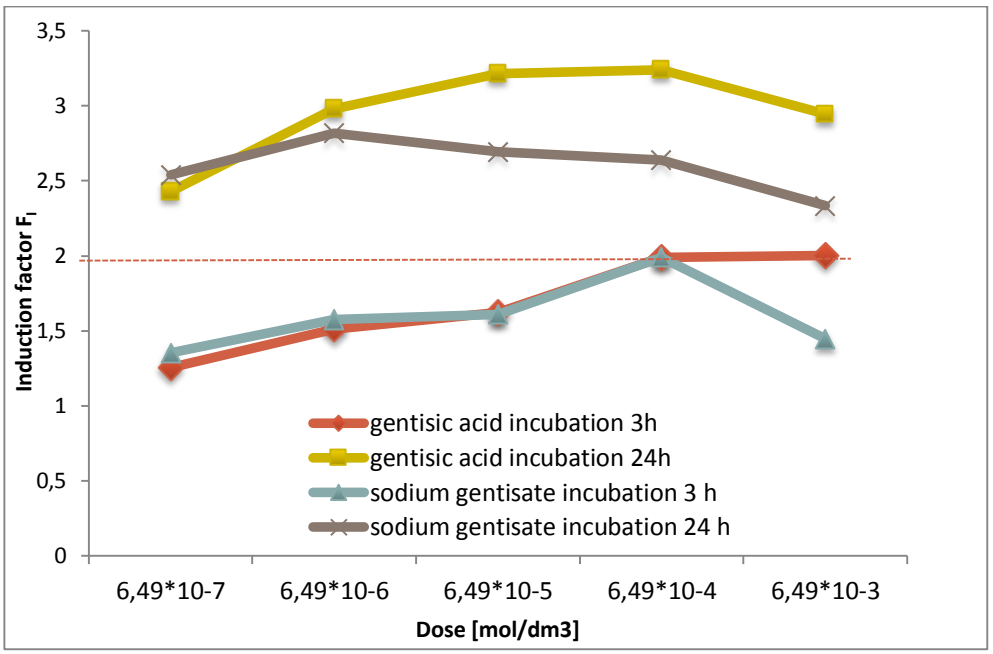


Figure 5. Induction of ssb::gfp mut2 by gentisic acid and its sodium salt after 3 hours and 24 hours incubation

Source: Own research

Obtained results show that in the case of using E. coli K-12 recA::gfp mut2 system for all tested substances FI stayed below 2 except for 24h incubation with gentisic acid in high dose where FI was slightly below treshold. Therefore, requirement for genotoxicity mentioned above was

not met. In the case of using E. coli K-12 ssb::gfp mut2 system only for 24h incubation with gentisic acid and sodium gentisate induction factor increased by a factor of 2 and more. Highest values of FI was noticed at 6.49x10-5 mol/dm3 (0.01 mg/ml) and 6.49x10-7 mol/dm3 (0.0001 mg/ml) respectively for gentisic acid and sodium gentisate. Incubation of E. coli K-12 ssb::gfp for shorter time period not met requirement for genotoxicity.

Results of the cytotoxic degree of gentistic acid and sodium gentisate with respect to dose of tested chemicals are shown in Figures 4 and 6.

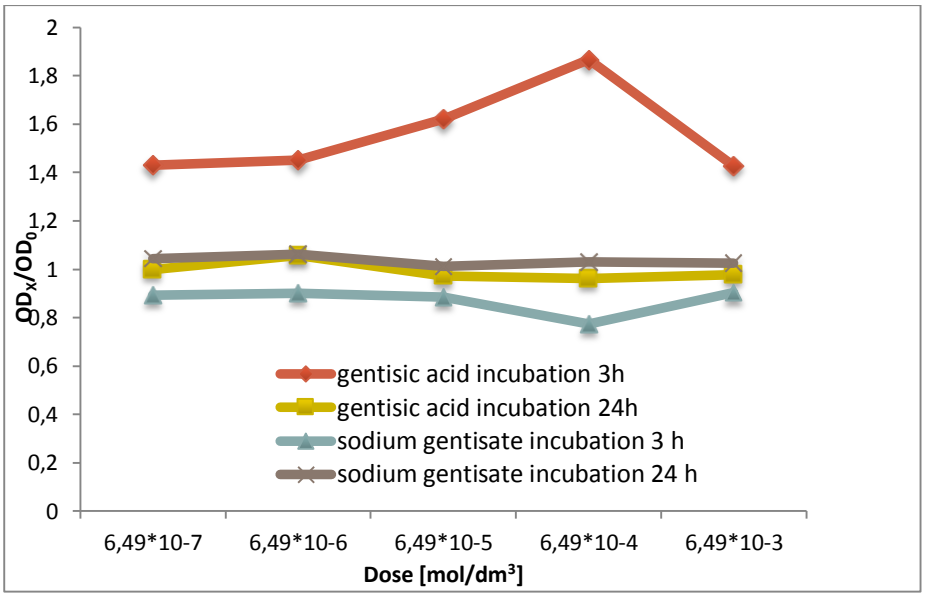


Figure 4. OD$_x$/OD$_0$ value changes depending on the dose and time of incubation (using E. coli K-12 recA::gfp mut2)

