RĪGAS TEHNISKĀ
UNIVERSITĀTE

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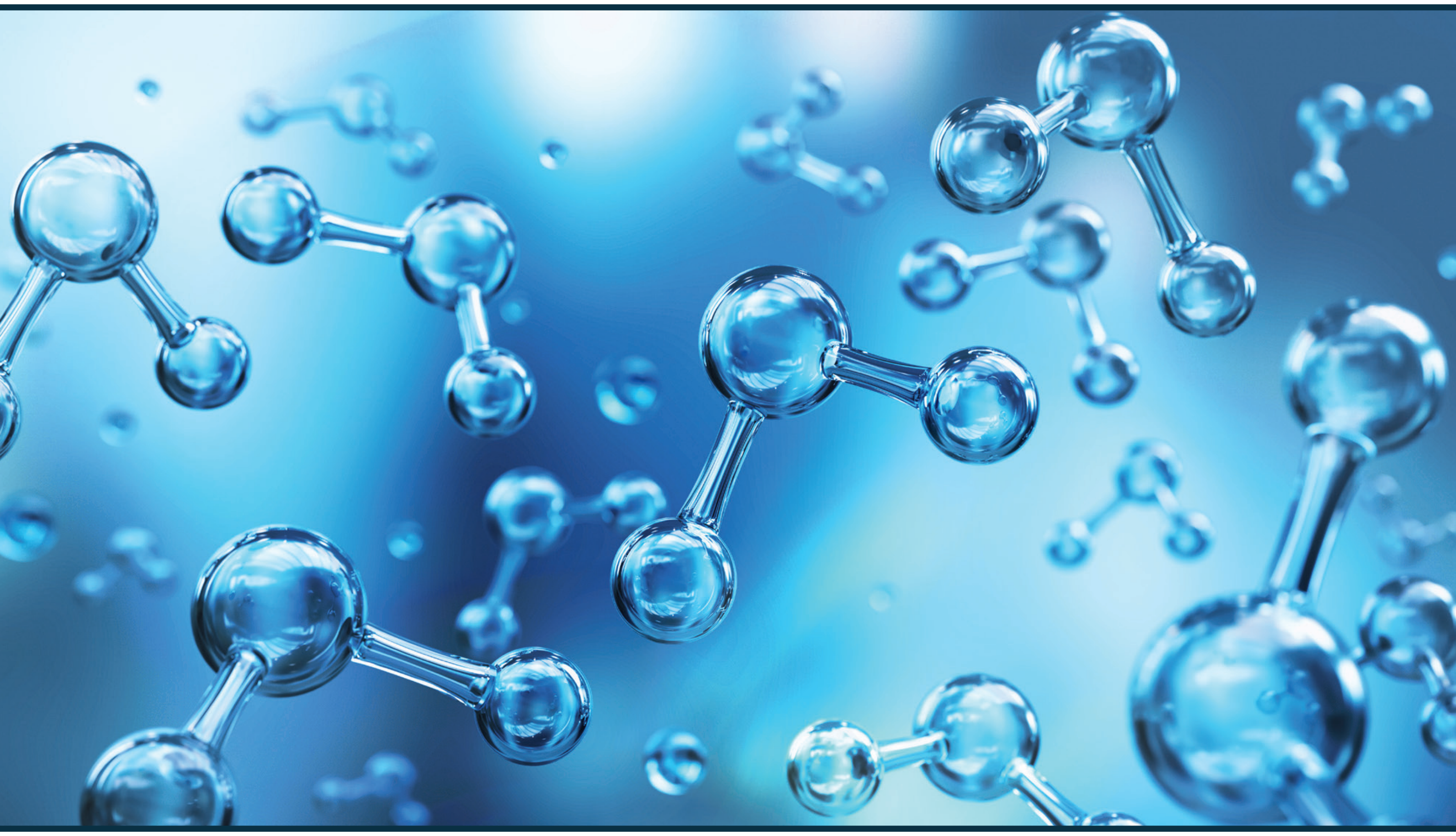
FARMACEITISKĀS RAŽOŠANAS ŪDEŅU BIOLOĢISKĀS ATTĪRĪŠANAS PROCESA OPTIMIZĒŠANA MULTISTRESA APSTĀKĻOS

Promocijas darbs

OPTIMIZATION OF THE BIOLOGICAL TREATMENT OF PHARMACEUTICAL PROCESSING WATERS UNDER MULTI-STRESS CONDITIONS

Doctoral Thesis



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Doctoral Thesis

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Apstiprinu, ka esmu izstrādājusi šo promocijas darbu, kas iesniegts izskatīšanai Rīgas Tehniskajā universitātē zinātnes doktora (*Ph. D.*) grāda iegūšanai. Promocijas darbs zinātniskā grāda iegūšanai nav iesniegts nevienā citā universitātē.

Elīna Strade ……………………………. (paraksts)
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Promocijas darbs sagatavots kā tematiski vienotu zinātnisko publikāciju kopa ar kopsavilkumu latviešu un angļu valodā. Tas ietver piecas zinātniskās publikācijas. Publikācijas zinātniskajos žurnālos uzrakstītas angļu valodā, to kopējais apjoms ir 65 lpp.

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2. pielikums: Neibergs, M., Strade, E., Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *Key Eng. Mater.* **2019**, 800, 122–131.
3. pielikums: Rozitis, D., Strade, E. COD Reduction Ability of Microorganisms Isolated from Highly Loaded Pharmaceutical Wastewater Pre-Treatment Process. *J. Mater. Environ. Sci.* **2015**, 6, 507–512.
4. pielikums: Smol, M., Preisner, M., Bianchini, A., Rossi, J., Hermann, L., Schaaf, T., Kruopienė, J., Pamakštys, K., Klavins, M., Ozola-Davidane, R., Kalnina, D., Strade, E., Voronova, V., Pachel, K., Yang, X., Steenari, B.-M., Svanström, M. Strategies for Sustainable and Circular Management of Phosphorus in the Baltic Sea Region: The Holistic Approach of the InPhos Project. *Sustainability* **2020**, 12, 2567.
5. pielikums: Strade, E., Kalnina, D., Kulczycka, J. Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry. *Water Resour. Ind.* **2020**, 24, 100132.

6. pielikums: Promocijas darbā pētīto farmācijas NAI raksturojums (pieejams pēc pieprasījuma). A: latviski, B: angliski.

SAĪSINĀJUMI

AEŠH-MS	augsti efektīvā šķidruma hromatogrāfija – masspektrometrija
AFV	aktīvā farmaceitiskā viela
BSP	bioķīmiskais skābekļa patēriņš
ES	Eiropas Savienība
GNAI	AS “Grindeks” notekūdeņu attīrīšanas iekārtas
GZF	gatavā zāļu forma
H	Šenona indekss
I. U.	starptautiskās vienības
KOO	kopējais organiskais ogleklis
KVV	koloniju veidojošās vienības
ĶSP	ķīmiskais skābekļa patēriņš
log K_{ow}	oktanola ūdens sadalījuma koeficienta logaritmiskā vērtība
NAI	notekūdeņu attīrīšanas iekārtas
N_{kop}	kopējais slāpeklis
P_{kop}	kopējais fosfors
PP	vājpiena pulveris
PVO	Pasaules Veselības organizācija
Q	plūsma, m^3/h

PROMOCIJAS DARBA VISPĀRĒJS RAKSTUROJUMS

Tēmas aktualitāte

Kā liecina Pasaules Veselības organizācijas (PVO) dati, ūdens ir visvairāk izmantotais resurss farmaceitiskajā rūpniecībā [1]. To lieto gan kā izejvielu un šķīdinātāju aktīvo farmaceitisko vielu (AFV) sintēzē un gatavo zāļu formu (GZF) ražošanā, gan iekārtu mazgāšanā, kā arī tehniskiem mērķiem – dzesēšanai un tvaika ražošanai. Ūdens daudzveidīgais lietojums liek farmaceitiskajām rūpnīcām efektīvi pārvaldīt gan ieejošās, gan izejošās ūdens plūsmas ražotnē un izmantot atšķirīgus attīrīšanas risinājumus ūdens sagatavošanā, lai garantētu tā kvalitātes atbilstību reglamentētajām prasībām.

Farmaceitiskās ražošanas tehnoloģiskajos procesos rodas ķīmiski piesārņotas ražošanas ūdeņu plūsmas, kas būtiski atšķiras pēc ķīmiskā sastāva, apjoma un toksicitātes [2]–[4]. Atkarībā no specifiskā AFV sintēzes procesa tās var būt piesārņotas ar dažādiem organiskajiem šķīdinātājiem, AFV atliekām, slāpekli saturošiem heterocikliskiem savienojumiem, kā arī dažādiem neorganiskiem sāļiem [2].

