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Application of Microbiological Methods in the Settlement Wastewater Treatment

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Additional information is available at the end of the chapter

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Abstract

The approach to solving the problem of water protection is changing in the world, and the opinion that wastewater is a resource instead of waste is now prevalent with research being directed in the direction of simpler, energetically more rational and more economically acceptable technological solutions for wastewater treatment, primarily in the field of biotechnology, especially there where favorable climate conditions and the use of large land areas are available. The mechanism of wastewater treatment by microbiological methods is simple and is reduced to extraction of certain substances from wastewater directly with plants or through the food chain with animals and their concentration into microbiological living stations. Microbiological living stations are extracted from the water in the form of biomass by simple mechanical methods, and in that way the final removal of nutrients and other substances from the water is completed. The produced biomass can be used as food or feed, with mandatory sanitary inspection, or as an emergent in biomass production. This paper presents the principles of application of macro biologic methods in wastewater treatment and the experience gained through the research at the Faculty of Civil Engineering of Niš and at the waste water treatment facilities in Sokobanja.

Keywords: wastewater treatment, microbiological methods, resource and energy potential

1. Introduction

The sudden technological and industrial development, tumultuous demographic growth and rapid urbanization especially in the last two decades pose humanity with four big problems: water, food, energy and environment. The problem of water is especially pronounced because

it is implicitly present in the remaining three problems, the production of food and energy are decisively dependent on water and the key problems of environment protection are protection of water quality and protection from the harmful effects of water.

Even today, the water crisis is well underway and according to predictions, by the middle of this century it will develop into a crisis of global proportions. The gap between available reserves of water and the increasing need for it on one and the pollution of water resources on the other hand are more and more pronounced with each day so rational use of water resources and their protection from further pollution, today and especially in the near future are developing as the main global problem.

The world today has many high technologies for wastewater treatment available, primarily of physicochemical nature, which allow for wastewater to be treated to a very high level and can satisfy all strict and more rigorous demands set regarding quality of treated effluents released into water recipients. However, the time of intensive development of these technologies was also the time of cheap energy, but today, when the evidence crisis is evident these technologies are too expensive even for the most developed countries of the world. That is why, in the world, the approach to solving the wastewater treatment problem is increasingly changing and intensive research is conducted in the direction of cheaper technologies for treatment of wastewater and protection of water from pollution.

In the last three decades, a special interest in the world is aroused by the potential of using the microbiological methods in the waste water treatment, whose application as of natural and not artificial waste water treatment processes provide effluents of demanded quality in an economically acceptable way in technically simple objects.

2. Microbiological methods in the wastewater treatment

Until the energy crisis which emerged in the 1970s the leading approach to secondary (biological) wastewater treatment was the philosophy of destruction of organic matter, and tertiary treatment, removal of nutrients from wastewaters and treated waters was mainly connected with complex and expensive chemical technological processes.

The hint of an energy crisis demanded directing towards cheaper technologies for treatment of wastewater and protection of water resources from eutrophication. The opinion that wastewater is a resource instead of waste became increasingly prevalent and research was intensively directed towards simpler and economically more acceptable technological solutions, primarily in the field of biotechnology especially in conditions where the use of large land areas is possible. The tendencies of questioning the philosophy of destruction of organic matter, or their mineralisation, have become more widespread, present in the previous technological practice of wastewater treatment and accepting the philosophy of synthesis of organic matter and nutrients into higher forms, which brings up the numerous matter in wastewaters [nitrogen (N), phosphorus (P), etc.] which can be interesting and useful as a raw material.

In accordance to the philosophy of the synthesis of organic matter and nutrients into higher forms, the possibility to use the macrobiological methods for the wastewater treatment attracted interest around the world.

Macrobiological methods of wastewater treatment present aerobic processes of synthesis in the direction of more complex organic matter which is easily removed from water in the form of biomass with noticeable reduction of energy needed for functioning of the system and encompass a whole series of macrobiological unit operations, the list of which, with constant research conducted around the world, continues to grow. These methods are applied as natural instead of artificial processes of wastewater treatment and they provide effluents of demanded quality in an economically acceptable manner in technically simple objects.

Intensive research in this area began in the 1970s in the world [1], and almost at the same time in Serbia [2, 3]. The starting results were obtained under the direction of Prof. Dr Lazar Ignjatović, in the period between 1975 and 1979, from the Faculty of Civil Engineering, University of Niš, Serbia and the wastewater treatment plant (WWTP) in Sokobanja, which was used as training ground for the staff of the faculty, through the project "The influence of accumulation on the change of ecosystem", with the research being continued in the period of 1978–1988, through the multidisciplinary project under the same name within the Fulbright program. In order to control the nutrient into effluent, numerous macrobiological unit operations were tested as laboratory models and then brought to the level of macro model, namely the part of an already existing wastewater treatment plant in Sokobanja, in cooperation with reputable experts from the USA for certain areas.

3. Treatment mechanism

In the wastewater treatment performed by the macrobiological methods the macrobiological living stations are used. The macrobiological living stations is the term for all the higher plants or animals with all the characteristics of living organisms, including with sexual and sexless procreation.

Living stations, either plant or animal, are mainly made of water starting from 73% in carp, 80% in terrestrial macrophytes, even up to 95% in hydrophytes. The exact percentage of water depends on the type of living station, the weight and the age of the station in the moment of sample processing.

The largest part of the living stations dry mass (usually over 90% in plants) is made of three basic elements: carbon (C), oxygen (O) and hydrogen (H), taken directly from the water or air, while a smaller part (around 10% in plants) is made of all the other elements.

