Current Trends in the Development of Fungal-Containing Consortia

Subjects: Environmental Sciences

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There is growing interest in the creation of artificial microbial consortia, especially in the field of developing and applying various bioremediation processes. Heavy metals, dyes, synthetic polymers (microplastics), pesticides, polycyclic aromatic hydrocarbons and pharmaceutical agents are among the pollutants that have been mainly targeted by bioremediation based on various consortia containing fungi and yeasts. Such consortia can be designed both for the treatment of soil and water.

Keywords: consortium composition ; main trends ; genetically modified cells ; multicomponence effect ; pollutant removal efficacy

1. Genetically Modified Microorganisms in Artificial Consortia with Fungi

Despite the high efficiency of degradation of various pollutants by natural microbial consortia [1], there has recently been a growing demand for strains that are improved by using advanced methods of synthetic biology and metabolic engineering, and numerical methods in the field of genetic engineering [2]. To date, the use of genetically modified strains in mixed microbial consortia for the decomposition of various pollutants is recognized (**Figure 1**). The percentage of such consortia is comparable to variants composed of yeast and bacterial cells (**Figure 1**). One example of the use of such an artificial consortium, as described in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**, is the heterologous expression of genes encoding MnP and LiP enzymes in non-ligninolytic fungi, which made it possible to complement the pathway of degradation of PAHs [3], without leaving toxic intermediates in the treated medium.

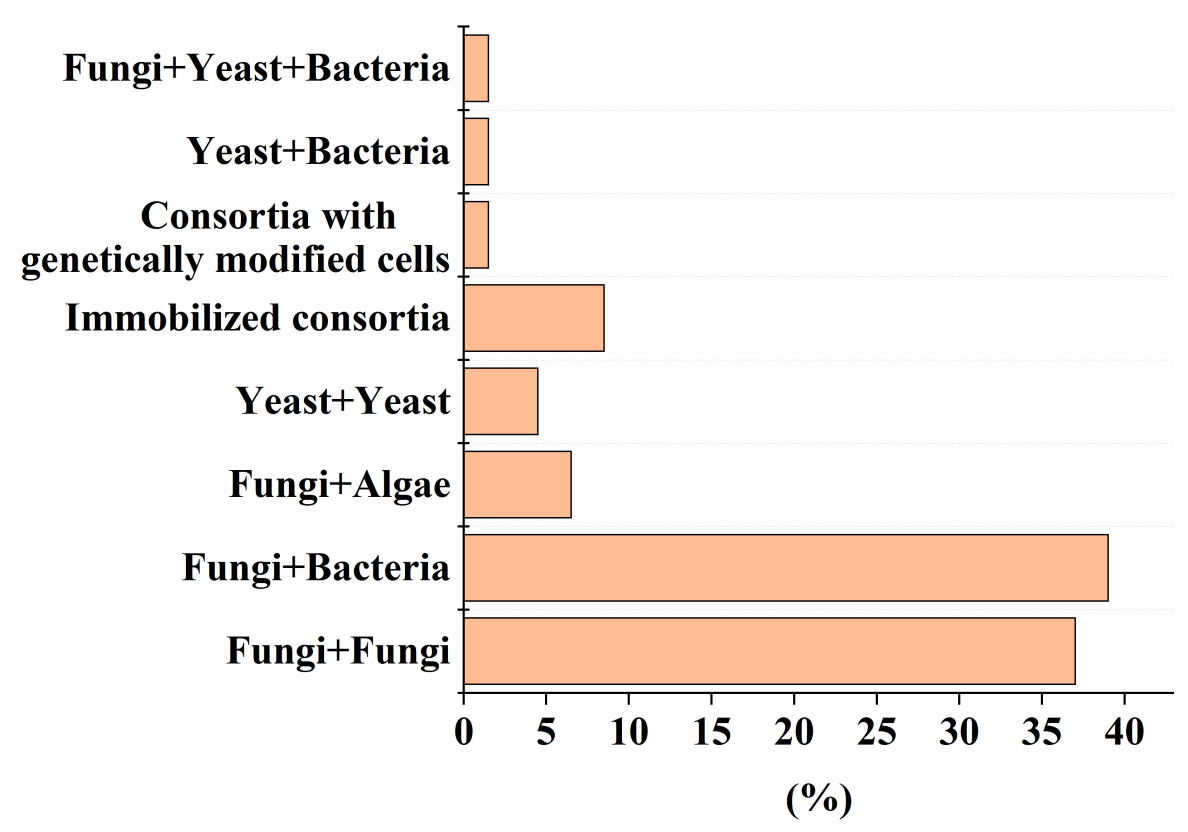

Figure 1. The percentage of artificial consortia of different composition of the total number (56) of reviewed studies, presented in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**.