Augstās piesārņojuma koncentrācijas un plūsmas fluktuācijas un daudzveidīgais ražošanas ūdeņu piesārņojuma sastāvs rada multistresa apstākļus un var izraisīt bioloģisko notekūdeņu attīrīšanas iekārtu (NAI) darbības traucējumus, negatīvi ietekmējot to spēju nodrošināt likumdošanas aktu prasībām atbilstošu notekūdeņu attīrīšanas pakāpi. Lai arī bioloģiskā attīrīšana ir viena no visbiežāk izmantotajām tehnoloģijām farmācijas notekūdeņu attīrīšanā [3], aizvien vairāk pētījumos uzsvērts, ka bioloģiskās NAI nepietiekami attīra notekūdeņus no AFV atliekām un kaitīgiem savienojumiem, tādēļ, lai panāktu augstu attīrīšanas efektivitāti, tā ir jākombinē ar fizikāli ķīmiskajām attīrīšanas tehnoloģijām [4]–[6]. Bet arī šādai pieejai ir savi ierobežojumi, jo, piemēram, ozonējot halogēnus saturošus ražošanas ūdeņus, toksicitāte var pieaugt [7]. Alternatīvs risinājums ražošanas ūdeņu bioloģiskās attīrīšanas efektivitātes paaugstināšanai var būt aktīvo dūņu sistēmu bioaugmentācija ar mikroorganismu kultūrām [8], kas savukārt prasa veikt priekšizpēti, nosakot bioaugmentācijai piemērotākās kultūras. Papildu izaicinājumus farmācijas rūpniecībā rada mainīgais pieprasījums pēc dažādām AFV, kas liek ātri pārorientēt ražošanu un prognozēt, kā izmaiņas ražošanā ietekmēs NAI spēju attīrīt jaunā veida piesārņojumu. Līdz ar to jaunu risinājumu, ar kuru palīdzību var prognozēt un preventīvi novērst vai mazināt dažādu stresa faktoru ietekmi uz bioloģiskās attīrīšanas procesu un uzlabot attīrīšanas pakāpi, izstrāde un ieviešana ir ļoti aktuāla.

Atšķirīgais AFV sintēzēs izmantoto izejvielu klāsts un tehnoloģisko procesu specifika nosaka, ka farmaceitiskās ražošanas ūdeņu piesārņojuma pakāpe, biogēno elementu un sāļu saturs dažādās ražotnēs var būtiski atšķirties. Savukārt atšķirīgie bioloģiskajās NAI lietotie inženiertehniskie risinājumi var dažādi ietekmēt sistēmas noturību pret dažādiem stresa faktoriem un izmaiņām notekūdeņu sastāvā [9]. Tas prasa izmantot individuālu pieeju arī bioloģisko attīrīšanu ietekmējošo stresa faktoru izvērtēšanā un piemērotāko procesu optimizācijas risinājumu izstrādē.

Pāreja uz aprites ekonomikas modeli notekūdeņu attīrīšanas jomā liek fokusēties ne tikai uz rūpnieciskā piesārņojuma samazināšanu un attīrīšanu, bet arī ūdens un ķīmisko vielu atgūšanu un otrreizēju izmantošanu [10]. Neraugoties uz to, ka ūdens atkārtota izmantošana ir atzīta kā prioritārā darbība, lai samazinātu ūdens patēriņu ražotnēs un veicinātu ilgtspējīgas attīstības mērķu sasniegšanu [11], [12], trūkst pētījumu, kuros, izmantojot visaptverošu pieeju, būtu analizēti aprites ekonomikas ieviešanas aspekti farmaceitiskajā ražotnē, vērtējot tos kontekstā ar specifiskajām farmācijas nozarei noteiktajām ūdens kvalitātes prasībām, līdz ar to tas ir aktuāls pētījumu virziens.

Pētījuma mērķi un uzdevumi

Promocijas darba mērķi ir:

1) izstrādāt risinājumus farmaceitiskās ražošanas ūdeņu bioloģiskās attīrīšanas efektivitātes paaugstināšanai multistresa apstākļos;

2) izvērtējot ūdens izmantošanu farmaceitiskajā ražotnē un labas ražošanas prakses vadlīnijās, farmakopejās un Eiropas Medicīnas aģentūras un Pasaules Veselības organizācijas vadlīnijās noteiktās farmaceitiskajā ražošanā izmantojamā ūdens kvalitātes prasības, identificēt ražošanas ūdeņu atkārtotas izmantošanas iespējas un ierobežojumus farmācijas rūpniecībā.

Mērķa sasniegšanai noteikti šādi darba uzdevumi:

1) balstoties AS "Grindeks" NAI (GNAI) darbības analīzē trīs gadu laikā, identificēt galvenos farmaceitiskās ražošanas ūdeņu bioloģisko attīrīšanu ietekmējošos stresa faktorus;

2) izstrādāt metodi ķīmiski piesārņotu ražošanas ūdeņu toksicitātes uz aktīvo dūņu mikroorganismiem novērtēšanai;

3) laboratorijas apstākļos noteikt bioaugmentācijai piemērotākās mikroorganismu kultūras AFV un ķīmiskā skābekļa patēriņa (ĶSP) degradācijas efektivitātes paaugstināšanai bioloģiskajās NAI, kas pakļautas multistresa apstākļiem;

4) rekomendēt alternatīvos P avotus optimālā barības vielu līmeņa nodrošināšanai farmācijas notekūdeņu bioloģiskās attīrīšanas procesā, lai mazinātu atkarību no komerciālās H_3PO_4.

Zinātniskā novitāte un galvenie rezultāti

Promocijas darba ietvaros izstrādāti risinājumi multistresa ietekmes mazināšanai farmācijas NAI, kas balstās uz individuālu plūsmu toksicitātes analīzi, bioaugmentācijas stratēģijas pielietojumu AFV un ĶSP degradācijas efektivitātes paaugstināšanai, un ietver arī priekšlikumus ķīmiski piesārņotu farmaceitiskās ražošanas ūdeņu plūsmu otrreizējai izmantošanai atbilstoši aprites ekonomikas principiem.

Promocijas darbā izstrādāta jauna metode ķīmiski piesārņotu notekūdeņu toksicitātes uz aktīvo dūņu biocenozi novērtēšanai, kas ieviesta GNAI un ir adaptējama arī citu ķīmiski piesārņotu rūpniecisko notekūdeņu bioloģisko NAI laboratoriju darbā, īpaši ražotnēs, kas rada mainīga ķīmiskā sastāva notekūdeņu plūsmas, kas satur potenciāli toksiskas ķīmiskās vielas.

Veiktā pētījuma rezultātā pirmo reizi identificēti farmaceitiskās ražošanas ūdeņu atkārtotas izmantošanas ierobežojumi farmācijas ražotnē, akcentējot farmaceitiskās rūpniecības īpašo lomu ūdensietilpīgo rūpniecības nozaru vidū. Darbā piedāvātie un praksē ieviestie risinājumi ražošanas ūdeņu otrreizējai izmantošanai kā ķīmiskajām vielām dažādās farmācijas notekūdeņu bioloģiskā attīrīšanas procesa stadijās parāda jaunu pieeju, kā veicināt cirkulāru materiālu plūsmu ražotnē, tādējādi mazinot ķīmisko vielu patēriņu un notekūdeņu attīrīšanas izmaksas.

Bioaugmentācijai piemērotu mikroorganismu kultūru atlase dod iespēju tālākiem NAI darbības optimizēšanas pasākumiem bioaugmentācijas ceļā, lai uzlabotu ķīmiskā piesārņojuma – īpaši AFV degradēšanas – efektivitāti. Tas var būt īpaši aktuāli, ieviešot jaunas normatīvo aktu prasības AFV emisiju kontrolē no farmācijas ražotnēm, ko pamato Eiropas Savienības (ES) stratēģiskā pieeja attiecībā uz farmaceitiskajām vielām vidē [13], un palielinot farmaceitisko ražotāju atbildību par AFV piesārņojuma, kas rodas no to produktu lietošanas, attīrīšanu kontekstā ar plānotajām izmaiņā Direktīvā 91/271/EEK par komunālo notekūdeņu attīrīšanu [14].

Darba struktūra un apjoms

Promocijas darbs sagatavots kā tematiski vienota zinātnisko publikāciju kopa par: a) ķīmiski piesārņotu farmaceitiskās ražošanas ūdeņu toksicitātes uz aktīvo dūņu biocenozi metodes izstrādi (**1. publikācija**); b) bioaugmentācijas stratēģijas lietojumu AFV attīrīšanas efektivitātes paaugstināšanai bioloģiskajās NAI (**2. publikācija**); c) ĶSP attīrīšanas efektivitātes paaugstināšanas iespējām, izmantojot bioaugmentāciju ar selektīvām mikroorganismu kultūrām (**3. publikācija**); d) aprites ekonomikas principos balstītu fosfora pārvaldību (**4. publikācija**); e) ražošanas ūdeņu atkārtotas izmantošanas iespējām un ierobežojumiem farmācijas rūpniecībā (**5. publikācija**).

Darba aprobācija un publikācijas

Promocijas darba galvenie rezultāti izklāstīti piecās zinātniskajās publikācijās. Pētījuma rezultāti prezentēti četrās zinātniskajās konferencēs.

Zinātniskās publikācijas

1. **Strade E.,** Kalnina, D. Cost Effective Method for Toxicity Screening of Pharmaceutical Wastewater Containing Inorganic Salts and Harmful Organic Compounds. *Environ. Clim. Technol.* **2019**, *23*, 52–63.
2. Neibergs, M., **Strade, E.**, Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *Key Eng. Mater.* **2019**, *800*, 122–131.
3. Rozitis, D., **Strade, E.** COD Reduction Ability of Microorganisms Isolated from Highly Loaded Pharmaceutical Wastewater Pre-Treatment Process. *J. Mater. Environ. Sci.* **2015**, 6, 507–512.
4. Smol, M., Preisner, M., Bianchini, A., Rossi, J., Hermann, L., Schaaf, T., Kruopienė, J., Pamakštys, K., Klavins, M., Ozola-Davidane, R., Kalnina, D., **Strade, E.**, Voronova, V., Pachel, K., Yang, X., Steenari, B.-M., Svanström, M. Strategies for Sustainable and Circular

Management of Phosphorus in the Baltic Sea Region: The Holistic Approach of the InPhos Project. *Sustainability* **2020**, *12*, 2567.