The plant or animal cannot complete its life cycle in the absence of any of the necessary elements, which must be available directly or through suitable enzyme activity, provided that there are no antagonistic or toxic effects of the other elements. From 92 natural mineral known elements around 60 are found in living stations. From the 60, around 16 are considered essential for growth of plants, and approximately the same number is necessary for growth of aquatic

animals of interest. It should be considered that although they do not need other elements, plants and animals accumulate some elements not significant for growth and development.

Wastewater treatment is completed through bioconcentration, or accumulation of substance from the environment and concentration in a biological station, directly in plants or indirectly, through the food chain, in animals. Because living matter is formed by a few biogenic elements either plastic: hydrogen (H), oxygen (O), carbon (C) and nitrogen (N) or oligo elements: zinc (Zn), copper (Cu), iron (Fe) and magnesium (Mg) in the presence of phosphorus (P), sodium (Na) and potassium (K), chloride (Cl) and manganese (Mn) the factor of bioconcentration of the substances from the environment in the organism is important along with the dynamic of the process, or the rate of bioconcentration of the substance of interest through time.

Numerous factors affect the growth, development and reproduction of the living stations. Some of them are crucial while others are less significant, depending on the phase of growth of the living station. The earliest developmental phases are the most sensitive in all living stations. Light and temperature, along with activation energies have crucial effect on the dynamic of the process. Plant species of areas with temperate continental climate are active when the temperature of the water reaches above 15–17°C, while tropical species are mainly active from 20 to 24°C.

If the natural conditions of the environment are favorable, application of macrobiological stations is possible in natural conditions, perfectly cheap because free energy of the Sun is used as an energy source, and the water itself serves as a collector with the least loss.

If the natural conditions of the environment are unfavorable, the influential factors can be put under control and then we have artificial environmental conditions. Application in greenhouses in periods of unfavorable conditions of the environment, with the addition of light and thermal energy, is possible. In those cases great economic effects of use in natural conditions decrease. There where geothermal energy is available, its use may be rational for extended work over the whole year.

It is clear that the wastewater treatment mechanism is simple. It consists of taking certain substances from the water (whereby the water is rid of this substance) and its bioconcentration into microbiological living stations. By removing larger macrobiological living stations in the form of biomass from the water with simple mechanical methods the nutrients and other substances are finally removed from the water.

The final disposition of biomass, depending on its nature, is performed by the standard transport means. If the biomass is used as a nutrient or food (with necessary sanitary control) it has market value which considerably exceeds the transportation costs.

It is a highly clean technology in wastewater treatment using clean energy (solar and/or geothermal) with the final product being usable biomass. The civil engineering objects are usually made of soil, relatively simple and followed by a minimal equipment fund, which significantly influences low investment costs. In investment costs the land makes as significant item but if there is unsuitable agricultural or commercial land close to the settlement available, ideal conditions for the application of these technologies are acquired.

4. Macrobiological living stations

For a living being to qualify as a macrobiological living station, respectively, a technological element in wastewater or sludge treatment it has to satisfy special criteria:

- It has to belong to a fast growing species with a short reproduction cycle, so the processes can run in an accelerated speed.
- It has to be easily removed from objects in which it is used with the purpose of wastewater treatment, namely it has to allow low manipulation costs with the produced biomass and the biomass should have value of use, which in turn affects the relief of wastewater treatment cost.

Great attention should be given to the question if the macrobiological living station can, for a longer period of time, survive and reproduce in natural habitat on its own and if the species is invasive. This is of great importance, from the aspect of possible ecological effects if a macrobiological living station finds its way into the natural environment, out of the object where it is used under control.

On the basis of the mentioned criteria for the macrobiological unit operations, only a small number of plant and animal species can be qualified. They predominantly originate in the tropical zones.



Figure 1. Floating macrophytes: *Eichornia crassipes*, *Pistia stratiotes* and *Salvinia* [3].

Macrobiological living stations, which qualify as a technological element in wastewater treatment technology, are classified by groups that are floating macrophytes, fish, mussels, earthworms, etc. The list of macrobiological living stations is very wide, but it does not encompass all possibilities because in this area intensive research is present.

The list of possible floating macrophytes should be made of hydrophytes without woody tissues, especially ones which float on the surface of the water. These plants cannot adapt to the change of the water level so for their normal growth and development the water level must be kept approximately constant.

The representatives of this plant group use food directly in the shape of dissolved nutrients in the water. Some do that only through the leaves, e.g., floating crystalwort (*Riccia fluitans* L.) to some extent lemna (*Lemna minor* L. and *Lemna trisulca* L.), while other especially larger species do that through their roots which hang in the water, e.g., water hyacinth (*Eichhornia crassipes* Martius), water lettuce (*Pistia stratiotes* L) and salvinia (*Salvinia natans* L. and *Salvinia auriculata* Aublet) (Figure 1).

In terms of fish, fast-growing species capable of consuming large quantities of food are of interest. Also of interest are food pyramids because of the choice of fish species, especially because of interrelationships in polyculture composition. Phytophagous species have a special role because they lean directly on the primary production in the aquatorium in the food chain.

Figure 2 shows a food chain and fish species of predominant interest: silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*) and common carp (*Cyprinus carpio*). The list can be expanded with some tropical fish species, e.g., tilapia (*Tilapia aurea*) and thai catfish (*Clarias batrachus* Linnaeus). The use of tropical fish species applies the same demands as the use of tropical plants.