Source: Own research

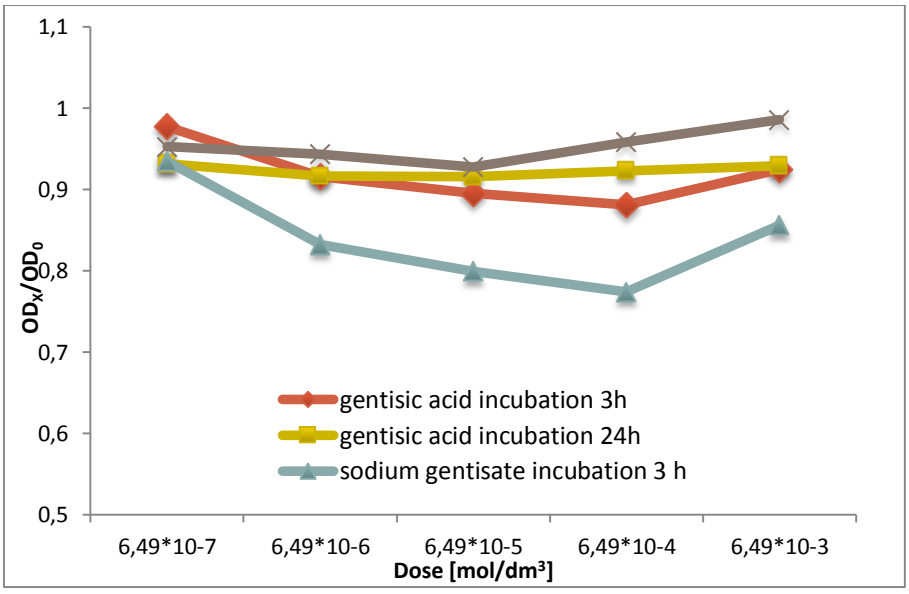


Figure 6. OD$_x$/OD$_0$ value changes depending on the dose and time of incubation (using E. coli K-12 ssb::gfp mut2)

Source: Own research

Incubation of Escherichia coli K-12 recA::gfpmut2 with sodium gentisate for 3 and 24h have not shown significant decrease of ODx/OD0. Similar results also shown incubation with gentisic acid for 24h. Significant decrease of ODx/OD0 was observed only from 6,49x10-4 mol/dm3 (0,1 mg/ml)to 6,49x10-7 mol/dm3 (0.0001 mg/ml)for 3h Escherichia coli K-12 recA::gfpmut2 incubation with gentisic acid. Results of E. coli K-12 ssb::gfp dose-response for tested chemicals with respect to induction factor (FI) are shown in figure 3. Only for 24h incubation with gentisic acid and sodium gentisate induction factor increased by a factor of 2 and more. Highest values of FI was noticed at 6,49x10-5 mol/dm3 and 6,49x10-7 mol/dm3 respectively for gentisic acid and sodium gentisate. Incubation of E. coli K-12 ssb::gfp for shorter time period not met requirement for genotoxicity.

Tukey's test ($p \leq 0,01$) indicated that gentisic acid and sodium gentisate in the tested concentrations using two different recA:;gfp mut2 and ssb::gfp mut2 genetic constructs in the applied time of bacteria incubation significantly influenced on the strength of genotoxic reactivity of Escherichia coli K-12 MG1655 cells.

The analysis of variance with Tukey's test for the gentisic acid and sodium gentisate for E. coli K-12 recA::gfp mut2 and E. coli K-12 ssb::gfp mut2 indicated significant differences in the values of Least Significant Differences (LSD) among tested groups: The values of LSD0,01 for gentisic acid/sodium gentisate (A): and for E. coli K-12 recA::gfp mut2 and E. coli K-12 ssb::gfp mut 2 strains (B) as well as for the time of incubation with chemical (C) amounted to 2.33; while the chemical concentration (D) equaled 6.68. The values of LSD0,05(AxC): and LSD0,01(BxC)were 4,72 while for LSD0,01(BxD), LSD0,01(CxD): and. LSD0,05(AxD) were 11.47. For (AxB), (AxBxC), (AxBxD) and for (AxCxD) there were no significant differences.

4. Results and discussion

Obtained results especially regarding 24 hours incubation showed that Escherichia coli K-12 ssb::gfp mut2 is more sensitive for genotoxic effect of gentisic acid and sodium gentisate in comparison with the Escherichia coli K-12 recA::gfp mut2.

To compare the genotoxic activity of gentisic acid and its sodium salt the stronger genotoxic effect in the case of gentisic acid was observed, particularly for longer time of incubation for the both genetic constructs in E. coli K-12. On the other hand 3 hours incubation showed stronger genotoxic effect of sodium gentistate except for 1 mg/ml concentration for Escherichia coli K-12 ssb::gfp mut2. In lowest concentration of tested chemicals in almost all cases sodium gentisate have had greater impact on genotoxic effect but in highest one it was inversely - gentisic acid had stronger genotoxic reactivity.

With use of E. coli K-12 recA::gfp mut2 FI≥2 was not noticed irrespective of concentration of chemicals and incubation period.The induction factor FI≥2 for tested chemicals were obtained for gentisic acid and sodium gentisate for the all applied concentration and for the 24 incubation of biosensors bacteria strains E. coli K-12 ssb::gfp with the both chemicals.

The above data suggest that gentisic acid and its sodium salt for applied in this experiment concentration could be qualified as a genotoxins.

Acknowledgements

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References

1. Su L., Jia W., Hou C., Lei Y., 2011. Microbial biosensors: A review. Biosens. Bioelectron. 26, 1788–1799.
2. Wei T., Zhang C., Xu X., Hanna M., Zhang X., Wang Y., Dai H., Xiao W., 2013. Construction and evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals. Biosens. Bioelectron. 44, 138–145.
3. Lei Y., W. Chen, A., 2006. Mulchandani. Microbial biosensors. Anal. Chim. Acta 568, 200–210.
4. Norman A., Hansen L. H., Sørensen S. J., 2006. A flow cytometry-optimized assay using an SOS-green fluorescent protein (SOS-GFP) whole-cell biosensor for the detection of genotoxins in complex environments. Mutat. Res. 603, 164-172.
5. D'Souza S. F., 2001. Microbial biosensors. Biosens. Bioelectron. 6, 337-353.
6. Stiner L., Halverson L. J., 2002. Development and characterisation of a green fluorescent protein-based bacterial biosensor for bioavailable toluene and related compounds. Appl. Environ. Microbiol. 68, 1962-1971.
7. Belkin S., 2003. Microbial whole-cell sensing systems of environmental pollutants. Curr. Opin. Microbiol. 4, 206-212.
8. Gu M. B., Mitchell R. J., Kim B. C., 2004. Whole-cell-based biosensors for environmental biomonitoring and application. Adv. Biochem. Engin. Biotechnol. 87, 269-305.
9. Hazen T.C., Stahl D. A., 2006. Using the stress response to monitor process control: pathways to more effective bioremediation. Curr. Opin. Biotechnol. 17, 285–290.