5. **Strade, E.**, Kalnina, D., Kulczycka, J. Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry. *Water Resour. Ind.* **2020**, *24*, 100132.

Publikācija kolektīvā monogrāfijā

Olsson, L.E., **Strade, E**., Ekenberg, E., Torresi, E, Quadri, L., Morgan-Sagastume F. Il Sistema MBBR per il trattamento degli scarichi da industrie farmaceutiche: aspetti tecnici ed esperienze gestionali. In *La gestione degli impianti di depurazione MBBR*; Vaccari, M., Favali, G., Eds.; Maggioli Editore: Santarcangelo di Romagna, **2021**, pp. 172–183 (italian).

Dalība zinātniskajās konferencēs

1. Neibergs, M., **Strade, E.**, Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *59th International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry*. Riga, Latvia, 26 October, **2018**.
2. **Strade, E**. The pollution of water with pharmaceutical residues: a growing environmental concern. *60th International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry.* Riga, Latvia, 24 October, **2019**.
3. **Strade, E**. Biological nitrogen removal from pharmaceutical wastewater. *61th International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry*. Riga, Latvia, 23 October, **2020**.
4. **Strade, E**., Kalnina, D., Kulczycka, J. Water Diversity and Problems in Water Re-use in Pharmaceutical Enterprises. *12th Eastern European Young Water Professionals Conference*. Riga, Latvia, 1–2 April, **2021**.

PROMOCIJAS DARBA GALVENIE REZULTĀTI

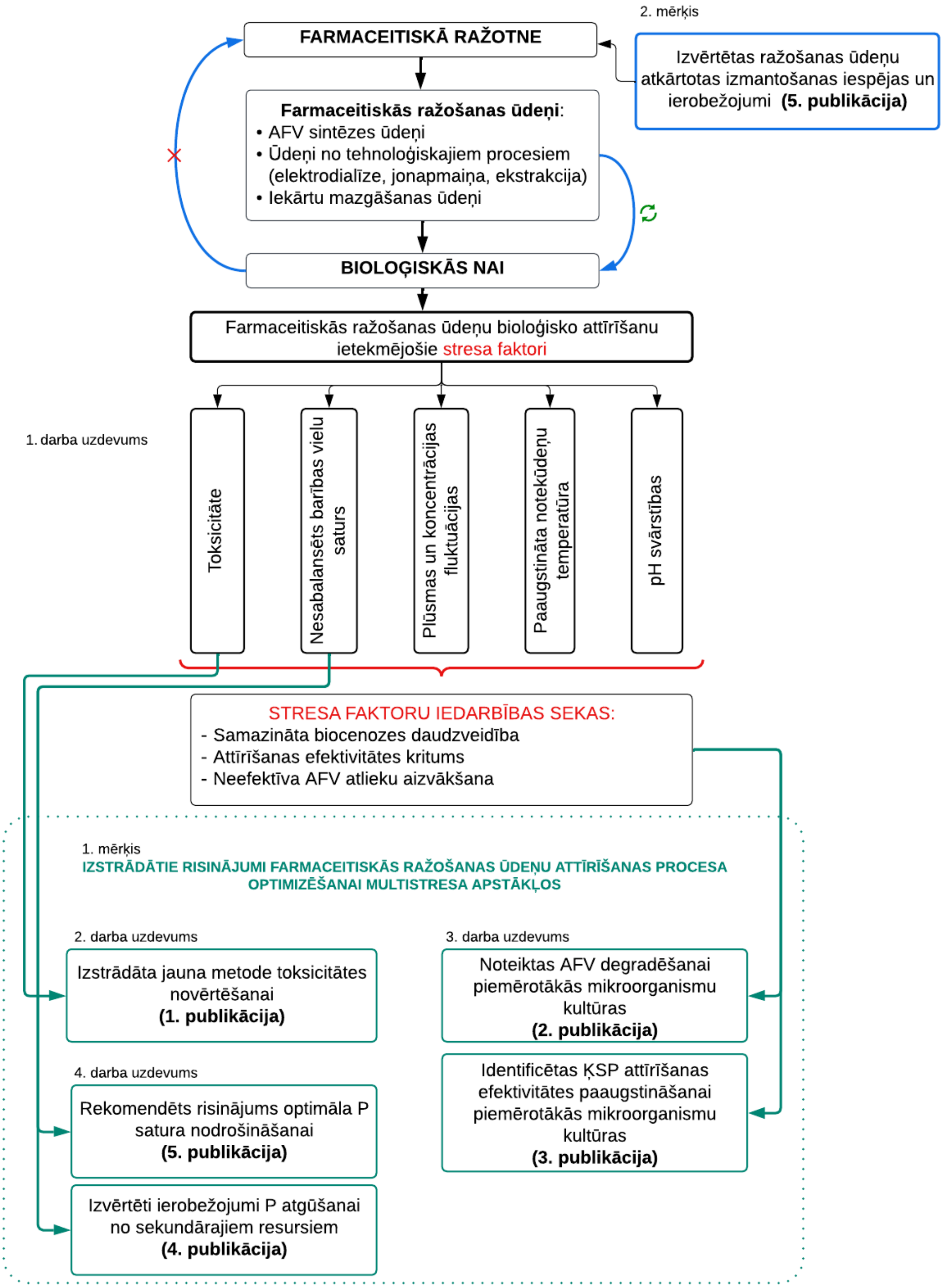
1. Farmaceitiskās ražošanas ūdeņu bioloģisko attīrīšanu ietekmējošo stresa faktoru identifikācija

Veicot GNAI tehnoloģiskā procesa izpēti un izvērtējot uzkrātos monitoringa datus par notekūdeņu ķīmisko un fizikālo parametru raksturlielumiem un aktīvo dūņu mikrobioloģiskajiem parametriem, tika identificēti pieci galvenie farmācijas notekūdeņu bioloģisko attīrīšanu ietekmējošie faktori: toksicitāte; nesabalansēts barības vielu saturs; plūsmas un koncentrācijas fluktuācijas; paaugstināta notekūdeņu temperatūra; pH svārstības (1. att.). Katrs no šiem faktoriem izraisa aktīvo dūņu mikroorganismu stresu un negatīvi ietekmē bioloģiskā attīrīšanas procesa darbību, bet visbiežāk šo faktoru iedarbība notiek vienlaikus, radot multistresa apstākļus.

Atsevišķu stresa faktoru, piemēram, pH svārstību, plūsmas un koncentrācijas fluktuāciju, ietekmi uz bioloģiskā notekūdeņu attīrīšanas procesa efektivitāti var mazināt, tehnoloģiski regulēt vai pilnībā novērst, paredzot atbilstošus tehnoloģiskos risinājumus jau NAI projektēšanas laikā [15], [16]. Tomēr tādus notekūdeņu raksturlielumus kā toksicitāte un barības vielu sastāvs nav iespējams prognozēt ilgtermiņā, jo ražošanas ūdeņu sastāvs var krasi mainīties atkarībā no izmaiņām ražoto produktu klāstā un globālā pieprasījuma pēc specifiskām zālēm.

Promocijas darbā izstrādātie risinājumi farmaceitiskās ražošanas ūdeņu bioloģiskā attīrīšanas procesa optimizēšanai multistresa apstākļos atspoguļoti zinātniskajās publikācijās, kuru sasaiste ar promocijas darba mērķiem un uzdevumiem atspoguļota 1. attēlā.

Dati par GNAI tehnoloģisko procesu, GNAI ieplūdes notekūdeņu ķīmiskajiem un fizikālajiem raksturlielumiem, kā arī aktīvo dūņu mikrobioloģiskajiem parametriem, kas izmantoti stresa faktoru identifikācijai, apkopoti pielikumā, kas pieejams pēc pieprasījuma.



1. att. Farmaceitiskās ražošanas ūdeņu bioloģisko attīrīšanu ietekmējošie stresa faktori un promocijas darba struktūra.

2. Farmaceitiskās ražošanas ūdeņu toksicitātes novērtēšana

Notekūdeņu ķīmiskie raksturlielumi nedod tiešu informāciju par notekūdeņu toksicitāti. Tādēļ, lai nepieļautu, ka toksisku vielu iedarbībā iet bojā aktīvo dūņu biocenoze un nav iespējams nodrošināt likumdošanas aktu prasībām atbilstošu notekūdeņu attīrīšanas pakāpi, svarīgi ķīmiskās analīzes papildināt ar toksicitātes testiem un veikt preventīvus pasākumus, ja toksicitāte konstatēta.

Lai objektīvi novērtētu NAI ieplūstošo ūdeņu toksicitāti, kā testa organismus svarīgi izmantot aktīvās dūņas no konkrētās attīrīšanas stacijas, jo toksicitātes testa rezultāts ir atkarīgs no izvēlētā testa organisma un atsauces funkcijas [17]. Pētījumi liecina, ka NAI sistēmai nespecifiski testa organismi, piemēram, luminiscējošās baktērijas *Vibrio qinghaiensis* un *Vibrio fischeri*, var uzrādīt paaugstināju jutību, neatspoguļojot notekūdeņu reālo toksicitāti uz NAI biocenozi [18]–[20], savukārt citos gadījumos īsais inkubācijas laiks var būt iemesls tam, ka tiek iegūti pazemināti toksicitātes rezultāti [21], neatbilstoši novērtējot potenciālo kaitējumu un toksicitātes risku.