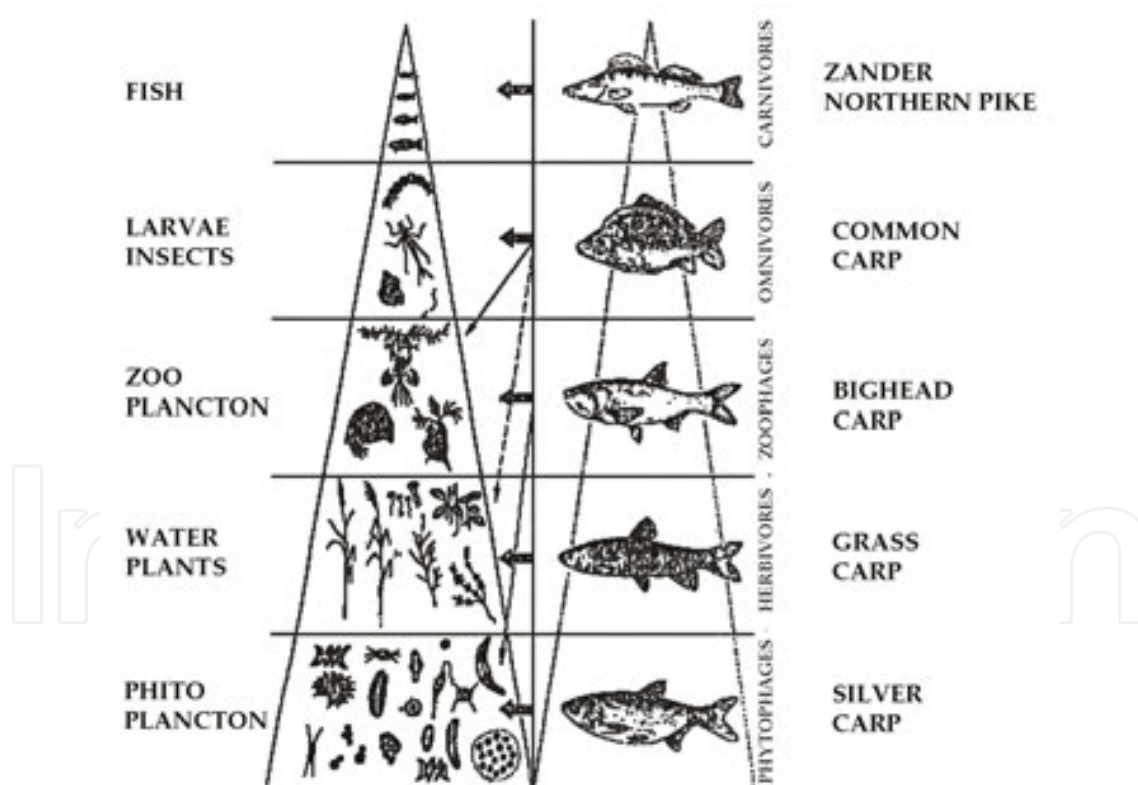


Figure 2. Fish-food pyramid and the primary interest species (based on Ref. [3]).

Fish, as poikilothermic animals, because of poor adjustment to sudden temperature changes, must not be rapidly transferred from one water environment to the other if the water temperature difference is greater than 2°C, because this leads to temperature shock and death in most

species. The fish must be transferred carefully, because being thrown in the water during transfer leads to bursting of the swim bladder.

The role of mussels in removal of suspended and colloid material from wastewater deserves great attention from the researchers. It was experimented only with one species of mussel from the temperate climate belt in the Faculty of Civil Engineering of Niš. It was experimented with the zebra mussel (*Dreissena polymorpha*) (Figure 3). It should be noted that this species of mussel may pose not only an ecological threat, but also great danger to the hydrotechnical systems and objects, so it should be used in strictly closed systems.



Figure 3. Mussels – *Dreissena polymorpha* [4], *Unio pictorum* [5] and *Anodonta cygnea* [6].

Species of interest would also probably be the painter's mussel (*Unio pictorum*), swan mussel (*Anodonta cygnea*) (Figure 3), Eastern Asiatic freshwater clam (*Anodonta (Sinanodonta) woodiana*) and large far eastern mussel species (order *Cristaria*) whose shell can grow over 30 cm. This area is open for research with warning if nonendemic species are in question.

Two species of earthworms are of interest as macrobiological living stations in the technology of sludge treatment: red Californian earthworm (*Eisenia fetida*) and red earthworm (*Lumbricus rubellus*) (Figure 4). For further research the red tiger earthworm (*Eisenia andrei*) (Figure 4) is interesting because it can treat rich organic waste in massive amounts. Probably the European nightcrawler (*Eisenia hortensis* or *Dendrobaena veneta*) would also be of interest but it is considered an invasive species which should be used in strictly controlled conditions without being allowed into the natural environment [7].



Figure 4. Earthworms – *Eisenia fetida* [8], *Lumbricus rubellus* [9] and *Eisenia andrei* [10].

For the needs of hydroponics unit terrestrial plants are used, such as tomatoes, e.g., the American flowerpot tomatoes (*Lycopersicum esculentum*), leafy vegetables, e.g., chard (*Beta vulgaris*), corn for silage and similar species. The list is very long because numerous fruits or vegetables can be used.

Carefully composed polycultures (bigger number of different species of macrobiological living stations in a unique aquatorium) have a bigger effect on the quality of effluents than a monoculture (a single species of a microbiological living station in a unique aquatorium). The reason being that a monoculture drains a narrow circle of substances and because of that it has limited effect in removing nutrients and wastewater treatment. The advantage is given to monocultures only in the case of final biomass derivation if it is used for human or animal consumption or partial wastewater treatment.

If ambient conditions favor some of the members of the polyculture, it spontaneously comes to suppression of the other members and the formation of a monoculture, namely population of a macrobiological living station which ambient and other factors provide the most suitable conditions. In those cases instead of insisting on polycultures the transition on a series of monoculture basins is expedient.