Table 1. Microbial consortia with fungal species for removal of heavy metals.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Aspergillus niveus, *A. flavus*, *A. niger* [4]	1.4×10^6 spore/mL of each strain; pH 5.0, 110 rpm, 30 °C, 96 h	Removal of Cr, Zn, Pb, Cd and Ni—70–90%
A. flavus, *A. fumigatus* [5]	Heavy metal concentration—100 mg/L, 1.2×10^6 spores/mL, pH 5.0, 30 °C, 144 h	Removal of Cr(VI)—81%, Cd(II)—82%, mixture of metals—73%
Ascomycota and *Basidiomycota* fungi [6]	Initial metal concentration (23–2347 mg/kg), pH 7.9, soil moisture 60–65%, 28 °C, 100 days	Removal of As—77%, Cr—60%, Cu—52%, Fe—52%, Mn—71%
Ascomycota and *Basidiomycota* fungi [7]	Initial metal concentration (400–800 mg/kg), pH 7.9, soil moisture 60–65%, 28 °C, 100 days	Removal efficiencies of Ni, Pb, Zn—52%, 44%, 32% respectively
A. fumigatus, *A. terreus*, *Paenibacillus dendritiformis* [8]	Cd—100 mg/L, pH 5.0, 30 °C, 120 h	Removal of Cd(II)—95%
A. terreus, *Talaromyces islandicus*, *Neurospora crassa*, *Aspergillus flavus* [9]	Pb(II)—20.5–293.23 mg/L, Ni(II)—12.1–164.7 mg/L, inoculum 8%, pH 5.0, 30 °C 120 h	Removal of Pb(II) and Ni(II)—95–97%

Table 2. Microbial consortia with fungal species for decolorization of dyes.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Yarrowia sp., *Barnettozyma californica*, *Sterigmatomyces halophilus* [10]	100 mg/L of dye,30 °C, static conditions, 6–12 h	Degradation of Scarlet GR, Red HE3B, Remazol Brilliant Blue R, Methyl Orange, Rubine GFL and Reactive Red 2—92–100%
Daldinia concentrica, *Xylaria polymorpha* [11]	50 mg/L of dye, pH 4.5, 30 °C, 150 rpm, 48 h.	Degradation of cibacron brilliant red 3B-A—99%
Rhodotorula sp., *Raoultella planticola* and *Staphylococcus xylosus* cells immobilized in Ca-alginate beads [12]	200 mg/L of methylene blue in municipal wastewater and industrial effluent, 144 h	Degradation of methylene blue—100% and 78.5% in municipal wastewater and industrial effluent, respectively
A. niger, *A. terrus*, *A. oryzae*, *A. fumigatus* [13]	20 mg/L of each dye, 150 rpm, 28 °C, 72 h	Degradation of reactive blue 4, fast green, methyl red, crystal violet, alura red AC, tartrazine, naphthol blue black, janus green B, alizarin yellow R, evans blue, brilliant green, pararosaniline, ponceau S, cibacron brilliant red 3B-A, direct violet 51—57–100%
Aspergillus sp., *Chlorella sorokiniana* [14]	Disperse Red—0.1 g/L, pH 6.0, 160 rpm, 25 °C, 4 days	Degradation/adsorption of disperse red 3B—98.1%
Daedalea dickinsii, *Pseudomonas aeruginosa* [15]	Methyl orange—100 mg/L, 30 °C, 7 days	Degradation of methyl orange—98%
Sterigmatomyces halophilus, *Meyerozyma guilliermondii* [16]	Reactive Black 5, Acid Orange 7; Reactive Green 19, Reactive Yellow, ABC, Atlantic Black C—50 mg/L, glucose as co-substrate, pH 7.0, 35 °C, 120 h	Degradation—88–97%
Penicillium oxalicum, *Aspergillus tubingensis* [17]	100 mg/L of congo red with dextrose (10 g/L), pH 5, 150 rpm, 28 °C, 12 h	Congo red degradation—97.1%