1. publikācijā [22] ir aprakstīta izstrādātā metode sistemātiskai ķīmiski piesārņotu ražošanas ūdeņu toksicitātes un biodegradācijas potenciāla novērtēšanai, balstoties uz bioķīmiskā skābekļa patēriņa (BSP) mērījumiem, kas veikti paplašinātā sākuma koncentrāciju diapazonā. Izmantojot šo metodi, tiek iegūtas eksperimentālās raksturlīknes, kas ļauj spriest par to, kā notekūdeņu biodegradācijas spēja un toksicitāte uz aktīvo dūņu mikroorganismiem mainās atkarībā no atšķaidījuma pakāpes. Ja BSP vērtības izteikti pieaug, pieaugot atšķaidījumam, tas liecina, ka ūdenī ir vielas, kas paaugstinātā koncentrācijā inhibējoši iedarbojas uz aktīvo dūņu biocenozi un var izraisīt attīrīšanas procesa traucējumus. Ja līkne ātri sasniedz plato, turklāt attiecība BSP/ĶSP ir augsta (≥ 50 %), tas liecina par labu biodegradācijas spēju aktīvo dūņu sistēmā un zemu toksicitātes līmeni [23]. Turpretī, ja attiecība BSP/ĶSP saglabājas zema (< 10 %) un nemainās atkarībā no atšķaidījuma pakāpes, tas liecina, ka testētie notekūdeņi ir toksiski un to sastāvā esošie organiskie savienojumi nav bioloģiski noārdāmi [24], tādēļ tos nevar novadīt bioloģiskajās NAI, lai neizraisītu mikroorganismu toksisko šoku.

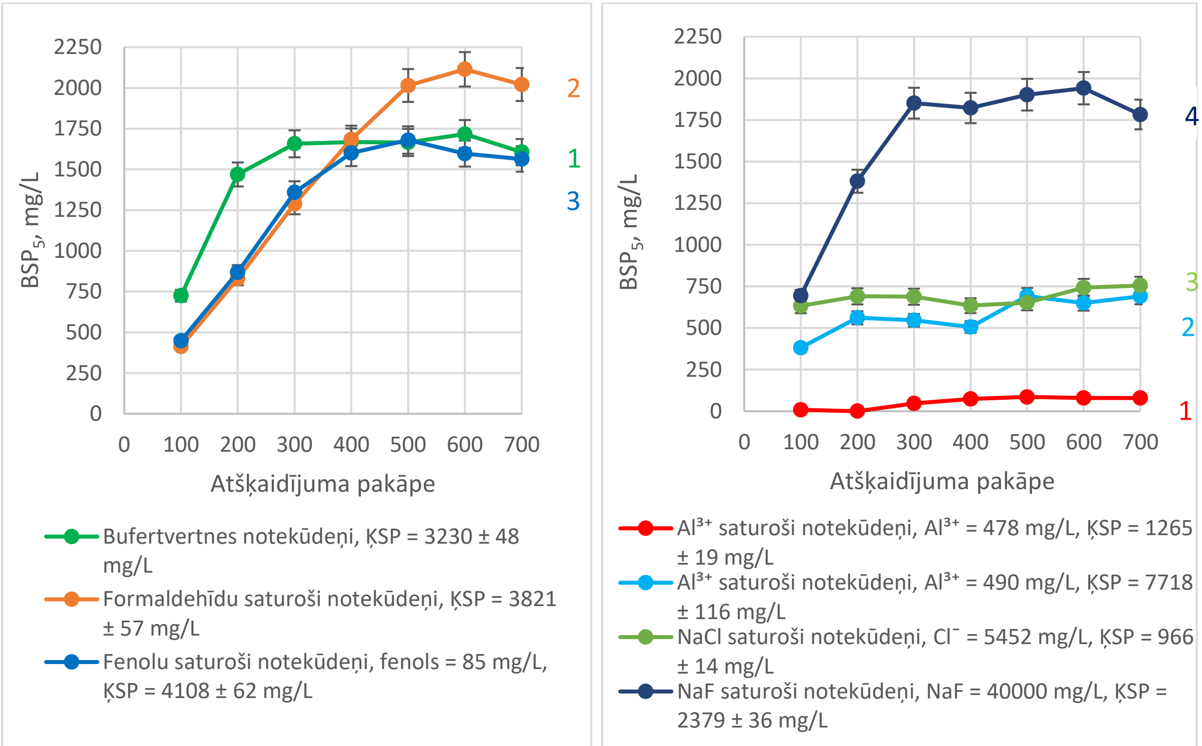
Metodes lietojums uzskatāmi demonstrēts, analizējot GNAI ieplūdes (bufertvertnes) notekūdeņus un specifiskas farmaceitiskās ražošanas ūdeņu plūsmas, kas piesārņotas ar organiskiem savienojumiem un neorganiskiem sāļiem. Notekūdeņu paraugi un informācija par galveno ķīmisko piesārņotāju un tā koncentrāciju notekūdeņos tika saņemta no ražošanas departamenta un izmantota notekūdeņu identifikācijai. Tādēļ ĶSP analīzes tika izmantotas kā piemērots indikators kopējā notekūdeņu organiskā piesārņojum raksturošanai.

Paraugi tika testēti pie atšķaidījuma pakāpes robežās no 100 līdz 700, kas atbilst reālajam dienā radītajam notekūdeņu daudzumam un to reāli iespējamajam atšķaidījumam GNAI bufertvertnē (izlīdzinātājtvertnē). Ūdens ar sējmateriālu tika izmantots kā kontroles paraugs. Skābekļa patēriņš kontroles paraugā piecu dienu inkubācijas periodā nedrīkst pārsniegt 1,5 mg/L. Testi tika veikti divos atkārtojumos.

2. a attēlā redzams, ka analizētā bufertvertnes notekūdeņu parauga BSP vērtības atkarībā no atšķaidījuma pakāpes mainās maz. Inhibējošā iedarbība uz aktīvo dūņu mikroorganismiem tiek

novērota, ja atšķaidījuma pakāpe ir 100, savukārt līkne sasniedz plato fāzi jau tad, kad atšķaidījuma pakāpe ir 200. Līknes raksturs liecina, ka notekūdeņi ir relatīvi labi biodegradējami un nav sagaidāma spēcīga toksiska iedarbība uz aktīvo dūņu mikroorganismiem. Atkarībā no atšķaidījuma pakāpes attiecība BSP/ĶSP variē 22–53 % robežās (1. tab.).

Iegūtās eksperimentālās līknes liecina, ka aktīvo dūņu mikroorganismus spēcīgi inhibē fenolu un formaldehīdu saturoši farmaceitiskās ražošanas ūdeņi. Kā redzams 2. a attēlā, abu notekūdeņu plūsmu testēšanas laikā iegūto toksicitātes līkņu raksturs ir līdzīgs – biodegradācijas spēja ir izteikti atkarīga no parauga atšķaidījuma pakāpes, un līkne sasniedz plato fāzi tikai tad, ja atšķaidījuma pakāpe ir 500, kas atbilst notekūdeņu atšķaidījuma pakāpei testa šķīdumā. Lai nepieļautu mikroorganismu intoksikācijas riskus, šāda tipa notekūdeņus, kas ir biodegradējami, bet augstās koncentrācijās toksiski aktīvo dūņu biocenozei, var uzkrāt atsevišķos rezervuāros un kontrolēti dozēt kopējā notekūdeņu plūsmā.



a) b)

2. att. Toksicitātes raksturlīknes: a) bufertvertnes notekūdeņiem, fenolu un formaldehīdu saturošiem ražošanas notekūdeņiem; b) neorganiskos sāļus saturošiem ražošanas notekūdeņiem.

Izvērtējot neorganiskos sāļus saturošu farmācijas notekūdeņu toksicitāti, secināts, ka izteikti toksiska iedarbība uz aktīvo dūņu biocenozi ir Al^{3+} jonus saturošiem ūdens šķīdumiem, kas rodas AFV milnaciprāna hidrogēnhlorīda sintēzē, kur kā Luisa skābi izmanto $AlCl_3$. Toksicitātes testi tika veikti notekūdeņu paraugiem ar atšķirīgu organisko vielu saturu (ĶSP koncentrāciju) un praktiski vienādu Al^{3+} koncentrāciju. Kā redzams 1. tabulā, attiecība BSP/ĶSP abiem paraugiem saglabājas zema arī tad, ja parauga atšķaidījuma pakāpe ir augsta un nepārsniedz 10 %, liecinot, ka šie notekūdeņi ir praktiski nedegradējami, turklāt toksicitāte nemainās atkarībā no ĶSP, kas apliecina, ka toksisko ietekmi izraisa Al^{3+}. Iegūtie rezultāti ļauj secināt, ka Al^{3+} saturošus notekūdeņus nedrīkst ievadīt bioloģiskajās attīrīšanas iekārtās, jo tas var izraisīt mikroorganismu toksisko šoku un iznīcināt aktīvo dūņu biocenozi. Šādām toksiskām un nebiodegradējamām notekūdeņu plūsmām ražošanas iecirkņos jānodrošina atsevišķa savākšana un pirms ievadīšanas bioloģiskajās NAI jāveic priekšapstrāde vai tie jānodod utilizācijai kā bīstamie atkritumi.