It should be mentioned that successive application of monoculture basins enlarges the investment costs. But continual additional introduction of macrobiological living stations from external sources, for polyculture maintenance, is usually more expensive than amortization of bigger investment costs in more basins.

In all cases parent clusters under optimal conditions must be ensured. This is optimally in the shape of a macrobiological living station bank on a regional level, for example botanical gardens or zoos, organized on a wider administrative area.

5. Objects and system design

Working on the choice of unit operations, their synthesis into the technological process and objects and system design is complex engineering work which demands professional experience along with team work of participants of the system design. Designing objects and the system for wastewater treatment starts from the available information on the wastewater quality, defining the type and concentration of the unwanted substances and the needed removable level. Based on the analysis, the technological scheme of the wastewater treatment system is defined.

Based on information on the amount of wastewater and its variation, hydraulic and process loads are defined. If the variations are big, the problem of synchronized hydraulic and process loads must be solved by choosing adequate modular object units. In this phase, decisions are made on the choice of macrobiological unit operations and the choice is made between mono and polycultures.

It should be kept in mind that for synthesis of macrobiological unit operations into the treatment processes, aside from macrobiological, standard (classic) unit operations are often incorporated with the purpose of bringing characteristics of wastewaters on the effluent of wanted quality. Although any wanted level of wastewater quality may be achieved through a planned combination of unit operations, the choice is made under clear economical conditions.

As with any modern biological system of wastewater treatment, primary treatment must always precede a system based on macrobiological methods. For primary treatment of wastewater the use of a highly efficient (tubular) settlement tank from which the primary sludge is processed by anaerobic decomposition in digesters and on vermiculture (VF) fields next to smaller wastewater treatment plants is recommended.

When secondary treatment is in question, unlike classic technologies with microbiological population with which secondary treatment is made of a microbiological unit and a secondary settlement tank, with macrobiological methods there is no need for a secondary settlement tank. The reason being the lack of secondary (biological) sludge because the transformations of materials from wastewaters, through the food chain, are done into the biomass of the macrobiological living station.

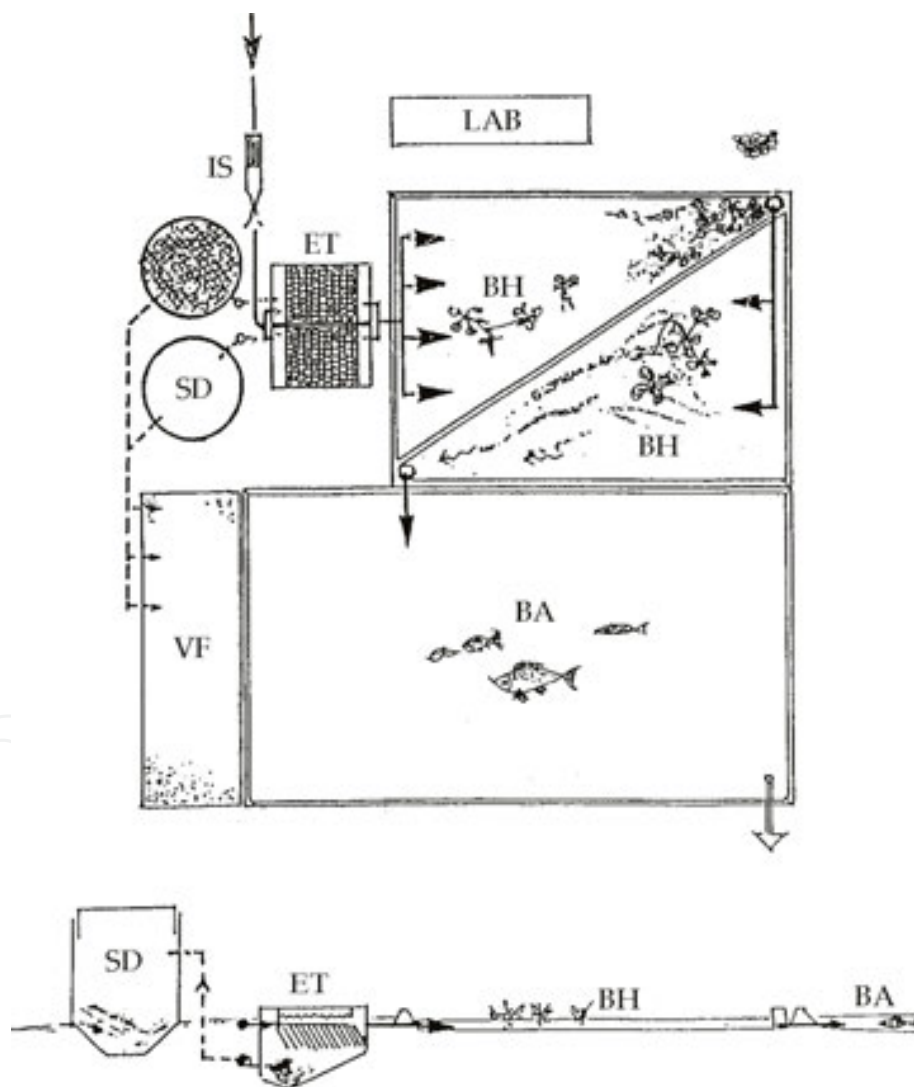


Figure 5. Scheme of the human settlement waste water treatment facility [3, 11]. IS – inlet structure; ET – efficient settlement tank; BH – basin for sanitary hydrophytocoltures; BA – basin for sanitary aquacultures; SD – sludge digester; VF – vermiculture fields.