Table 3. Microbial consortia with fungal species for degradation of synthetic polymers.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Sterigmatomyces halophilus, *Meyerozyma guilliermondii*, *M. caribbica* [18]	30 °C, 45 days	Low-density polyethylene (LDPE) mass reduction—33.2%

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
A. niger, *P. aeruginosa* [19]	37 °C, 30 days	Polyurethane weight loss—20%
Curvularia lunata, *Alternaria alternata*, *Penicillium simplicissimum*, *Fusarium* sp. [20]	90 days	Polyethylene weight loss—27%
A. niger, *A. flavus*, *A. oryzae* [21]	55 days	Polyethylene weight loss—26.2%
Microorganisms isolated from activated sludge and river sediments (*Lysinibacillus massiliensis*, *Bacillus licheniformis*, *B. indicus*, *B. megaterium*, *B. cereus*, *Pseudomonas alcaligenes*, *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., *Candida parapsilosis* [22]	160 rpm, 56 days at room temperature, 10 mL of bacterial and fungi suspension, and one film sample (1 cm^2) of polymer materials	Weight loss of sample (LDPE & thermoplastic starch & styrene-ethylene-styrene)—16%
Microorganisms isolated from compost (*B. sonorensis*, *B. subtilis*, *Aspergillus* sp. *Trichoderma* sp., *Rhizopus* sp.) [22]		Weight loss—21.9%
Microorganisms of enriched landfill soil (*Achromobacter xylosoxidans*, *Trichosporon chiropterorum*, *Penicillium chalabudae)* [23]	pH 7.2, 150 rpm, 30 °C, 90 days	LDPE weight loss—55.6%
Aspergillus sp., *Penicillium* sp. [24]	29 °C, 85% humidity, 30 days	Polypropylene/poly (butylene adipate-*co*-terephthalate)/thermoplastic starch weight loss—1.0–2.3%
Bacillus sp., *Aspergillus* sp. [25]	30 °C, 150 rpm, 30 days	LDPE weight loss—12%

Table 4. Microbial consortia with fungal species for degradation of pesticides.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Fomitopsis pinicola, *B. subtilis* [26]	30 °C, 7 days	DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane) degradation—86%
Pleurotus ostreatus, *P. aeruginosa* [27]	25 °C, 7 days	DDT degradation—86%
A. niger, *Chlorella vulgaris* [28]	38 pesticides in mixture—total concentration—72.7µg/L, biomass—181.6 mg dry weight/L, pH 4.0, 100 rpm, 68 h	Degradation—23%
Verticilium sp., *Metacordyceps* sp. [29]	Concentration of each pesticide—50 mg/L, 100 rpm, pH 5.5, 27 °C, 21 days	Degradation of atrazine—81%, iprodione—96%; chlorpyrifos—99%
Verticilium sp., *Metacordyceps* sp. immobilized in Ca-alginate beads [29]	Concentration of each pesticide—50 mg/L, flow rate—90 mL/h, inoculum concentration—30 *w/v*, 100 rpm, 27 °C	Degradation of atrazine—64%, iprodione—96%; chlorpyrifos—85% (11–15 days)
Consortium of microorganisms present in coconut fiber, garden compost and agricultural soil and *Trametes versicolor* [30]	Mixture of pesticides—30–40 mg/kg, pH 6.4, 25 °C, 16 days	Degradation of atrazine—72.2%, carbendazim—96.7%, carbofuran—98.7%, metalaxyl—96.7%
Fomitopsis pinicola, *Ralstonia pickettii* [31]	DDT—5 mM, 30 °C, 7 days	DDT degradation—61%

Table 5. Microbial consortia with fungal species for degradation of PAHs.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Acremonium sp, *B. subtilis* [32]	Concentration of each PAH in mixture—50 mg/L, 28 °C, 160 rpm, 10 days	Degradation of naphthalene—100%, fluorine—89%, phenanthrene—82%, anthracene—71%, fluoranthene—61%
Pleurotus ostreatus, *Penicillium chrysogenum* [33]	30 °C, 30 days	Degradation of benzo[a]pyrene—86%
P. ostreatus, *P. aeruginosa* [33]		Degradation of benzo[a]pyrene—75%