Analizējot NaCl saturošu notekūdens plūsmu, toksiska ietekme uz aktīvo dūņu mikroorganismiem netika konstatēta, veicot salīdzināšanu pie vienādiem atšķaidījumiem (2.b att.). To var skaidrot ar faktu, ka NaCl saturs testētajā notekūdeņu paraugā atbilst sāls koncentrācijai fizioloģiskajā šķīdumā un BSP mērījumi tika veikti lielā atšķaidījumā. Eksperimentālie rezultāti ļauj secināt, ka notekūdeņos esošie organiskie savienojumi ir ar labu biodegradācijas spēju – attiecība BSP/ĶSP ir lielāka par 65 % (1. tab.). Veicot toksicitātes testu NaF saturošai notekūdens plūsmai, inhibējošais efekts uz aktīvo dūņu mikroorganismiem tika novērots tikai tad, ja atšķaidījuma pakāpe ir 100 (2. b att.), savukārt, ja atšķaidījuma pakāpe ir 200, attiecība BSP/ĶSP jau sasniedz 58 % (1. tab.), kas liecina, ka šādā atšķaidījumā fluorīdi vairs neietekmē notekūdenī esošo organisko vielu biodegradāciju.

1. tabula

Attiecība BSP/ĶSP (%) atkarībā no notekūdeņu atšķaidījuma pakāpes

Notekūdeņu veids	ĶSP, mg/L	Attiecība BSP/ĶSP, %						
		Atšķaidījuma pakāpe						
		100	200	300	400	500	600	700
Bufertvertnes notekūdeņi	3230 ± 48	22,4	45,4	51,3	51,6	51,5	53,2	49,7
Formaldehīdu saturoši notekūdeņi	3821 ± 57	10,8	21,7	33,7	44,1	52,7	55,3	52,9
Fenolu saturoši notekūdeņi fenols = 85 mg/L	4108 ± 62	10,9	21,2	33,1	38,9	40,9	38,9	38,1
Al^{3+} saturoši notekūdeņi Al^{3+} = 478 mg/L	1265 ± 19	0,6	0,5	3,6	5,8	6,8	6,3	6,3
Al^{3+} saturoši notekūdeņi Al^{3+} = 490 mg/L	7718 ± 116	5,0	7,3	7,1	6,6	9,0	8,4	9,0
NaCl saturoši notekūdeņi Cl^- = 5452 mg/L	966 ± 14	65,5	71,4	71,3	65,6	67,5	76,9	78,2
NaF saturoši notekūdeņi NaF = 40000 mg/L	2379 ± 36	29,2	58,1	77,8	76,6	79,9	81,6	75,0

Eksperimentāli iegūtie dati uzskatāmi parāda, ka farmaceitiskās ražošanas ūdeņiem attiecība BSP/ĶSP vienādas atšķaidījuma pakāpes gadījumā var atšķirties pat desmitkārtīgi (1. tab.). Tas apstiprina, ka ūdeņi no dažādiem tehnoloģiskajiem procesiem būtiski atšķiras pēc to biodegradācijas spējas un toksicitātes uz aktīvo dūņu mikroorganismiem, ko tieši ietekmē atšķirīgais piesārņojošo vielu sastāvs un savienojumu ekotoksicitāte.

Rezultāti liecina, ka izstrādātā toksicitātes metode ir pietiekami jutīga, ļauj salīdzināt atšķirīgas ražošanas ūdeņu plūsmas un identificēt NAI biocenozei toksiskos savienojumus. Testēšanā izmantotais paplašinātais sākuma koncentrāciju diapazons parāda, kādā atšķaidījuma pakāpē notekūdeņu toksicitāte mainās, un to var izmantot, lai prognozētu, kādu ietekmi uz attīrīšanas procesu var radīt produktu ražošanas apjomu palielināšana. Būtiska metodes priekšrocība ir salīdzinoši zemās izmaksas un spēja noteikt toksisko vielu iedarbību uz konkrētu attīrīšanas iekārtu biocenozi, izmantojot to kā sējmateriālu.

3. Bioaugmentācijai piemērotu mikroorganismu kultūru atlase mērķa AFV un ĶSP degradācijas paaugstināšanai farmācijas NAI

AFV atliekām, kas ar nepietiekami attīrītiem farmaceitiskās ražošanas notekūdeņiem nonāk apkārtējā vidē, pēdējās desmitgadēs tiek pievērsta pastiprināta uzmanība, jo pētījumi liecina, ka zāļu ražotnes var būt nozīmīgs punktveida AFV emisiju avots [25], [26], bet šīs vielas ir bioloģiski aktīvas arī ļoti zemās koncentrācijās, un vairāki pētījumi apstiprina, ka to nonākšana ūdens ekosistēmā izraisa negatīvas sekas gan uz ūdens organismiem, gan cilvēku veselību [27].

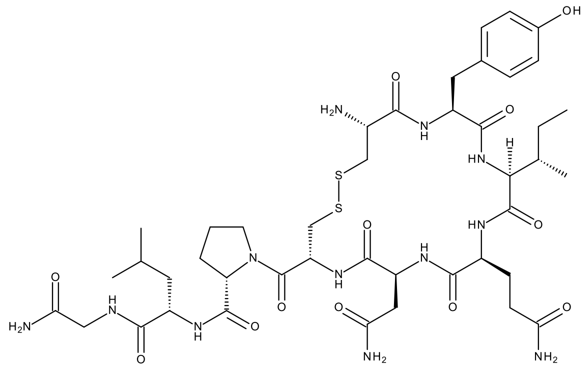
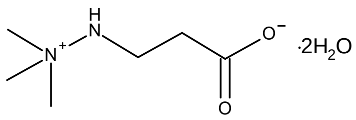
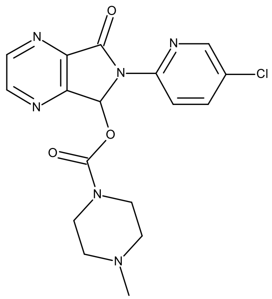
Aktīvo dūņu sistēmu bioaugmentācija ar selektīvām mikroorganismu kultūrām ir viena no tehnoloģijām, ko var lietot AFV mikropiesārņojuma degradācijas efektivitātes paaugstināšanai gan sadzīves, gan farmaceitiskās ražošanas ūdeņu bioloģiskajās NAI [28], [29]. Bioaugmentācijai piemērotu kultūru atlase ir viens no galvenajiem aspektiem, kas nosaka bioaugmentācijas stratēģijas efektivitāti. Kultūrām ir jānodrošina ne tikai augsta mērķa savienojumu degradēšanas spēja, bet arī jābūt noturīgām pret notekūdeņu vides apstākļiem, toksicitāti un jāspēj izdzīvot konkurencē ar citiem aktīvo dūņu mikroorganismiem [30].

Farmācijas notekūdeņos piesārņojošo vielu klāsts ir ļoti plašs. Tādēļ, lai bioaugmentācija būtu efektīva un tiktu nodrošināts, ka summārie ūdens piesārņojuma rādītāji NAI izplūdē atbilst likumdošanas aktu prasībām, izmantotajām mikroorganismu kultūrām jānodrošina ne tikai augsta AFV attīrīšanas pakāpe, bet arī jāspēj efektīvi degradēt pārējie ūdenī esošie ķīmiskie savienojumi. Citu autoru pētījumi apliecina, ka bioaugmentācijas stratēģija ļauj intensificēt farmācijas notekūdeņu attīrīšanas pakāpi un uzlabot ĶSP degradācijas efektivitāti [31], tādēļ promocijas darba izstrādes gaitā tika meklēti bioaugmentācijai piemērotākie mikroorganismi, kas spēj nodrošināt gan augstu mērķa AFV degradācijas pakāpi, gan efektīvi samazina ĶSP un būtu piemēroti kandidāti notekūdeņu attīrīšanas efektivitātes paaugstināšanai GNAI.

Promocijas darba **2. publikācijā** [32] prezentēti skrīninga rezultāti, kas iegūti, testējot desmit baktēriju, desmit raugu un trīs mikroskopisko sēņu, kas izdalītas no GNAI aktīvajām dūņām, spēju degradēt trīs AFV ar atšķirīgu ķīmisko uzbūvi un terapeitisko iedarbību – ciklisko nonapeptīdu oksitocīnu, heterociklisku slāpekļa savienojumu zopiklonu un hidrazīna atvasinājumu meldonija dihidrātu (2. tab.). Šīs AFV pētījumam izvēlētas kā modeļa savienojumi, jo to mikrobioloģiskā degradācija līdz šim ir maz pētīta, bet pēc ražošanas apjoma tās ieņem būtisku vietu AS “Grindeks” produktu klāstā, turklāt to ražošanas laikā rodas liels apjoms ķīmiski piesārņotu ražošanas ūdeņu. 2. tabulā uzskatāmi redzams, ka izvēlētie savienojumi būtiski atšķiras pēc ķīmiskajām īpašībām, kas nosaka to uzvedību ūdenī, t. i., šķīdības ūdenī un oktanola ūdens sadalījuma koeficienta logaritmiskās vērtības log K_{ow}.