10. Kuang Y., Biran I., Walt D. R., 2004. Living bacterial cell array for genotoxins monitoring. Anal. Chem. 76, 2902-2909.
11. Girotti S., Feeri E. N., Fumo M. G. Maiolini E., 2008. Monitoring of environmental pollutants by bioluminescent bacteria. Anal. Chim. Acta 608, 2-29.
12. Errampalli D., Leung K., Cassidy M. B., Kostrzyńska M., Blears M., Lee H., Trevors J. T., 1999. Applications of green fluorescent protein as a molecular marker in environmental microorganism. J. Microbiol. Meth. 35, 187-199.
13. Bischoff P. L., Holl V., Coelho D., Dufour P., Weltin D., Luu B., 2001. Apoptosis at the interface of immunosuppressive and anticancer activities: the examples of two classes of chemical inducers, oxysterols and alkylating agents, Curr. Med. Chem. 7, 693–713.
14. Martineau, R.L., Stout, V., Towe, B.C., 2009. Whole cell biosensing via recA::mCherry and LED-based flow-through fluorometry. Biosens. Bioelectron. 25, 759–766.
15. Kain S. R., 1999. Green fluorescent protein (GFP): applications in cell-based assays for drug discovery. Drug Discov. Tod. 4, 304-312.
16. Jansson J. K., 2003. Marker and reporter genes: illuminating tools for environmental microbiologists. Curr. Opin. Microbiol. 6, 310-316.
17. Kostrzyńska M., Leung K. T, Lee H., Trevors J. T., 2002. Green fluorescence protein based biosensor for detecting SOS-inducing activity of genotoxic compounds. J. Microbiol. Methods, 48 43-51.
18. Albano C. R., Lu A., Bentley W. E., Rao G., 2001. High Throughput studies of gene expression using green fluorescent protein-oxidative strees promoter probe constructs. J Biomolec. Screen. 1, 421-428.
19. Pestryakov P. E., Lavrik O. I., 2008. Mechanisms of single-stranded DNA-binding protein functioning in cellular DNA metabolisms. Biochemistry (Moscow), 13, 1388-1404.
20. Xu T., Close D. M., Sayler G. S., Ripp S., 2013. Genetically modified whole-cell bioreporters for environmental assessment. Ecolog. Indic. 28, 125-141.
21. Park M., Tsai S. L., Chen W., 2013. Microbial Biosensors: Engineered microorganisms as the sensing machinery. Sensors 13, 5777-5795.
22. Khadem S., Marles R. J., 2010. Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: occurrence and recent bioactivity studies. Molecules, 15 7985-8005.
23. Sakushima A., Coskun M., Maoka T., 1995. Hydroxybenzoic acids from Boreva orientalis. Phytochemistry 40, 257-261.

24. Yang C., Tang Q., Liu J., Zhang Z., Liu W., 2008. Preparative isolation and purification of phenolic acids from Smilax china by high-speed counter-courrent chromatography. Separat. Purific. Technol. 61, 474-478.
25. Nayaka M.A.H., Sathisha U.V., Dharmesh S.M., 2010. Cytoprotective and antioxidant activity of free, conjugated and insoluble-bound phenolic acids from swallow root (Deecalepis hamiltonii). Food Chem. 119, 1307-1312
26. Stochmal A., Kowalska I., Janda B., Perrone A., Piacente S., Oleszek W., 2009. Gentisic acid conjugates of Medicago truncatula roots. Phytochemistry 70, 1272-1276.
27. Fayos J., Bellés J. M., López-Gresa M. P., 2006. Induction of gentisic acid 5-β-D-xylopyranoside in tomato and cucumber plants infected by different pathogens. Phytochemistry 67, 142-148.
28. Belles J. M., Garro R., Fayos J., Navarro P., Primo J., Conejero V., 1999. Gentisic acid s a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. Mol. Plant-Microbe Interact. 12, 227-235.
29. El-Mousallamy A. M. D., Hussein S.A.M., Merfort I, Nawwar M. A.M., 2000. Unusual phenolic glycosides from Cotoneaster orbicularis, Phytochemistry 53, 699-704.
30. Kamaya Y., Fukaya Y., Suzuki K., 2005. Acute toxicity of benzoic acid to the crustacean Daphnia magna. Chemosphere 59, 255-261.
31. Hutt A.J., Caldwell J., Smith R.L., 1986. The metabolism of aspirin in man: a population study. Xenobiotica 16, 239–49.
32. Imbenotte M., Azaroual N., Cartigny B., Vermeersch G., Lhermitte M., 2003. Identification and quantification of xenobiotics by 1H NMR spectroscopy in poisoning cases. Forensic Sci. Int. 133, 132-135.
33. Huang W.E., Hopper D., Goodacre R., Beckmann M., Singer A., Draper J., 2006. Rapid characterization of microbial biodegradation pathways by FT-IR spectroscopy. J. Microbiol. Methods 67, 273-280.
34. Rabus, R., Kube, M., Heider, J., Beck, A., Heitmann, K., Widdel, F., and Reinhardt, R. (2005). The genome sequence of an anaerobic aromatic-degrading denitrifying bacterium, strain EbN1. Arch. Microbiol. 183, 27-36.
35. Wöhlbrand L., Kallerhoff B., Lange D., Hufnagel P., Thiermann J., Reinhardt R., Rabus R., 2007. Proteomics. Supporting Information for Proteomics DOI 10.1002/pmic.200600987. Functional proteomic view of metabolic regulation in “Aromatoleum aromaticum” strain EbN1. WILEY-VCH Verlag GmbH, Co. KGaA, Weinheim, pp.3-34,3-35