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Consortium (*Proteobacteria*, *Bacteroidota*, *Fusarium*) immobilized on biochar [34]	Mixture of 50 mg/L of phenanthrene and 150 mg/L of Cd^{2+}, 150 rpm, 30 °C, 7 days	Degradation of phenanthrene—92–98%, removing of Cd^{2+}—94–99%
Consortium with two genetically modified strains of *A. niger* [3]	Mixture of pyrene and benzo(a)pyrene—1000 mg/kg soil, pH of 8.4, 30 °C, 14 days	Degradation efficiency of phenanthrene—92%, pyrene—64%, benzo(a)pyrene—65%
Kocuria rosea and *A. sydowii* immobilized in guargum-nanobentonite composite water dispersible granules [35]	Mixture of naphthalene, fluorene, phenanthrene, anthracene, and pyrene—100 µg of each PHA/g of soil, pH 8.3, 27 °C, 30 days	Degradation efficiency—85–100%
P. putida, yeast *Basidioascus persicus* [36]	800 mg/L of pyrene, rhamnolipid biosurfactant 100 µL, 28 °C, 21 days	Degradation efficiency—78%
Ochrobactrum intermedium and white rot fungus *Pleurotus ostreatus* [37]	Concentrations of different PAH—138.2–268.0 mg/kg of soil, moisture—70%, 30 °C, 110 days	Degradation of fluoranthene, indene[1,2,3-cd]pyrene and benzo[g,h,i]perylene—100%; Anthracene, pyrene, chrysene and benzo[a]anthracene—96%, 86%, 98% and 98%, respectively
Pleurotus ostreatus, *Azospirillum brasilense* [38]	A mixture of anthracene, phenanthrene, fluorene, pyrene, and fluoranthene—50 mg/L, 130 rpm, 24 °C, 14 days	Degradation efficiency > 70%

Table 6. Microbial consortia with fungal species for degradation of pharmaceutical pollutants.

Consortia [Reference]	Conditions	Pollutant/Process Efficiency
Pycnoporus sanguineus, *Phanerochaete chrysosporium* [39]	Each antibiotic concentration—10 mg/L, biomass of each strain—0.15 g dry weight/L), pH 4.5, 30 °C, 4 days	Removal efficiency of ciprofloxacin, norfloxacin and sulfamethoxazole in their mixture—100%
Pycnoporus sanguineus, *Alcaligenes faecalis* [40]	Sulfamethoxazole (50 mg/L) and vitamins mixture (VB2, VB6, VB12 and VC), 28 °C, 120 rpm, 24 h	Sulfamethoxazole degradation—93%
Ganoderma applanatum, *Laetiporus sulphureus* [41]	Concentration of each of three pollutants—10 mg/L, pH 6.4, ambient temperature, 150 rpm, 72 h	Degradation (mixture of celecoxib, diclofenac and ibuprofen)—99.5%
A. niger, *Mucor circinelloides*, *Trichoderma longibrachiatum*, *Trametes polyzona* and *Rhizopus microsporus* [42]	Pollutants concentration—1 mg/L, pH 4.6, 30 °C, 7 days, consortium concentration—30% (*v*/*v*)	Degradation of carbamazepine—90%, diclofenac sodium—96% and ibuprofen—91%
A. niger, *C. vulgaris* [43]	Pharmaceutical substances—8–11 µg/L, microalgae-fungus biomass—75 mg dry weight/L, 72 h	Relative removal of initial ranitidine concentrations—50%
Penicillium rastrickii, *P. oxalicum*, *Cladosporium cladosporoides*, *Micrococcus yunnanensis*, *Oligella ureolytica*, *Sphingobacterium jejuense* [44]	Mixture of diclofenac, carbamazepine and ketoprofen with 100 µM of each compound, 28 °C, 10 days	Degradation of diclofenac—99%, ketoprofen—80%
Chlorella vulgaris, *Aspergillus oryzae* [45]	Simulated swine wastewater with addition of 0.1–0.5 mg/L Cu (II), 0.4 mg/L of mixture of antimicrobial agents, pH 7.2, 28 °C, 14 days	Removal efficiency of sulfamonomethoxine, sulfamethoxazole and sulfamethazine—58.8%, 63.5%, and 63.9%, respectively

Table 7. Microbial consortia with fungal species for degradation of various pollutants not mentioned in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5** and **Table 6**.