2. tabula

Bioaugmentācijas pētījumos izmantotās AFV

AFV	Ķīmiskā formula un struktūra	Molmasa, g/mol	CAS Nr.	Šķīdība ūdenī, g/L	log K_{ow}	Terapeitiskā klase
Oksitocīns	$C_{43}H_{66}N_{12}O_{12}S_2$ 	1007,19	50-56-6	12	–6,27	Hormons
Meldonija dihidrāts	$C_6H_{14}O_2N_2 \cdot 2\ H_2O$ 	182,26	86426-17-7	20,2	0,45	Sirds un asinsvadu slimību līdzeklis
Zopiklons	$C_{17}H_{17}ClN_6O_3$ 	388,81	43200-80-2	0,151	1,54	Sedatīvs līdzeklis

Pētījumi tika veikti laboratorijas mēroga eksperimentos, testējot mikroorganismu spēju aizvākt AFV kā vienīgo oglekļa avotu un kometabolisma ceļā, kā papildu barības vielu pievienojot vājpiena pulveri (PP). PP tika izvēlēts kā papildu barības viela, balstoties *Quintina* u. c. publicētajos rezultātos, kas liecina, ka piena pulvera pievienošana ļauj kometaboliski degradēt tās AFV, kas nevar tikt utilizētas kā vienīgais oglekļa un enerģijas avots [33]. AFV aizvākšanas efektivitāte % 168 h inkubācijas periodā, kas noteikta, veicot AEŠH-MS analīzes, apkopota 3.–4. tabulā. Izvēlētais inkubācijas laiks atbilst ilgākajam iespējamajam hidrauliskās aiztures laikam GNAI. AFV aizvākšana no šķīduma ir sorbcijas un biodegradācijas kopējais rezultāts. Promocijas darba pētījumā šie abi procesi netika diferencēti, tomēr oktanola-ūdens sadalījuma koeficienta logaritmiskās vērtības log K_{ow} izvēlētajām AFV ir zemas (2. tab.), kas liecina, ka sorbcijai uz aktīvajām dūņām attīrīšanas procesā ir nebūtiska loma [34].

Lielākā daļa pārbaudīto mikroorganismu kultūru – t. sk. baktērijas *Acinetobacter schindleri, Bacillus cereus, Chryseobacterium balustinum, Myroides odoratus, Sphingobacterium thalpophilum*; raugi *Apiotrichum montevideense, Cutaneotrichosporon arboriforme, Trichosporon asahii* un mikroskopiskā sēne *Fusarium udum* – 168 h laikā pilnībā (> 99 %) utilizēja visu oksitocīnu šķīdumā, kur AFV bija vienīgais oglekļa avots. Vājpiena pulvera pievienošana attiecībā 1 : 10 pret AFV sākuma koncentrāciju būtiski uzlaboja oksitocīna aizvākšanas efektivitāti biodegradācijas testos ar raugiem *Candida inconspicua 1, Cutaneotrichosporon cutaneum, Farysia acheniorum* un mikroskopisko sēni *Talaromyces radicus*.

Pētījumi liecina, ka oksitocīna molekula ūdens šķīdumos nav stabila, paaugstinātā temperatūrā notiek tās degradācija un zūd bioloģiskā aktivitāte [35], [36]. Bioaugmentācijas eksperimentu laikā oksitocīna degradācija kontroles paraugā bez mikroorganismu pievienošanas 168 h laikā bija 12 %, kas apstiprina, ka mikrobioloģiskā degradācija veiktajos pētījumos tomēr bija primārais oksitocīna degradācijas veids.

Literatūrā pieejama limitēta informācija par heterocikliskā slāpekļa savienojuma zopiklona biodegradācijas spēju. Attīrot slimnīcas notekūdeņus membrānu bioreaktorā, tā aizvākšanas efektivitāte bija mazāka par 10 % [37]. Promocijas darba pētījumos zopiklona degradācijas efektivitāte būtiski variēja starp dažādām mikroorganismu grupām. Raugs *Apiotrichum domesticum* aizvāca > 99 % zopiklona no testa šķīduma vājpiena pulvera klātbūtnē, savukārt pārējie raugi uzrādīja limitētu zopiklona degradēšanas spēju. *Pseudomonas putida* un *Moraxella osloensis* tika identificētas kā efektīvākās baktēriju kultūras selektīvai zopiklona degradācijai un uzrādīja attiecīgi 85 % un 89 % attīrīšanas efektivitāti 168 h inkubācijas periodā. Savukārt mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum* aizvāca vairāk nekā 90 % zopiklona gan testos, kur šī AFV bija kā vienīgais oglekļa avots, gan vājpiena pulvera klātbūtnē, liecinot par šo kultūru tālākas lietošanas augsto potenciālu AFV degradēšanas mērķim.

3. tabula

AFV aizvākšanas efektivitāte (%) 168 h inkubācijas periodā testa šķīdumos, kur AFV ir vienīgais oglekļa avots (AFV sākuma koncentrācija 20 mg/L), un PP klātbūtnē (AFV sākuma koncentrācija 5 mg/L), izmantojot bioaugmentāciju ar baktēriju kultūrām

	Oksito-cīns	Oksito-cīns + PP	Zopik-lons	Zopik-lons + PP	Meldonija dihidrāts	Meldonija dihidrāts + PP
Acinetobacter schindleri	> 99	> 99	54	82	4,7	35
Aeromonas caviae	99	99	59	71	1,3	1,8
Bacillus cereus	> 99	> 99	63	60	3,0	12
Chryseobacterium balustinum	> 99	> 99	68	81	5,2	13
Comamonas testosteroni	99	> 99	63	67	12	23
Moraxella osloensis	69	57	89	91	14	40
Myroides odoratus	> 99	> 99	64	60	24	27
Pseudomonas aeruginosa	58	57	50	79	10	18
Pseudomonas putida	73	68	85	87	19	34
Sphingobacterium thalpophilum	> 99	> 99	75	84	17	91
Kontroles paraugs bez mikroorganismiem	12	12	25	17	17	10

Meldonija dihidrāts tika identificēts kā noturīgākais savienojums pret biodegradāciju. Lielākā daļa testēto mikroorganismu kultūru nespēja degradēt meldonija dihidrātu kā vienīgo oglekļa avotu. Raugs *Apiotrichum domesticum* kometabolisma ceļā degradēja 65 % meldonija dihidrāta 168 h laikā, savukārt citas raugu kultūras uzrādīja zemu attīrīšanas efektivitāti (< 20 %). No visām 10 pārbaudītajām baktēriju sugām tikai *Sphingobacterium thalpophilum* uzrādīja augstu meldonija dihidrāta degradācijas efektivitāti vājpiena pulvera klātbūtnē – 91 % 168 h laikā, savukārt pārējās baktērijas utilizēja meldonija dihidrātu 1,3–40 % robežās 168 h inkubācijas periodā gan testa šķīdumos, kur meldonija dihidrāts bija vienīgais oglekļa avots, gan kopā ar vājpiena pulveri. Mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum* testa šķīdumos, kur meldonija dihidrāts bija vienīgais oglekļa avots, uzrādīja attiecīgi 21 % un 46 %

degradācijas efektivitāti, savukārt vājpiena pulvera klātbūtnē degradācijas efektivitāte būtiski pieauga, sasniedzot attiecīgi 91 % un 94 %.

4. tabula

AFV aizvākšanas efektivitāte (%) 168 h inkubācijas periodā testa šķīdumos, kur AFV ir vienīgais oglekļa avots (AFV sākuma koncentrācija 20 mg/L), un PP klātbūtnē (AFV sākuma koncentrācija 5 mg/L), izmantojot bioaugmentāciju ar raugu un mikroskopisko sēņu kultūrām

	Oksitocīns	Oksitocīns + PP	Zopiklons	Zopiklons + PP	Meldonija dihidrāts	Meldonija dihidrāts + PP
Raugi						
Apiotrichum domesticum	98	> 99	42	> 99	17	65
Apiotrichum montevideense	> 99	> 99	21	44	14	11
Candida inconspicua 1	13	51	29	35	6,7	8,5
Candida inconspicua 2	98	> 99	56	26	12	3,5
Cutaneotrichosporon arboriforme	> 99	> 99	0,7	32	6,0	8,0
Cutaneotrichosporon cutaneum	1,0	7,1	16	29	1,6	15
Farysia acheniorum	11	> 99	14	28	13	25
Rhodotorula mucilaginosa	91	61	16	47	13	14
Saprochaete gigas	45	81	64	59	7,6	16
Trichosporon asahii	> 99	> 99	7,7	24	4,4	2,8
Mikroskopiskās sēnes						
Fusarium solani	99	> 99	98	> 99	21	91
Fusarium udum	> 99	> 99	> 99	91	46	94
Talaromyces radicus	12	98	2,3	26	5,8	11
Kontroles paraugs bez mikroorganismiem	12	12	25	17	17	10

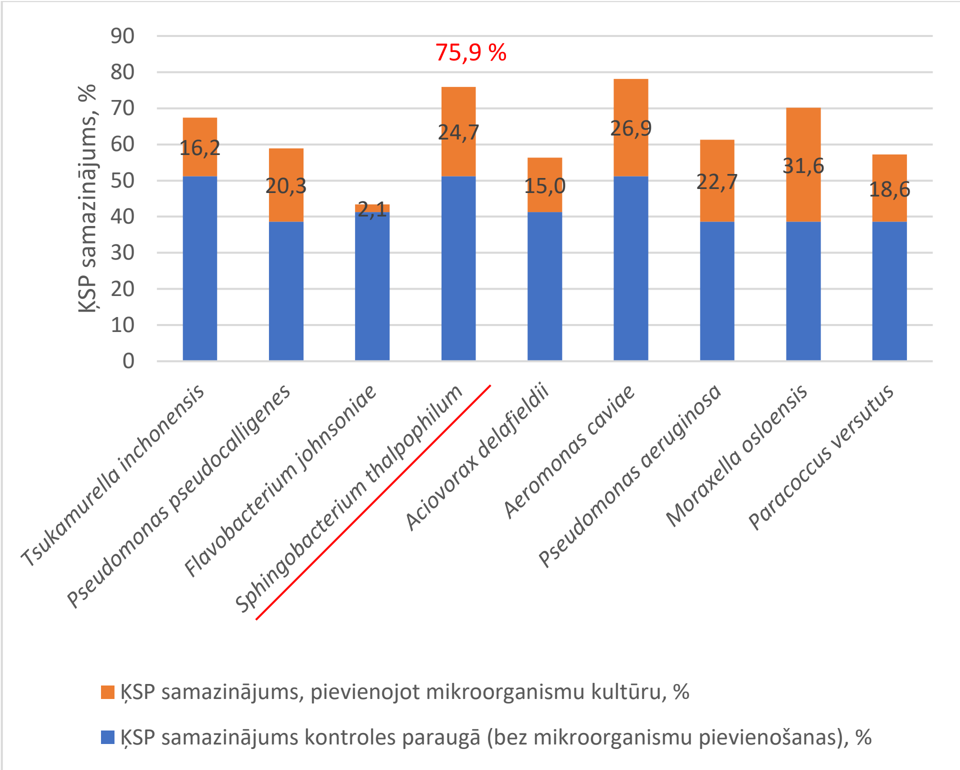
Lai arī meldonija degradācijas procesi un metabolīti cilvēka un dzīvnieku organismā ir salīdzinoši plaši pētīti [38]–[41], literatūrā nav atrodami pētījumi, kuros būtu analizēti šīs AFV mikrobioloģiskās degradācijas mehānismi aktīvo dūņu sistēmās vai ar selektīvām mikroorganismu kultūrām un vērtēta kometabolisma loma degradācijas procesā. Zemie meldonija degradācijas rezultāti liecina, ka vairumam pētīto mikroorganismu nav atbilstošu enzīmu, lai veiktu šīs AFV noārdīšanu, bet mikroorganismi *Sphingobacterium thalpophilum*, *Fusarium solani* un *Fusarium udum* spēj efektīvi degradēt meldoniju tikai papildu oglekļa avota klātbūtnē.