Based on the technological scheme, after choosing unit operations and defining modular units, the technological scheme of the system with the basic hydraulic and technological calculations is designed. This results in a horizontal plan of the objects and their height scheme from the entrance to the exit of the treatment plant.

After the place and the role of some objects, their sizes and height positions are defined, the design of the objects for application of macrobiological unit operations is reduced to civil engineering design of objects. For object design, the knowledge of unit of macrobiological living station, design information and characteristic technical details of the object is required. Knowledge of civil engineering design, stability and dimension of constructions and civil engineering regulations is also required.

For application of macrobiological unit operations, two tendencies are present:

- For smaller agglomerations, especially with seasonal problems, macrobiological unit operations are synthesized into complex, with cheaper investment and maintenance objects.
- For bigger agglomerations behind classical treatment plants these methods are used for polishing of effluent quality with nutrient removal (tertiary treatment).

The example for the first approach for smaller settlements is given in the follow-up. The scheme of a wastewater treatment plant is given in **Figure 5** with object marks. Each of the objects is described in more detail with needed design information.

The shown scheme is applicable for settlements without industrial and toxic wastewater. The scheme incorporates wastewater treatment and sludge stabilization so that they can be disposed into the natural environment without negative ecological effects behind the treatment plant. This is ecologically clean technology.

Depending on the ability and concert of the operator the removal of suspended solids is from 80% to above 95%, and this applies for putrescible matter too. The reduction of bacteria is above 99% so the water can be used for irrigation in semiarid areas without danger.

This technological scheme is more favorable in the level of efficiency and the produced biomass if the climate conditions are warmer and insolation is more intensive. In areas of temperate continental climate the starting hypothesis is the disposal of solar energy during the whole year or geothermal energy, if continued work of the system with low cost investment and maintenance is desired during the whole year.

The technological scheme and all unit operations are checked on the wastewater treatment plant in Sokobanja, Serbia which served as a pilot treatment plant with the process scale of 1:1, under realistic conditions. In the follow-up, description and instructions for some objects are given.

5.1. IS – inlet structure

Inlet structure (**Figure 6**) serves for removal of large suspended matter and measurement of flow of wastewater. It is made of a channel with a grid which continues to the Parshall flume. The space between iron flat bars is 2–5 cm. The slope is 1:2 to 1:3 for easier cleaning. For smaller

treatment plants the cleaning is done manually with loading of handcarts and daily transport to burial of the material from the grid to a suitable location in the treatment plant area. For bigger treatment plants the cleaning of the grid is automatic and the transport of material is off the grid to the landfill.



Figure 6. Inlet structure and efficient settlement tank on the WWTP Sokobanja.

Based on the known, defined hydraulic load, standard hydraulic calculation of width of the channel for defined level and loss is completed.

5.2. ET – efficient settlement tank

The primary settlement tank is based on the system of a highly efficient tubular settlement tank (**Figure 6**) which includes a separator of oil and grease into compact construction.

Domestic wastewater treated on this type of settlement tank with a process load not greater than 0.6 l/s po m^2 (horizontal area of settlement tank) is of such quality that without further treatment it can go on microbiological units. Water is kept in the settlement tank shortly, 15–25 minutes and there is no danger of transit into septic state which is of great importance for the effluent quality. The sludge from the settlement tank is pumped into the sludge digester (SD) for further treatment.

Based on the known, defined hydraulic load, the calculation is completed by standard procedure for primary, mechanical wastewater treatment.

In the case of different primary treatment or no treatment, the quality of wastewater should be brought to an acceptable one for microbiological living stations which will be used in further treatment.

5.3. BH – basin for sanitary hydrophyticultures

In this basin (**Figure 7**), the dissolved and colloid matter from the wastewater is transformed into biomass of floating macrophytes under the influence of solar energy. Basin depth of 0.4–

0.6 m with a protective bank or edge of 0.2 m above the water level is recommended. The insertion of young macrophytes can be done with monocultures or polycultures depending on whether there is previous experience with the wastewater being treated. In highly polluted wastewater, *Eichhornia crassipes* is the most active species; in medium-polluted wastewaters, *Pistia stratiotes* should be given advantage and in the least polluted wastewaters, *Salvinia* is most appropriate. The quality of the wastewater, the choice of macrobiological living station and process loads define the dimensions of the basin. For domestic wastewater and daily specific consumption of 250 l/person from 3 to 5 m²/PE of area under the hydrophytoculture *Eichhornia crassipes* is needed, while *Pistia stratiotes* demands double of that value.



Figure 7. Basin for sanitary hydrophytocultures and basin for sanitary aquacultures on the WWTP Sokobanja.

The growth dynamic of green biomass of floating macrophytes and area coverage of the basin in green biomass is in function of plant quality, insolation and temperature. For temperate climate conditions, based on the research done in the wastewater treatment plant in Sokobanja, the basin area coverage for the *Pistia stratiotes* biomass is 2–25 kg/m² and for *Eichhornia crassipes* biomass it is 5–35 kg/m² [3, 12]. The growth dynamic of green biomass of larger floating macrophytes is well presented by the exponential equation $B = B_0 \cdot e^{kt}$ in which B is the probable green biomass after a certain time t in kg, B_0 is initial green biomass in kg, k is the rate of growth in 1 day and t is time in days [3, 12]. The rates of growth for *Eichhornia crassipes* are 0.130 for 30°C, 0.052 for 20°C and 0.015 for 15°C and for *Pistia stratiotes* 0.061 for 30°C, 0.026 for 20°C and 0.010 for 15°C [3, 12].