36. Merkl R., Hrádková I., Filip V., Šmidrkal J., 2010. Antimicrobial and antioxidant properties of phenolic acids alkyl esters. Czech J. Food Sci. 28, 275–279.
37. Wang, W.H., Lay, J.P., 1989. Fate and effects of salicylic acid compounds in freshwater systems. Ecotoxicol. Environ. Safety 17, 308–316.
38. Marques C.R., Abrantes N., Gonçalves F., 2004. Life-history traits of standard and autochthonous cladocerans: II. Acute and chronic effects of acetylsalicylic acid metabolites. Environ Toxicol 19, 527–540.
39. Zaslaver A., Mayo A. E., Rosemberg R., Bashkin P., Sberro H., Tsalyuk M., Surette M. G., Alon U., 2004. Just-in-time transcription program in metabolic pathways. Nat. Genet. 36, 486 – 491.
40. Ptitsyn L. R., Horneck G., Komova O., Kozubek S., Krasavin E. A., Bonev M., Rettberg P., 1997. A biosensor for environmental genotoxin screening based on an SOSlux assay in recombinant Escherichia coli cells. Appl. Environm. Microbiol. 63, 4377–4384.

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Tank heat pumps

Keywords: *heat pump, air,* warm usable water, Podlachia

Abstract: Heat pumps are the solution which combines both saving and ecology. Heat pumps with built-in warm usable water tank are the commonly known and more often applied. Tank heat pumps often do not require applying additional, supporting conventional energy sources for heating up the warm usable water. The heat pumps were applied in some public utility buildings in Podlasie province.

1. Introduction

Still growing fossil fuels prices and sustained just for some years interest in ecology resulted in interest in alternative energy sources. Average user takes care of the financial aspect, that is why the heat, which is free or costs little, is a dream of a lot of investors.

Replacing applied traditional energy sources with ecological solution is a sensible solution for expenses minimizing which also gives the cheap air from the environment. Heat pump can be this solution. Heat pump is the device which collects energy from unconventional energy source, for example water, ground, air etc., and use it for heating up warm usable water or for other heating purposes (and not only).

2. Classification of compressor heat pumps due to low-grade heat sources

The types of natural (renewable) low-grade heat sources, i.e. water, ground, air, solar radiation matches the classification of the compressor heat pumps. Therefore due to low-grade heat sources we can distinguish four basic types of the heat pumps [1]:

- water heat pumps (water/water),
- ground heat pumps (brine/water),
- heat pumps using direct evaporation (direct evaporation/water),
- air heat pumps (air/water).

2.1. Water heat pumps (water-water)

Water, because of its physical properties, for example high specific heat capacity, is a good heat accumulator. water from the natural environment is the most available and the easiest to collect. However, wastewater from the technological processes has got the highest energy potential.

Surface water deposits, which are lakes, rivers and ponds, can transmit great amounts of thermal energy, but not every building is located in a favorable place.

Low-grade source intake, which are heat exchangers can be supplied with a pump or can be immersed directly in water deposits (river, lake, pond). Heat removal in heat exchangers immersed in water can be done in two ways. One of the ways is plate heat exchanger which is located in a canal giving a proper water flow. The second way is pipe coil exchanger located permanently at the bottom of the tank. The collector pipes are located at the bottom and are attached in order not to swim out to the surface. The distance between the pipes should be about 60 cm.

The water can be collected through the whole river, because its temperature is reach by the heat exchange with the surroundings. The heat pumps of this type are used the most often in motels and resorts [2, 3].

Investment and exploitation costs are lower than in the other types of used water, which is a advantages of surface water sources. The set of this type has got also disadvantages:
-relatively high temperature fluctuation (0-15 C),
-the lowest temperatures coincide with the highest heat demand - bad coherence,
-there is a necessity of using additional heat source,
-when the temperature is 0 C, heat exchanger surface IS covered with ice.

Ground water sources are the most used type of water sources in this type of heat pumps. It is the most profitable and the most demanding heat accumulator.

The average yearly temperature of the ground water sources is maintained at 5-12 C. In order to allow collect the heat from the ground water sources, delivery well and damping well should be done. The water is collected with the delivery well and is piped away to the ground with the damping well. The distance between delivery well and damping well should be about 30-50 m.

The distance depends on the level of ground water and properties of the ground. The ground water and the deep water before the use should be analyzed, because there is a possibility that the ground water or the deep water cannot be directly used for evaporation. If the level of

mineralization of the ground water or the deep water is not too high or the water is not aggressive, it is allowed to press the water directly to evaporator. When the water does not meet the requirements, indirect heat exchanger should be used. The unitary amount of heat unitary in evaporator used in this type of heat pump is 4500-5900 Wh/m^2.

The use of this type of low temperature heat source depends on the type and the location of the ground water sources. These factors have got a huge influence on the amount of investment costs [3, 4]:

Advantages of the ground water and the deep water:

-relatively high and constant temperature 5-12°C

-good coherence,

-the possibility to piping the water away back to the ground,

-low investment costs,

During the exploitation of heat pumps with the system of heat collection from the ground water sources adverse occurrences can happen:

-biological pollution ,

-slime accumulation on the well side

-limited fresh water supply ,

-iron precipitation

-silting (water in circulation cannot contact with the air)

-damping well framing damages.