Apkopojot iegūtos AFV degradācijas skrīninga rezultātus, secināts, ka efektīvākās kultūras mērķa AFV degradācijai ir baktērija *Sphingobacterium thalpophilum* un mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum.* Šīs mikroorganismu kultūras spēja degradēt visas trīs pārbaudītās AFV ar efektivitāti virs 84 % 168 h inkubācijas periodā.

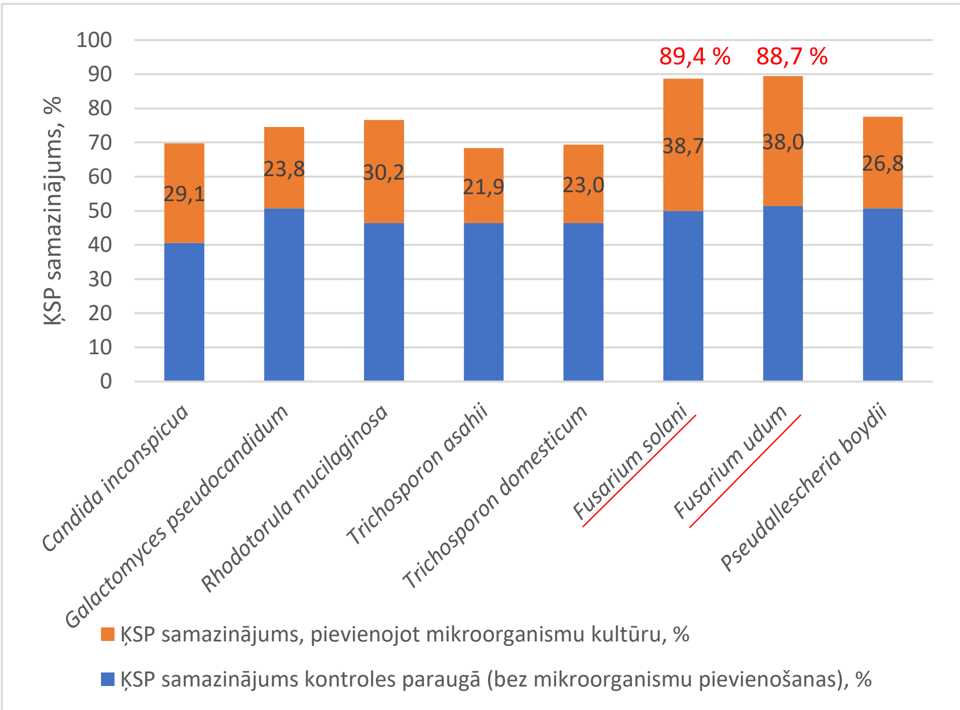
Citos AFV degradācijas pētījumos sēne *Fusarium solani* ir identificēta kā piemērota kultūra AFV diklofenaka un ketoprofēna aizvākšanai no notekūdeņiem [42], savukārt baktērija *Sphingobacterium thalpophilum* uzrādījusi antibiotikas nitrofurantoīna degradācijas spēju [43]. Saskaņā ar analizētajiem literatūras avotiem, *Fusarium udum* loma AFV degradācijas efektivitātes paaugstināšanā līdz šim nav pētīta.

3. publikācijā [44] apkopotie eksperimentālie dati liecina, ka starp 65 pārbaudītajiem mikroorganismu izolātiem, baktērija *Sphingobacterium thalpophilum* un mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum* ir starp tām kultūrām, kas uzrādījušas arī augstāko ĶSP samazinājumu, attīrot farmācijas notekūdeņus. Farmācijas notekūdeņu paraugi testiem tika ņemti no GNAI bufertvertnes, kurā nonāk un samaisās ražošanas ūdeņi no visiem ražošanas iecirkņiem, pirms tie tiek novadīti attīrīšanai pirmajā bioreaktorā. Izmantojot šādu kompleksu paraugu, ir iespējams pārliecināties par mikroorganismu spēju degradēt plaša spektra ķīmiskos savienojumus, kas ir būtisks nosacījums, lai mainīgajos ražošanas apstākļos bioaugmentācija būtu efektīva.

Kā redzams 3.–4. attēlā apkopotajos rezultātos, 120 h inkubācijas laikā baktērija *Sphingobacterium thalpophilum* un mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum* samazināja ĶSP attiecīgi par 75,9 %; 89,4 % un 88,7 %. ĶSP ir summārs notekūdeņu organiskā piesārņojuma līmeņa rādītājs, kas iekļauts arī normatīvo aktu prasībās, kas reglamentē maksimāli pieļaujamās piesārņojuma koncentrācijas NAI izplūdē, tāpēc augstie ĶSP samazinājuma rādītāji, kas iegūti mikroorganismu skrīninga testos, apstiprina, ka *Sphingobacterium thalpophilum, Fusarium solani* un *Fusarium udum* kultūras var būt piemēroti kandidāti farmācijas notekūdeņu attīrīšanas efektivitātes paaugstināšanai bioaugmentācijas ceļā.



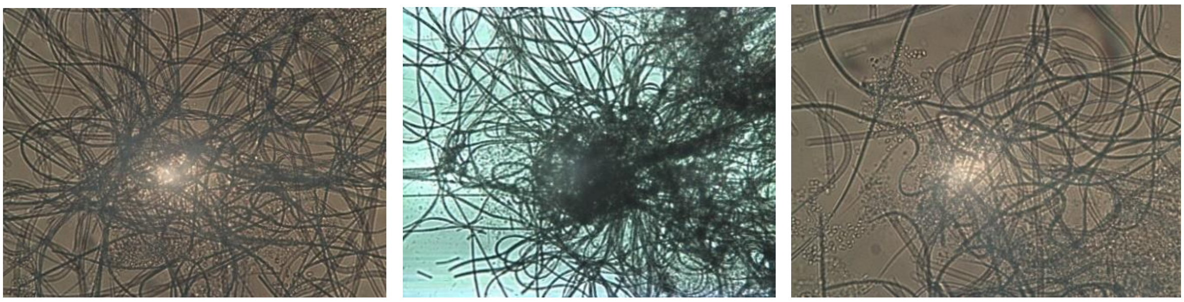
3. att. ĶSP samazināšanas efektivitāte, izmantojot bioaugmentāciju ar baktēriju kultūrām.



4. att. ĶSP samazināšanas efektivitāte, izmantojot bioaugmentāciju ar raugu un mikroskopisko sēņu kultūrām.

4. Fosfora trūkuma problēmas risinājumi farmācijas notekūdeņu bioloģiskajās attīrīšanas sistēmās

Saskaņā ar GNAI notekūdeņu piesārņojuma monitoringa datiem, ĶSP un N_{kop} koncentrācijas mediāna GNAI ieplūdē ir attiecīgi 5500 mg/L un 160 mg/L, savukārt vidējais PO_4-P saturs ir tikai 3 mg/L, kas ir nepietiekami, lai nodrošinātu attīrīšanas procesam optimālo ĶSP : N : P attiecību 100 : 5 : 1. Fosfora trūkums notekūdeņos pasliktina bioloģiskās attīrīšanas efektivitāti, veicina filamento baktēriju attīstību (5. att.), izraisa dūņu tilpuma pieaugumu un struktūras izmaiņas, kas negatīvi ietekmē to nostādināšanas un atūdeņošanas kvalitāti [45]–[48]. Lai nodrošinātu optimālu P līmeni bioloģiskās attīrīšanas procesā, kā papildu P avots parasti tiek izmantota H_3PO_4.



5. att. P deficīta izraisīta filamento baktēriju dominance GNAI aktīvajās dūņās (palielinājums 100 x, gaismas mikroskops *Optika B-600 Tiph*).