For basin coverage with green biomass of 20 kg/m² and average temperature of 20°C, daily wet biomass growth about 5 t/ha for *Pistia stratiotes* and about 10 t/ha for *Eichhornia crassipes*. Biomass extraction in smaller treatment plants is manual. The biomass dries on a bank if fresh, green biomass is not used. Mechanized extraction of biomass is possible and economically justified in bigger treatment plants.

The work of this part of the treatment plant is connected to temperature and insolation conditions and under natural climate conditions in areas of temperate continental climate is

possible in the period between May and October. In the case of continued process during the year greenhouses and introduction of additional thermal and solar energy are needed.

5.4. BA – basin for sanitary aquacultures

Under the effect of solar energy, through primary production the process of nutrient removal and transformation into the biomass of fish is completed. Phytoplankton and zooplankton which have used the nutrients are transformed through the food chain of herbivore and carnivore fish into a high value protein.

Average basin depth of 0.3–0.7 m is recommended (**Figure 7**). Young fish are inserted into the basin in spring, most often with polycultures of herbivore fish with the addition of carp. Depending on the input water quality, dimensioning of the basin and aquapolyculture composition are completed.

If primary water treatment is done through tubular settlement tanks by the usual specific consumption, hydraulic load of $Q \leq 50 \text{ m}^3/\text{ha}$ for a day is recommended. Stocking is done with 200–400 kg/ha by polyculture (silver carp 65–50%, bighead carp 22–30%, grass carp 8–10% and river and ponds common carp 5–10%).

Production of biomass for a season depends on the success of plant management, and it ranges from 1.200 to 2.000 kg/ha for a season of 200 days.

5.5. SD – sludge digester

Sludge digestion (**Figure 8**) is desirable for hygienic and esthetic reasons, although it is not necessary if microbiological treatment of the sludge is completed.



Figure 8. Sludge digester and vermiculture field on the WWTP Sokobanja.

The amount of sludge and digester dimensions is calculated by the process for classical problem solutions. The calculation of sludge pumps and pipes, with notice that the pipes should not be under $\text{Ø } 200 \text{ mm}$ because of sludge flow resistance, is done by standard procedure. The same applies for the use of biogas.

If hydrophytocoltures are used as an emergent for biogas production, the volume of the digester is to be increased by 20%. Construction of a lateral opening with a nonreturn flap for insertion of the biomass into the digester is mandatory, along with a spiral access ramp or a lift.

5.6. VFs – vermiculture fields

On the vermiculture field (**Figure 8**) sludge treatment into highly valued hummus is completed, using *Eisenia fetida* or *Lumbricus rubellus* living stations.

If fresh sludge was put on vermiculture fields, no matter the addition of wood chips or cut paper for moisture reduction, odor and insects may appear.

The excess of earthworms is returned to the basin for sanitary aquacultures and can be used as food for the fish with a goal of increasing their growth.

Turning plant biomass into hummus is by far more rational than gasification and it gives a valuable commercial product, it especially increases the quality of the total produced material.

Macrobiological unit operations are especially important for a greater number of smaller agglomerations, in which rational expansion of wastewater treatment systems is possible so they give an effluent of high quality. The use of macrobiological unit operations, along with other classic operations in the technological scheme, allows not only a cheap but a technically simple and safe solution to nutrient (nitrogen, phosphorus) removal and BOD₅ reduction from wastewater of settlements without industrial wastewater.

6. Usage of the resource and energy potential of waste waters

The macrobiological living stations use from the waste water the nutrients and other elements which are a part of the biomass for their growth and development. The produced biomass has a practical value, so the nutrients and other matter in the waste water are not only harmful matter to be removed from the waste water, but are also the resource for production of the biomass. Regarding that the macrobiological living stations should belong to the fast-growing species with the short reproductive cycle, the quantity of biomass produced in the waste water treatment process, are huge, as a rule.

Based on previous research some possibilities for the use of biomass were noticed, but this is an area which is yet to be thoroughly researched.

Floating macrophytes can be widely used as biomass [3], especially when water hyacinth (*Eichornia crassipes*) and water lettuce (*Pistia stratiotes*) are in question [13–15].

Water hyacinth and water lettuce mixed with sludge are great material for hummus production, especially for winter cover and thermal protection of the vermiculture.

Water hyacinth combined and composted with manure gives better quality material for vermiculture nutrition with acceleration of the population dynamic because the root system is the ideal habitat for laying cocoons and reproduction.

Water hyacinth is ideal for nutrition of nutria (coypu) and they would rather be fed the hyacinth than beet leaves. Detailed information about the possibilities of application of floating macrophytes in livestock keeping, including its possible application in silage, is not available.

Fish as biomass can be used as food in human nutrition, especially if higher quality species are in question: grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), walking catfish (*Clarias batrachus*) and common carp (*Cyprinus carpio*). The possible danger from bioconcentration of heavy metals and pesticide, as well as quarantine in connection with epidemiological control of food quality should be mentioned.

Mussel meat, which is easily removed with hot water, is rich in proteins and is eaten by poultry and pigs in dried form. The shell of some mussels, e.g., painter's mussel, can be used as material for nacre products.

Worms, both species: red Californian earthworms (*Eisenia fetida*) and red earthworms (*Lumbricus rubellus*) which are interesting as macrobiological living stations in the sludge and manure treatment technology can be widely used as a biomass.

Earthworms can directly be used as food for poultry, pigs and pets (birds, tortoises, iguanas, snakes, fish in aquariums). They can also be used for production of protein flour, a high quality protein component in dry condition which is added to fish flour or feed. Commercially, the use of earthworms for the nutrition of aquacultures of fish is very favorable, especially for cultivation and fattening of fastgrowing *Clarias* catfish and cultivation of trout.

Even more profitable is placement of earthworms as bait (in fishing or for attracting wild birds) through specialized stores for hunter and anglers.