2.2 Ground heat pumps (brine/water)

Heat pumps collecting the energy from the ground are currently the most widespread pumps on the market because of their very good exploitation parameter and independence of the system on external temperature [3].

The ground is a very good thermal energy accumulator. The energy originated in the Sun and the exchange the heat with the atmosphere and is accumulated in the lower ground layer at the depth of about 10 m. On this depth the temperature is equal to the average yearly air temperature.

The energy in the ground which source is the Sun is called shallow geothermal energy which appears at the depth to 350 m. The layers of thermal energy on the higher depth comes from the Earth's kernel and these are this is the geothermal energy [2].

The course of air temperature changes is not the same as the course of ground temperature changes on the higher depth. This occurrence is beneficial, because during the

highest heat demand period, the ground temperature is higher than the temperature of air as a heat source. The ground structure and humidity result in ground conductivity. The value of heat conductivity coefficient of damp ground is higher than the value of heat conductivity coefficient of little damp ground (damp ground = 2,1 W/mK, little damp ground =1,4 W/mK). The temperature deviations from the average yearly temperature of the dry ground are much smaller than the temperature deviations of more damp ground. The heat conductivity coefficient depends on the kind of ground.

The disadvantages of this heat sources are first of all high investment costs. The costs of ground collector is 25-50% of total heat pumps installation costs (in Polish conditions).
Low temperature heat source with horizontal collector is cheaper than low temperature heat source with vertical collector.
The main advantages of ground as low temperature heat source are:

- high heat capacity,
- low exploitation costs,
- stable temperature,
- accessibility,
- silent work,
- good exploitation parameters,
- a little influence of external temperature on exchanger capacity,
- the use of ground exchanger in the ways: as a evaporator in winter and as condenser in summer.

Brine/water heat pump works with ground collector. Working medium (brine) flows through collector which removes heat from low temperature heat source.

Heat pumps of this type are devices which are very similar to water/water heat pumps. According to Zawadzki and in [1], the main difference between two types of these pumps is the fact that antifreeze working medium called brine, instead of water, flows in closed circuit in system. Brine, which is cooled in evaporator, can reach the temperature lowe than 0°C, that is why the use of antifreeze liquid is very important.

Heating system efficiency with the use of heat pump of type brine/water depends mainly on [1]:

- well assorted size of ground exchanger,
- ground thermal efficiency,
- properly done excavation.

In the heat pumps of this type as a ground exchanger can be used[1]:

- horizontal ground collector,
- flat ground collector,
- spiral ground collector.
- vertical ground collector (submersible probes).

The shape and the location of the collector significantly affect the heat exchange processes. The type of ground exchanger is chosen depending on the kind of ground, ground water sources level, the size and management of plot.

Flat ground collector

Flat ground collector allows to obtain the solar radiation energy accumulated in the ground. The collector consists of some sections. The collection sections are some pipe sections which are about a hundred-meter long and their diameter is 20-40 mm. The particular sections length should be the same. It results in gaining the same flow resistance and flow intensity. The collector pipes are most often made of polythene (sometimes they are made of polypropylene or polybutylene) and their thermal conductivity is high [1, 4].

The collector pipes are lain about 30 cm under local freezing zone (at the depth of from 1,5 to 2 m). Usually the distance between the pipes is from 50 to 80 cm. Two pipe ends are located in cumulative manhole, where they are connected with the water main with the use of dividers. In the case of failure there should be a possibility of severance of particular branches next to dividers, so shut-off valves should be installed.

There are some condition according to the field where installation of flat ground collectors is planned. First of all, there should not be any buildings, shrubs and trees with the deep rooting. The collectors should not be installed in the field where the high slope occurs, because it can the reason of the complication during the collector installation [1, 4].

The advantages of use of the collector of this type is their low cost and ease of installation because the collectors are located shallowly under the surface of ground. The disadvantages are spending a lot of surface for installation of collector, ground corrosivity and difficult access to exchangers during the failure. The surface of field where investor wants to install the collector of this type should be big, because the collector surface required for heating the building is from 2 to 5 times bigger than the heated building surface [1, 4, 5].

Table 1. Heat transmission ground ability in dependence on ground cohesion and ground humidity

Kind of ground	Ground thermal efficiency
dry sandy soil	10 – 15 W/m^2
wet sandy soil	15 – 20 W/m^2
medium-dry clay	20 – 25 W/m^2
wet clay	25 – 30 W/m^2
water moistened soil	30 – 40 W/m^2

Source: Self-elobaration based on [1]

Ground thermal efficiency depends on some factors: the kind of soil, the soil humidity, mineral ingredients content, the soil structure [1].

Spiral ground collector

The collectors of this type work in a similar way like the flat ones do. The collector pipes are made with polythene and are 125 cm long and their diameter is 1 inch. Similarly like flat collectors, the spiral ones are divided into sections which are spiral coils. They are located in ditches which are from 15 to 20 m long, at least 0,8 m wide and at most 2,0 m deep. The distances between the ditches with the particular sections should be at least 3 m long. According to Zawadzki and in [1] the spiral collector is an alternative solution relative to the flat one. Usually removing 2-meter ground layer is more cumbersome than digging some ditches which are at most 20 m deep.

The surface which is required for spiral collector installation, because of enforced distances between sections, is similar to flat collector retention surface. The main difference between the flat collector and the spiral one is possibility of trees and shrubs planting in the free space between the ditches. While having the flat collector installing, trees and shrubs planting is not possible. The plants which are planted next to and above the sections should have flat roots system, so that the collector is protected against damage and the roots system is protected against freezing [1, 4].