Consortia [Reference]	* Conditions	Pollutant/Process Efficiency
Acinetobacter baumannii, *Talaromyces* sp. [46]	The initial concentration of petroleum in soil—1220 mg/kg, pH 8.3, 30 °C, 28 days	Degradation of petroleum—65.6%
Paraburkholderia sp., *Paraburkholderia tropica*, *Scedosporium boydii* [47]	1% *v*/*v* crude oil, 120 rpm, 30 °C, 7 days	Degradation of crude oil—81.5%

Consortia [Reference]	* Conditions	Pollutant/Process Efficiency
Scedosporium sp., *Acinetobacter* sp. [48]	Crude oil—200 mg/L, pH 7.0, 150 rpm, 30 °C, 7 days	Crude oil degradation—58.6%
Micrococcus luteus, *Rhodococcus equi*, *A. niger* [49]	Greywater—COD—1165.6 mg/L, oil and grease—58 mg/L, sulphate—95.6 mg/L, pH 7, 35 °C, 96 h	Degradation of COD, oil and grease and sulphate were 78.7, 82.6 and 89.7%, respectively
Aspergillus versicolor and bacterial species (*Pseudomonas*, *Klebsiella* species, *B. subtilis*) [50]	Greywater with 100 µg/L of carbendazim and thiamethoxam, 80 rpm, 30 °C, 240 h	Degradation of carbendazim and thiamethoxam 94.4 and 93.6%, respectively
A. flavus, *Fusarium oxysporium* [51]	Real textile effluent pH 8.7, COD—611 mg/L, pH 6.0–8.0, 28 °C, 7 days	Degradation—78.1%, COD removal—77.6%
Consortium of *Brevibacillus laterosporus* and *Galactomyces geotrichum* immobilized into Ca-alginate or polyvinyl alcohol-alginate beads [52]	Textile industry effluent pH 8.8, COD—2400 mg/L, 48–60 h	Degradation during 5 repeated cycles—76–95%
Ralstonia pickettii, *Trichoderma viride* [53]	Chlorobenzene—220 mg/L, 160 rpm, 28 °C, 60 h	Chlorobenzene degradation—100%
Chaetomium globosum, *A. niger*, *Rhizopus oryzae* [54]	Poly(vinyl acetate) processing wastewater pH 7.1, COD—23.48 g/L; pH 5.5, 150 rpm, 28 °C, 10 days	COD, poly(vinyl acetate) and color removal yields—97.8%, 98.5% and 99.8%, respectively.
Phanerochaete chrysosporium, *Delftia lacustris* [55]	Phenol (1000 mg/L) and selenite concentration—10 mg/L, 180 rpm, pH 6.5, 30 °C, 120 h	Phenol degradation—97.8% with the simultaneous reduction of selenite to Se(0)

* COD—chemical oxygen demand.

However, the traditional method of random mutagenesis is a time-consuming approach, and searching for key amino acid mutations is extremely complex and requires operating across a large space. With the help of computer modeling, it is possible to “calculate” enzymes with improved binding affinity and greater specificity of action in relation to the substrates destruction, which are both necessary [56]; it is also necessary to successfully introduce producers of “improved” enzymes into consortia, which is another serious task that confronts researchers.

Another difficulty is that genetically modified strains often have to survive high concentrations of pollutants [2][57]. Several strategies have been used to improve cellular tolerance, including changing the composition of membrane lipids, phenotypic screening through adaptive laboratory evolution, modification of global gene expression, genome shuffling, directional evolution, and others.

Although genetically modified strains are effective in bioremediation processes [58], their use is limited to laboratory studies due to minimizing the risks of environmental impact. Since there are no globally accepted regulatory documents that concern the spread of genetically modified organisms, the regulation of the development and release of genetically modified organisms varies in different countries, depending on the purposes of their use, extending from a complete ban on their import, release or use to allowing their use, subject to varying degrees of regulation. However, despite this, methods of genetically engineering filamentous fungi continue to be actively developed and used in research around the world, making it possible to overcome many of the shortcomings of classical methods for improving strains [57][59][60]. To eliminate the negative effect of such cells, it is possible to use several genetic tools, including cell self-destruction systems [61]. Such approaches to realization of programmed cell death in a certain period of their functioning can be used and activated after the completion of bioremediation or after accumulation of certain concentrations of the cells.