Earthworms feed on detritus, decomposing organic matter, and as secretion finely crushed material appears, relatively stable vermicompost, namely humus. Humus has great value as a natural fertilizer because it improves the structure of the ground and reduces/eliminates the need for chemical fertilizers. It is used in flower and vegetable cultivation, for nursery gardens, orchards and lawns, for topsoiling of surfaces or as a component in soil devastated because of the use of chemical fertilizers. If there was no pesticide, heavy metals or toxic substances in the starting material, the humus can be used in production of healthy food because, aside from having a positive effect on the ground, it has a positive effect on various plants and crop plants.

Regarding that, when larger floating macrophytes are in question, the amounts of biomass produced daily in the process of wastewater treatment are quite large, the possibility of using said biomass as raw material for production of biogas is of great importance. The produced biogas can further be used for combined production of electrical and heat energy.

Combined production of electrical and heat energy [combined heat and power (CHP)], also called cogeneration, is the production of electrical power out of the natural gas, biogas and

waste matter disposal site gas, with the simultaneous usage of waste heat which is otherwise lost in the industrial process. Modern cogeneration systems today reach efficiency above 90%, that is why cogeneration presents the most efficient and economically most justified way of reducing high energy costs in industrial plants and municipal objects.

In wastewater treatment plants with an anaerobic reactor (digester) for sludge stabilization biogas occurs as a mixture of combustible and noncombustible gases with the average composition of (in cubic %): methane 55–75%, carbon-dioxide 25–45%, other gases like hydrogen, oxygen, carbon-monoxide, nitrogen, hydrogen-sulfide, ammonia and water vapor [16]. The efficiency of biogas production is provided by maintaining temperature, pH value, by mixing and removal of oxygen and toxic matter.

Production of biogas can be assessed based on the following practical and experimental information [16]:

- on municipal wastewater treatment plants the average production of biogas 25 l/PE per day;
- with industrial wastewater (sugar refineries, molasses processing, potato processing, fruit juice production, dairy farms, breweries, paper and cellulose) the average methane production is 0.20–0.30 m³/kg CSB with the methane fraction in biogas being 60–80%.

The heat power of biogas depends on the methane content and for the average content of 65% methane it is equal to 6.4 kWh/m³ [16]. That is how it is possible to produce 2.5 kWh of electrical and 3.3 kWh of heat energy from 1 m³ of biogas with the reduction of CO₂ emission above 50% in a practical operation on a cogeneration plant with gas motors (**Figure 9**) [16].

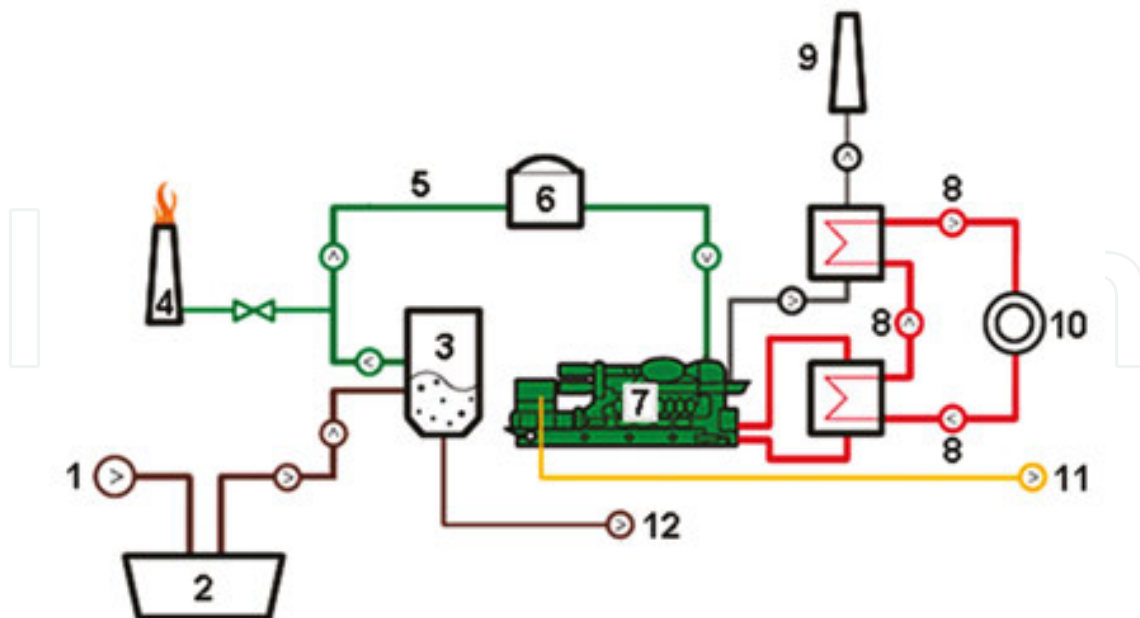


Figure 9. Usage of biogas at the facilities for waste water treatment (based on Ref. [17]). 1 – biomass; 2 – sludge thickening; 3 – anaerobic digester; 4 – gas torch; 5 – biogas; 6 – gas tank; 7 – gas engine; 8 – heat exchanger; 9 – exhaust; 10 – heat energy; 11 – electrical energy; 12 – agricultural fertilizers.

In wastewater treatment plants with a basin for sanitary phytocultures, the produced biomass of floating macrophytes, either processed through a digester for biogas production increase, used in cogeneration plants with a gas motor, or directly burned in cogeneration plants with an indirect gas turbine process, the amounts of produced electrical or heat energy can be multiply increased relative to plants with classic technologies, which of course directly depends from the available basin area for sanitary hydrophytocultures and the daily growth of the biomass of floating macrophytes.