In order to calculate the collector pipes length, geological ground structure and ground average temperature distribution should be known [2].

$$L = \frac{Q_0 \cdot \left(R_p + \tau_h \cdot R_s\right)}{\Delta T_g} \ [m] \qquad ((1)$$

where:

Q_o- thermal power collected from the ground, W

R_p- pipe unitary thermal resistance, (m·K)/W

R_s- ground thermal resistance, (m·K)/W

$\boldsymbol{\tau}_h$- correction taking into account the heat pump work period

ΔT_g- difference between intact structure ground temperature and heat carrier on supply to heat pump evaporator temperature, K

R_p- unitary pipe thermal resistance can be calculated with the formula:

$$R_p = \frac{1}{2\pi\lambda_p} \ln \frac{D_z}{D_w} \qquad ((2)$$

where:

λ_p- pipe material thermal conductivity coefficient, W/(m·K)

D_z- pipe external diameter,

D_w- pipe internal diameter.

Vertical ground collector

There are some types of vertical ground collector, depending on the way of realization [2]:

- „U" type
- with concentric flow,
- with countercurrent flow.

„U" type probes are the easiest to make and most often used.

When the our field surface appropriate, which means that there is nowhere to install the flat or spiral collector, there is a possibility to collecting the heat from the ground with the submersible probes. Zalewski writes that easy installation and not big surface caused that ground collectors of this type spread out widely on the market. Similarly like the other ground exchangers, the collector is divided into sections. Most often they are „U"'-shaped and are located in some vertical bores which are from 20 to about 150 m deep. The distance between the particular sections should be at least 5 m long [2].

The disadvantage of ground probes is costly enough realization because of obtainment of appropriate permissions, bores and geodesic project making. The advantages is constant, high ground temperature at the high depth. The temperature does not change whole the year [1].

Table 2. Possible unitary powers collected for ground probes (double „U" pipe probes according to VDI 4640 sheet 2

Kind of ground	Proper unitary power collected from the source with vertical exchangers
General approximate values Unfavourable ground Normal mineral ground and waterlogged sediment High hermal conductivity rocks	 20 W/m 50 W/m 70 W/m
Particular kinds of rocks	
gravel, sand, dry	<20 W/m
gravel, sand, water-bearing	55 – 65 W/m
damp clay and damp loam	30 – 40 W/m
limestone	45 – 60 W/m
sandstone	55 – 65 W/m
acid migmatites	55 – 70 W/m
alkaline migmatites	35 – 55 W/m
gneiss	60 – 70 W/m

Source: Self-elobaration based on [6]

Before the beginning of installation designing, detailed ground analysis should be made. Knowledge of soil properties, layers configuration, kind of soil, water presence etc. is necessary.

Usually real thermal power obtained from 1 m-long bore does not coincide with thermal power which was established during installation designing, that is the project should be corrected after making the first bore.

2.3. Heat pumps using direct evaporation

In heat pump of this type with low-grade heat source working medium circulates in ground collector as medium transferring the heat and it evaporates there, too. This solution results in the highest efficiency coefficient and even highest exploitation safety than in other variants, because in this case there is no indirect heat exchanger and brine circulation pump.

In heat pumps using direct evaporation flat ground collector made of copper pipes without seams and covered with protective layer made of plastic can be used as a low-grade

heat source. This collector can be used as a very efficient evaporator. Collector pipes diameter is 10-12 mm, one loop is from 60 to 75 m long.

Copper pipes are thermodynamic circulation element where thermodynamic sensor is located. The sensor, after contacting the ground, evaporates through pipe walls. The heat, which is collected from the ground, is transferred directly to compressor. The increase of pump heating capacity is caused by elimination of brine, which is indirect heat carrier [1].

2.4. Air heat pumps (air/water)

Air heat pumps are the devices which use sun energy accumulated in the air. The external is easily accessible heat source and can be collected in every amount, but has some disadvantages such as:

- low specific heat,
- low heat reception coefficients,
- low heat penetration in heat pumps evaporators coefficients,
- high temperature fluctuation.

Using used air from ventilating devices (for example staircases, factory halls, etc.), instead of atmospheric air, is much more economically profitable [1].

In air/water heat pump heating power and capacity coefficient COP decreases when air temperature drops. This pumps work the most profitable in summer, during severe winter power and pump efficiency drop is so high that additional heat source use is necessary. Usually high power heating boiler, fireplace or electric heater is used for this purpose.

The advantages of heat pumps of this type is low investment cost, because there is no necessity of making low-grade heat source. Water wells or ground collectors, which are costly, are not necessary in this case. There are known three versions of air-water heat pumps [1]:

- external air/water type pumps – monoblock

They are devices which have big sizes and have big ventilator (horizontally or vertically installed), which leads the heat at once to evaporator. Heat pumps, because of hard work conditions many times (the installation of device outside the building), have got solidly made and painted casing, that is why they are resistant to atmospheric factors influence. One of the negative phenomena which could appear when the temperature is below zero is hoarfrost formation on evaporator surface. The pumps of this type work, when the temperature is below zero, is possible, because evaporator defrosting automatic system is installed with pumps.

- separated air/water type pumps – with external evaporator called Split

In air/water pumps of this type evaporator and compressor are located out of main device. Thermodynamic circulation pipeline joins these two pump parts with condenser. Evaporator in pumps of this type, because of location (atmospheric hard conditions), should be equipped with defrosting system and ventilator [1].

- internal air/water type pumps – monoblock

The pump of this type does not have to be protected against atmospheric factors, because it is located inside the building. Collected air flows to the pump through canals made of galvanized steel sheet or through special air sleeve [1].