2. Role of Composition in Artificial Consortia with Fungal Cells

Microbial consortia have a high self-organization, which allows them to carry out the catalytic conversion of substrates with high efficiency, providing an intensive exchange of metabolites. Moreover, in consortia, cells have higher adaptability and viability in relation to environmental factors (pH, temperature, concentration of pollutants, etc.) [62]. Despite this, most artificial microbial consortia face problems of non-sustainable functioning [63]. Even minor fluctuations in the composition and activities of consortia can have a negative impact on the effectiveness of the processes taking place with their participation. The difficulty of managing the bioremediation characteristics of artificial consortia is actually determined by the qualitative (**Figure 1**) and quantitative composition (**Figure 2**) of the participants in the artificial biosystems being formed.

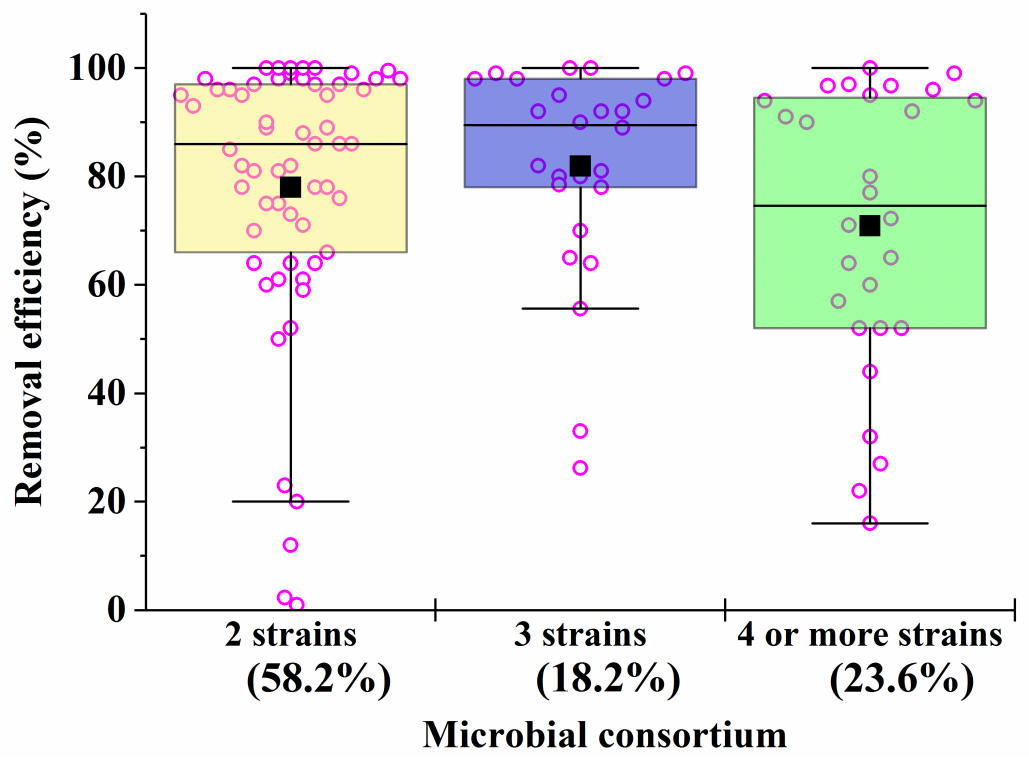

Figure 2. The average efficiency of degradation of pollutants by consortia with two, three, four and more strains in the composition. The percentage of the total number of all options presented in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7** (56 studies reviewed) was calculated. Each point corresponds to the research results listed in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**.

As the analysis of the data in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7** shows that, despite the diversity of the consortia being developed (**Figure 1**), the ones most widely used to create effective bioremediation microbial systems, that have demonstrated their advantages over natural systems, consist of two or three microorganisms, of which one is a fungal culture (**Figure 2**). The maximum part of all artificial consortia currently being developed combines mycelial fungi with each other and with cells of different bacteria (**Figure 1**).

It is known that fungi and bacteria are characterized by different rates of synthesis of enzymes that are necessary for the catalysis of different processes in the bioremediation of pollutants. Consequently, the rates of different processes may be compared by varying the biomass of certain cells introduced into heterogeneous consortia in order to ensure their maximum effectiveness of action. This is the basis for the unification of certain microorganisms into artificial consortia (**Figure 3**).