The dry mass of *Pistia stratiotes* is 4.9% and for *Eichhornia crassipes* 4.6% from the green mass for the leaf part of the plant (the variations of the root mass are great). Based on the literature information each kilogram of *Eichhornia crassipes* dry mass gives 370 l of biogas, whose heating value is around 6.1 kWh/m³ [13]. For *Pistia stratiotes*, keep in mind that the structure of biomass is similar to the previously mentioned plant.

In **Table 1**, the values of biomass growth, biogas amount and electrical and heat energy, which can be produced from the biogas are shown for *Pistia stratiotes* and *Eichhornia crassipes*, calculated based on the previously stated experimental data. All values are given in ha of basin area under sanitary hydrophytocultures by day.

| | Air temperature (°C) | <i>Pistia stratiotes</i> | | | <i>Eichhornia crassipes</i> | | |
|---|----------------------|------------------------------------|---------|---------|------------------------------------|----------|----------|
| | | Area coverage (kg/m ²) | | | Area coverage (kg/m ²) | | |
| | | 2 | 20 | 25 | 5 | 20 | 35 |
| Green biomass growth (t/ha by day) | 15 | 0.201 | 2.010 | 2.513 | 0.756 | 3.023 | 5.290 |
| | 20 | 0.527 | 5.268 | 6.585 | 2.669 | 10.675 | 18.682 |
| | 30 | 1.258 | 12.580 | 15.725 | 6.941 | 27.766 | 48.590 |
| Dry biomass growth (t/ha by day) | 15 | 0.010 | 0.098 | 0.123 | 0.035 | 0.139 | 0.243 |
| | 20 | 0.026 | 0.258 | 0.323 | 0.123 | 0.491 | 0.859 |
| | 30 | 0.062 | 0.616 | 0.771 | 0.319 | 1.277 | 2.235 |
| Biogas amount (m ³ /ha by day) | 15 | 3.644 | 36.442 | 45.552 | 12.861 | 51.445 | 90.029 |
| | 20 | 9.551 | 95.512 | 119.390 | 45.423 | 181.691 | 317.959 |
| | 30 | 22.807 | 228.071 | 285.089 | 118.143 | 472.572 | 827.001 |
| Electrical energy amount (KWh/ha by day) | 15 | 9.110 | 91.105 | 113.881 | 32.153 | 128.612 | 225.071 |
| | 20 | 23.878 | 238.781 | 298.476 | 113.557 | 454.228 | 794.898 |
| | 30 | 57.018 | 570.179 | 712.723 | 295.357 | 1181.430 | 2067.502 |
| Heat energy amount (KWh/ha by day) | 15 | 12.026 | 120.258 | 150.323 | 42.442 | 169.768 | 297.094 |
| | 20 | 31.519 | 315.191 | 393.988 | 149.895 | 599.580 | 1049.266 |
| | 30 | 75.264 | 752.636 | 940.795 | 389.872 | 1559.487 | 2729.102 |

Table 1. Biomass growth, biogas amount and energies which can be produced for larger floating macrophytes [18].

As it may be concluded on the basis of the displayed values, at the facilities for waste water treatment with the basin for sanitary hydrophyticultures, significant quantities of electric and thermal energy can be obtained through the cogeneration.

Part of the produced electrical energy can be used for the plants' own needs, and extras can be forwarded into the ED network, while the produced heat energy can be used for maintaining the temperature in the digester, to ensure the efficiency of biogas production. The heat energy can also be used for providing favorable conditions (air temperature from min. 20°C and area coverage from min. 20 kg/m²) in greenhouses for application of these technologies in our climate conditions during the whole year, which in turn provides constant growth of floating macrophyte biomass and annuls the seasonal character of microbiological methods.

Considering the global climate changes, the Kyoto protocol predicts the possibility that developed countries invest in modernization of industrial and energy power plants and reduction of carbon dioxide emission and other gases which cause the greenhouse effect on the territories of undeveloped and developing countries. As introduction of cogeneration in wastewater treatment plants by microbiological methods and the usage of surpluses of electrical and heat energy in the energy system is in accordance with the Kyoto protocol, it would allow receiving of exceptionally favorable credits and investments for energy and ecology sector, and is as such of great importance, especially for developing countries, which are yet to solve the problem of settlement and industrial wastewater and the building of plants for their treatment.

Considering that the yields, which amount to a few dozen tons by ha daily with larger floating macrophytes in favorable insolation and temperature conditions, are of fantastic size, research of their value of use in animal husbandry, and even more in energetics is extremely significant.

7. Conclusion

Based on previously achieved results, it is evident that microbiological unit operations will in the future find their place in the technology of wastewater treatment for multiple reasons:

- instead of destruction of material, namely the stopping of natural processes we are going towards the philosophy of synthesis of organic matter into higher levels of biomass;
- the processes of synthesis use natural energy sources (sun, heat) and on that basis they present ecologically highly "clean" technologies;
- the objects are relatively simple civil engineering objects made of land followed by a minimal equipment fond, which greatly impacts low investment costs;
- there is no biological sludge and no parts of the object which represent the secondary settlement tank and sludge line, which significantly affects the relief of wastewater treatment costs;

- the final product is biomass which has value of use as food or feed, or as an emergent in biogas production, which in turn affects the reduction, even complete annulment of wastewater treatment costs.

The energy crisis, which is deepening day by day, high prices of energy, materials and the workforce, the demand for low investment and operation costs, and the sharper requests set in regards of discharge treated wastewater into recipients make the application of macrobiological methods in wastewater treatment around the world even today, and especially in the near future come to the fore and intensify the research in this extremely important area.

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