Tank heat pumps are an interesting solution. They are internal air water type heat pumps connected with warm usable water tank. They are modern devices which are usually able to protect warm usable water demand for detached house in 100%. They should not be applied as independent energy source for heating up usable water in places where usable water demand is very high. Air intake can occur both from inside and from outside the building. The air intake from inside the building allows to get notable savings. However, it often results in installation of additional elements for clean-up and filtering collected air because the air is polluted [7].

Tank heat pump, as the name says, consists of heat pump and warm water tank. The basic structural elements are tank made of stainless steel, pump condenser submerged inside the tank, built-in additional coil, electric heater, electronic maneuvering with software and all these elements are located in case made of polished stainless steel [7].

The way of tank heat pump operation is the same like the way of the other heat pumps operation, so it is based on thermodynamic processes in cooling circulation.

The heat collected from surroundings is transferred to the evaporator, where working medium evaporation occurs and steam flows to compressor. In the compressor working medium is compressed to high pressure, during this phenomenon the temperature also increases. With the compressor medium is pressed to heat pump hot side to the condenser, which is submerged inside the tank. In this place working medium steam is cooled and is again condensed. The heat collected from surroundings with additional energy piped during the compression is given to the water in the tank. Working medium flows to the evaporator and is decompressed at decompression valve on the way. The circulation is closed [1, 7].

3. Heat pumps application

Heat pumps are used for buildings heating and warm usable water heater preparation mainly in buildings where gas boiler cannot be used. Heat pumps are very interesting alternative for oil heaters, because of very high heating oil prices, and for constant action boilers, because of

maintenance-free and system safety. Their biggest disadvantage is for sure high investment cost - compared to traditional constant action boilers even 5-6 times higher. On the other hand, the heat pumps exploitation costs are low and are mainly connected with electric energy costs. High investment costs are the main factor which restraints on dissemination of solutions of this type, that is why air heat pump of warm usable water heater is an interesting option which purchase, installation adaptation and exploitation cost is relatively low.

Figure 1. Tank heat pumps for the puproses of warm usable water heater

Source: Own source

This pump exploitation costs compared to water heating with electric heater costs are presented below. Analysis was made for the purposes of one of the Białystok companies.

Enclosed prices are in accordance with valid in September-October 2014 period price list in Białystok.

Daily institution warm usable water heater demand was about 400 l, electric energy price according to PGNiG price list was 0.56 zł/kWh.

Average pump efficiency coefficient, which was given by producer, was COP = 3.5.
Heating this amount of water with electric heater means that the energy consumption is within 16,26 kWh – according to previous calculations.

Monthly heating up the water with electric heater cost:

$$D_{ke} = X \cdot C_e \cdot n[zł/day] \quad (3)$$

where:

X - the amount of energy necessary for heating up 400l of water [kWh]

C_e – electric energy price according to PGNiG price list

n – analysis duration period – 30-day period = 1-month period was assumed [month]

$D_{ke} = 16.26 \cdot 0.56 \cdot 30 = 273.3$ zł/month

Monthly heating up the water with heat pump cost:

$$D_{kp} = (X \cdot C_e \cdot n)/COP \ [zł/day] \quad (4)$$

where:

X - the amount of energy necessary for heating up 400 l of water [kWh]

C_e – electric energy price according to PGNiG price list

n – analysis duration period – 30-day period = 1-month period was assumed [month]

COP – pump efficiency coefficient. [-]

$D_{kp} = (16.26 \cdot 0.56 \cdot 30)/ 3.5 = 78$ zł/month

Above-mentioned analysis shows that the difference in two types of costs is about 196 zł per month during daily 400l of water consumption, but if the consumption was higher, the difference would be even higher. Per annum it gives about 2300 zł savings, which is about the difference between heat pump purchase costs (about 7500 zł) and costs of buying the tank, with similar parameters, made of stainless steel (4900 zł), so payback period is about 14 months, what is very good result.

However, water demand in detached house, which are the main consumers of pumps of this type, is remarkably lower, so the payback period can be even two times longer.

Above-mentioned specification does not take into consideration service costs which can occur while using heat pumps and which are practically equal zero in the second solution. Furthermore, additional exploitation costs are not taken into consideration, for example filters

exchange on suction wires costs, which is mainly dependent on conditions the pumps works in. These costs in this case are low and does not affect the general comparison.

Above-mentioned specification shows the main advantage of using air heat pumps – low purchase and exploitation costs. However, it should be remembered that in comparison to other energy sources (for example gas, oil), profitability and payback period will drop. However, during the trial of making this kind of analysis, for example with gas boiler, it should be remembered that both electric energy necessary for bolier work, what practically is not mentioned by producers, and gas consumption are exploitation costs.

The other advantage of using tank heat pump is possibility of rooms cooling and drainage with the appropriate intake and eruptive wires disposition. It is not sure alternative for ventilating devices, but in a measure during heat pump work, it can be similar to mechanical ventilation system. Device in air supply wire collect the air from the room, and cooled air is given to the room with jet wire. In this system, the air circulation occurs and this process is similar to mechanical ventilation. The wires obviously does not have to be directed into the same room, intake and outlet can be located independently.

Air heat pumps efficiency is the highest when intake air temperature is the highest. However, the temperature should not be higher than about 43°C, because working medium overheating in the pump can occur which results in device damage. Therefore, the intake wires should be appropriately disposed, so that the air temperature during possibly the longest period could possibly be the highest. In summer this problem practically does not occur, but in winter it is not so easy. The easiest and also the most often met solution is air collecting from outside the building. Unfortunately, in summer it has a logical application, but in winter the pump efficiency drops and electric heater must support the pump work. The air intake from housing space, for example living-room, is equally often met solution. In summer we can additionally drop the room temperature and in winter pump works without electric heater support, but warm air collected by pump is warmed by central heating system, so the energy is indirectly transferred to the pump.