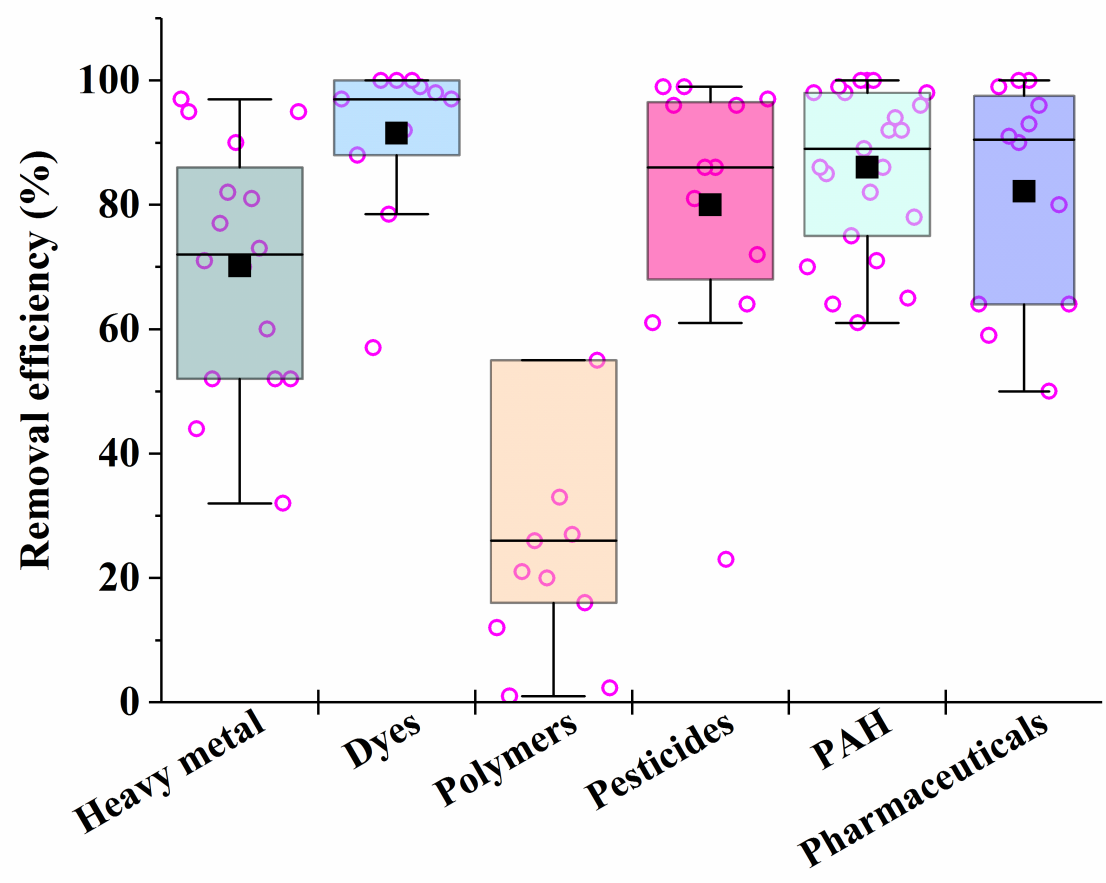

Figure 3. The average efficiency of the consortia for elimination of different types of pollutants, calculated on the basis of sources performed in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**. Each point corresponds to the research results listed in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**. The six differently colored squares correspond to the six pollutants shown on the abscissa axis.

After analyzing the data in **Table 1**, **Table 2**, **Table 3**, **Table 4**, **Table 5**, **Table 6** and **Table 7**, it is possible to conclude that there are consortia consisting of two–three strains of various fungi, mainly mycelial types, that are most effective in removing 70–90% of heavy metals in mixtures from various media.

For the removal of dyes, the most effective are the consortia consisting of yeast cells, which provide bioremediation efficiency of up to 100%; the destruction of pesticides is the most successful under the action of consortia consisting of fungal cultures, such as *Verticilium* sp. and *Metacordyceps* sp.

Synthetic polymers undergo the most difficult microbial degradation, but the maximum weight loss of synthetic polymers (microplastics) is achieved under the action of consortia that combine bacteria, mycelial fungi or yeast.

In the case of the presence of mixtures of several PAHs in media subjected to bioremediation, the most effective (in terms of the degree of degradation of the pollutant (86–100%)) were consortia that included bacteria and white rot fungus *Pleurotus ostreatus*, as well as an immobilized consortium consisting of the bacteria *Kocuria rosea* and the fungi *Aspergillus sydowii*.

Among the most effective in the removal of pharmaceutical pollutants were selected consortia from cells of white rot saprobic fungus *Pycnoporus sanguineus* and *Phanerochaete chrysosporium* (the removal efficiency of ciprofloxacin-, norfloxacin and sulfamethoxazole in their mixture was 100%), as well as tinder fungi *Ganoderma applanatum* and *Laetiporus sulphureus* (efficiency of degradation of a mixture containing celecoxib, diclofenac and ibuprofen was 99.5%).

Bacterial and fungal consortia proved to be the most effective for the treatment of real wastewater from industrial enterprises and oil pollution. The fungal consortium consisting of *Phanerochaete chrysosporium* and *Delftia lacustris* can be used to remove phenol, with an efficiency of over 90%.

Of course, in order to achieve the effective functioning of synthetic microbial consortia, it is important to predict the possible types of interactions of all microorganisms involved in the functioning of artificially created biosystems [64][65].

Most often, these interactions are based on mutualism or competition [66][67]. Mutualism suggests that jointly cultivated microorganisms have a beneficial effect on each other, while the composition of artificial consortia is stabilized, but only at a certain cell density [55][68].

In case of competition for a substrate, consortium members can release metabolites into the environment that negatively affect other consortium members (organic acids, mycotoxins, antibiotics, antimicrobial peptides, enzymes) [69][70][71][72][73]. As a rule, these processes are regulated by cell Quorum Sensing (QS) [74]. QS molecules and the conditions of their formation can be used to control or regulate the expression of certain genes, control the composition of consortia, and ensure intercellular connections between certain consortium members [63][68][75]. In considering this, modern studies of artificial consortia should enable the creation of model systems for the accumulation of toxic metabolites of a number of microorganisms (fungi, microalgae, bacteria) and develop effective ways to detoxify them.

Cell immobilization can also solve the instability problems of microbial consortia, since cellular communication can be more active in a confined space and positively affects the speed of bioremediation processes. At the same time, the inclusion of cells in gel matrices [13][29][52] simulates the development of a stabilized state of cells and the additional formation of biofilms [37][47][55][58][68].

Immobilized fungal consortia can be used in non-sterile conditions at a separate stage, and then be integrated into conventional waste treatment systems before the action of aerobic sludge at water processing stations [76][77][78]. In some cases, the self-stabilization of the artificial fungi-containing consortia is sufficient for their use in non-immobilized form in laboratory conditions to treat various real contaminated industrial and environmental water and soil samples [4][9][10][12][51][52][54][79]. While such studies bring the introduction of fungal consortia closer to practice, there are not many known examples of pilot or industrial tests of artificial consortia [80][81][82]. Some of them follow:

- microbial consortium containing *T. versicolor*, *P. ostreatus*, *Phanerochaete* sp., *Pseudomonas fluorescens* and *B. subtilis* cells was applied for the treatment of non-domestic wastewater. This fungal/bacterial consortium was prepared by mixing fungal biomass pellets with suspensions of bacterial cells. The removal of colored substances (2700 Color $Units_{550nm}$), COD (1.75 g/L) and nitrate (3 mg/L) was 91 ± 2%, 90 ± 4% and 17 ± 2%, respectively, after 15 days of water treatment at a pilot plant [80];

- consortium of *A. niger*, *Mucor hiemalis* and *Galactomyces geotrichum*, has been tested for the treatment of real wastewater from industry at a pilot scale station (110 L) and industrial wastewater treatment plant (1000 L). The efficiency of COD removal in the industrial reactor was 50% under the influence of this consortium [81];

- consortium containing *Acinetobacter oleivorans*, *Corynebacterium* sp., *Pseudomonas* sp, *Rhodococcus* sp., *Micrococcus* sp. and yeast *Yarrowia* sp. was tested by Ecophile Co., Ltd. (Korea) in the biodegradation of hydrocarbons in soil (2300 mg/kg) contaminated with diesel fuel. This large-scale experiment involved two samples of 100 metric tons of contaminated soil, both without (control) and with consortium treatment (10^9 cells/kg of soil). The introduction of consortium reduced pollution by 57.7% within 2 weeks, whereas in the control (without the consortium), degradation was only 10.1% [82].

Thus, such positive samples of scaling use of artificial fungal consortia not only demonstrate their real-world efficacy, but also addresses potential solutions encountered during practical applications.

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