When heat source, for example constant action or gas boiler, is installed in building for the purposes of central heating, we can use waste heat. Air in the room, which was warmed by adopted heat source, can be sufficient for economical heat pump work in winter. In some cases the pump work can be supported by the boiler work or the other external heat source, so that the electric heater does not have to be used.

Figure 2. Heat pump cooperating with the boiler

Source: Own source

The other rooms, where waste heat occurs, can be used similarly to this solution. Therefore, every room, where there are devices producing the heat while their work, for example computer service rooms, the rooms with ventilating central point etc. can be used, too. This solution, besides assurance optimal pump work condition for heat pumps, ensures also good conditions of surroundings of the devices, which the heat is collected from, by dropping temperature in the rooms, where the devices are located. However, it should be remembered

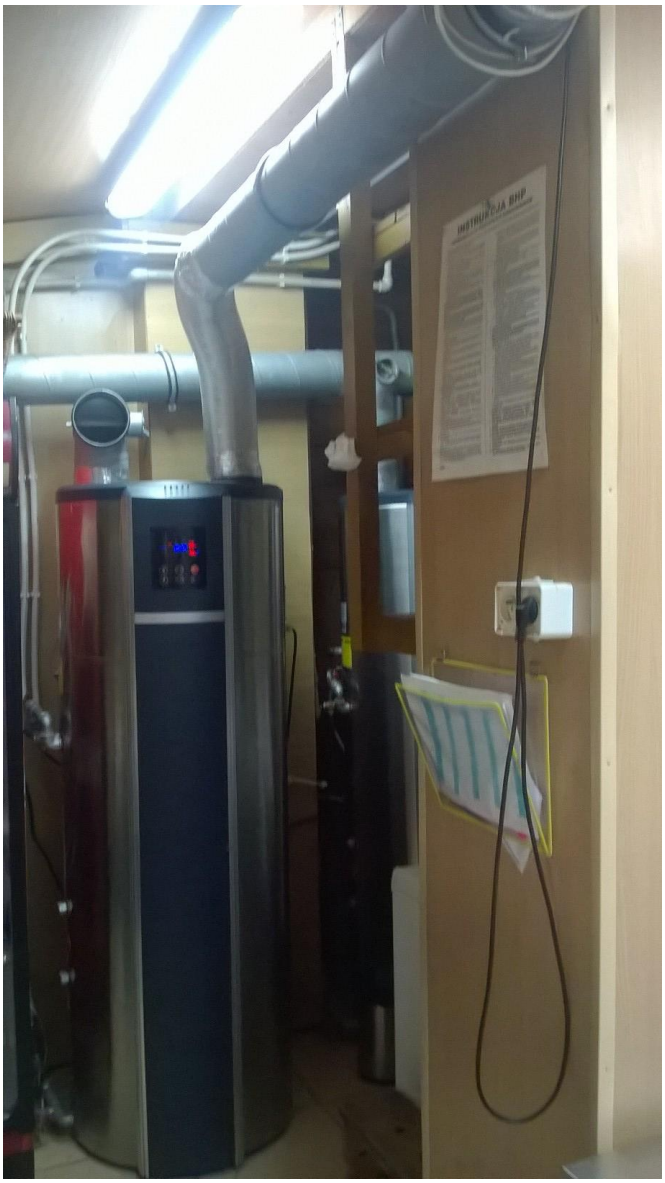
that the proper quality air, that means the air without mechanical pollution which can affect on device, mainly evaporator, damage, should be assured for the heat pump. In the picture below the heat collection with the pump from the room with the ventilating devices in one of the Podlasie hotels is shown.

Figure 3. The heat pump in the room with the ventilating central point. The heat intake was made directly in the room, reproach – outside the room

Source: Own source

Intake wire disposal, for example in kitchens, grill rooms etc., is the other way of heat collection by the device. This solution is notable mainly in food making building, where the rooms temperature is high enough and the same temperature values are practically available whole the year. Unfortunately, it results in additional device protecting filters application and periodic evaporator cleaning, because in this solution the air is heavily polluted, mainly by fats. This solution can result in higher people work comfort with the room temperature drop.

Figure 4. The heat pump located in kitchen in one the Podlasie hotels



Source: Own source

Figure 5. The intake wire, collecting the air from the kitchen

Source: Own source

4. Conclusions

1. Nowadays heat pumps are not an interesting solution in economic matters when it comes to heating the buildings.
2. Investment and exploitation costs of heating warm usable water for tank heat pumps, in comparison to costs for the other heat pumps, are low, what results in higher interest in this product in the last years.

References

1. Praca zbiorowa pod kierownictwem Zawadzkiego M.: Kolektory Słoneczne. Pompy ciepła- Na Tak. Polska Ekologia 2003
2. Rubik M.: Pompy ciepła- Poradnik. Ośrodek Informacji „Technika instalacyjna w budownictwie", Warszawa 2006
3. Oszczak W.: Ogrzewanie domów z zastosowaniem pomp ciepła. Wydaw. Komunikacji i Łączności, Warszawa 2009
4. Zalewski W.: Pompy ciepła sprężarkowe, sorpcyjne i termoelektryczne. Podstawy teoretyczne. Przykłady obliczeniowe. Wydaw. IPPU MASTA 2001.
5. Zeszyty fachowe, Pompy ciepła, Viessmann 11/2009
6. Brodowicz K., Dyakowski T.: Pompy ciepła. Państwowe Wydawnictwo Naukowe, Warszawa 1990
7. http://www.wolfpolska.pl/fileadmin/content/PL/Do_pobrania/Instrukcje/Pompy_ciepla/instrukcja_rickenbacher_hpw.pdf