Tomēr fosforskābes pieejamība pēdējo gadu laikā ir būtiski samazinājusies. Viens no šādas situācijas galvenajiem iemesliem ir dabisko P resursu straujais izsīkums [49] un fakts, ka fosfātiežu atradnes pasaulē ir izvietotas tikai dažos reģionos ārpus Eiropas Savienības (ES) galvenokārt, Ķīnā, Marokā, ASV un Krievijā, kas padara ES valstis pilnībā atkarīgas no P savienojumu importa un pieaugošām cenām. Šo apstākļu dēļ H_3PO_4 iegūšanā izmantotie fosfātieži un fosfors ir iekļauti Eiropas ekonomikai kritisko izejvielu sarakstā [50], īpaši uzsverot arī nepieciešamību atgūt P savienojumus no dažādām atkritumu plūsmām un meklēt optimālākos risinājumus pārejai uz cirkulāru P apriti ne tikai valsts, bet arī reģionālā līmenī.

4. publikācijā [51], analizējot dažādas P plūsmas un to līdzšinējās pārvaldības praksi Baltijas jūras reģiona valstīs, pasaulē pieejamos tehnoloģiskos risinājumus P savienojumu atgūšanai no sekundārajiem resursiem un reģionam specifiskos apstākļus, secināts, ka, neraugoties uz to, ka ir identificēti vairāki P saturoši sekundārie resursi – tajā skaitā notekūdeņi, notekūdeņu dūņas un dūņu pelni (5. tab.), P atgūšana no tiem Baltijas jūras reģiona valstīs netiek plaši izmantota galvenokārt sarežģītās pārstrādes un augsto P atgūšanas tehnoloģiju uzstādīšanas un ekspluatācijas izmaksu, kā arī atbilstoša likumdošanas regulējuma trūkuma dēļ [51].

5. publikācijā [52] norādīts alternatīvs risinājums P trūkuma problēmas novēršanai GNAI. Noteikts, ka vienīgais tehnoloģiskais process, kurā rodas ar ortofosfātiem bagāti ražošanas ūdeņi (C = 10 g/L PO_4-P), AS "Grindeks" ražotnē ir AFV ipidakrīna sintēze. Lai novērstu ekstrēmus fosfātu koncentrācijas pieaugumus un fosfora pārdozēšanu šīs AFV ražošanas stadijas laikā, kas ilgst tikai dažas nedēļas gadā, saskaņā ar darbā sniegtajām rekomendācijām ražošanas cehā šie ūdeņi (~10 m^3 jeb ~ 50 % no gada apjoma) tiek savākti atsevišķi un atkārtoti izmantoti kā P avots periodos, kad ortofosfātu saturs GNAI ieplūdes ūdeņos ir zems vai nepietiekams. Šāda prakse ļauj samazināt gada vidējo H_3PO_4 patēriņu par ~ 30 %. Patlaban ipidakrīna sintēzes ūdeņu izmantošanu pilnā apjomā limitē GNAI ierobežotā platība un iespēja ilgstoši uzglabāt atsevišķos konteineros savāktos ražošanas ūdeņus tālākai izmantošanai.

5. tabula

Atkritumu plūsmas, kas identificētas kā iespējamie sekundārie P resursi

Nozare	**Sekundārie P resursi**
Rūpniecība	Rūpnieciskie notekūdeņi, rūpniecisko notekūdeņu dūņas, rūpniecisko notekūdeņu dūņu pelni, fosforģipsis, biomasas pelni
Lauksaimniecība	Kūtsmēsli, gaļas un kaulu milti, zivjaudzētavu dūņas
Komunālais sektors	Sadzīves notekūdeņi, sadzīves notekūdeņu dūņas, sadzīves notekūdeņu dūņu pelni, pārtikas atkritumi

Literatūras dati liecina, ka AFV sintēzēs var rasties arī ar fosfora organiskajiem savienojumiem piesārņoti ūdeņi, ko tiešā veidā kā biopieejamu fosfora avotu aktīvo dūņu mikroorganismiem izmantot nevar. Veiktie pētījumi ar antibiotikas fosfomicīna sintēzes ūdeņiem liecina, ka piemērots risinājums P atgūšanai no šādām farmaceitiskās ražošanas ūdeņu plūsmām ir mitrā gaisa oksidācija, lai sašķeltu organiskos fosfora savienojumus neorganiskos fosfātos, kam seko izgulsnēšana hidroksiapatīta un struvīta formā [53].

Neraugoties uz to, ir secināts, ka P trūkumu nevar attiecināt uz visām farmācijas NAI. Pretstatā GNAI raksturīgajam nepietiekamajam P saturam ražošanas ūdeņos citi pētījumi rāda, ka var būt arī pretēja situācija un ortofosfātu saturs farmaceitiskās ražošanas ūdeņos var sasniegt pat 380 mg/L (ja ĶSP un N_{kop} koncentrācija ir attiecīgi 4200 mg/L un 410 mg/L), kas izslēdz nepieciešamību dozēt papildu P avotu bioloģiskās attīrīšanas reaktoros [54]. Taču ar fosfātiem bagātu ražošanas ūdeņu no vienas ražotnes izmantošana citas ražotnes NAI nav ekonomiski pamatota augsto ūdens transportēšanas izmaksu un sarežģītās loģistikas dēļ.

5. Ražošanas ūdeņu atkārtotas izmantošanas iespējas un ierobežojumi farmācijas rūpniecībā

Pāreja uz aprites ekonomikas modeli notekūdeņu attīrīšanas jomā liek izvērtēt iespēju no notekūdeņiem atgūt ne tikai P, bet arī citas ķīmiskās vielas, vienlaikus iegūstot otrreizēji izmantojamu ūdeni, lai tādā veidā mazinātu resursu patēriņu un ražošanas procesu ietekmi uz apkārtējo vidi.

Sērijveida vairākpakāpju ražošanas procesi, ražošanas ūdeņu plūsmu dažādība un tehnoloģisko procesu fragmentācija neļauj piemērot universālu risinājumu ūdens attīrīšanai un otrreizējai izmantošanai farmācijas rūpniecībā. Savukārt individuālu risinājumu piemērošana katrai ūdens plūsmai ir tehniski sarežģīta un dārga, tāpēc limitē aprites ekonomikā balstītu tehnoloģiju ieviešanu plašā mērogā.

Farmācijas nozares specifika nosaka arī to, ka jebkādām izmaiņām ražošanas procesos ir jābūt validētām, saskaņotām ar atbildīgajām institūcijām un klientu apstiprinātām [55], kas ir laikietilpīgi, dārgi un nemotivē farmaceitiskās ražotnes ieviest izmaiņas farmaceitisko produktu ražošanas tehnoloģiskajos procesos.

5. publikācijā veiktās izpētes rezultātā secināts, ka augsto ūdenim noteikto ķīmiskās un mikrobioloģiskās tīrības prasību dēļ attīrītu ražošanas ūdeņu otrreizēja izmantošana nav iespējama ražošanas procesos, kur ūdens nonāk tiešā kontaktā ar farmaceitisko produktu. Atkarībā no ražošanas stadijas, kurā ūdeni izmanto, kā arī formulācijas un ievadīšanas veida, ūdenim jāatbilst dzeramā ūdens vai Farmakopejās definētajām farmaceitiskās tīrības pakāpes ūdens kvalitātes prasībām (6. tab.). Pieejamās notekūdeņu attīrīšanas tehnoloģijas nevar garantēt tik augstu ūdens attīrīšanas pakāpi, bet farmaceitisko produktu kontaminācija var radīt nopietnus riskus pacientu veselībai, tāpēc tā netiek pieļauta atbilstoši nozarei noteiktajām labas ražošanas prakses prasībām [56]. Īpaši jāņem vērā tas, ka multiproduktu farmaceitiskajās rūpnīcās ražošanas ūdeņos nonāk arī farmaceitisko vielu atliekas, kas, atkārtoti izmantojot nepietiekami attīrītu ūdeni, var radīt AFV šķērskontaminācijas riskus. Ņemot vērā to, ka AFV ir bioloģiski aktīvas arī ļoti zemā koncentrācijā, produktu šķērskontaminācija var kritiski ietekmēt zāļu kvalitāti un drošību [57].

6. tabula

Farmaceitiskajā ražošanā izmantotajam ūdenim noteiktās kvalitātes prasības un atbilstošais izmantošanas veids [58]

Ūdens klase	Kvalitātes prasības atbilstoši Eiropas farmakopejai	Lietotās attīrīšanas tehnoloģijas	Izmantošana ražošanā
Dzeramais ūdens	Atbilstība PVO vadlīnijām dzeramā ūdens kvalitātei	Netiek specificētas	AFV starpproduktu sintēze Ražošanas iekārtu, telpu mazgāšana Farmaceitiskās klases ūdens sagatavošana
Attīrītais ūdens	KOO < 500 µg/L Elektrovadītspēja ≤ 4,3 µS/cm (20 ºC) Nitrāti ≤ 0,2 mg/L Aerobās baktērijas ≤ 100 KVV/mL	Jonapmaiņa, ultrafiltrācija, reversā osmoze, destilācija, aktīvās ogles filtrācija	AFV gala produkta izdalīšana un attīrīšana Nesterilu GZF ražošana Ražošanas iekārtu, taras beigu skalošana
Ūdens injekcijām	KOO < 500 µg/L Elektrovadītspēja ≤ 1,1 µS/cm (20 ºC) Nitrāti ≤ 0,2 mg/L Aerobās baktērijas ≤ 10 KVV/mL Bakteriālie endotoksīni ≤ *I. U.*/mL	Reversā osmoze, elektro-dejonizācija, ultrafiltrācija, nanofiltrācija	Sterilu farmaceitisko produktu ražošana

Pētījumā secināts, ka pašas farmaceitiskās tīrības pakāpes ūdens sagatavošanas sistēmas rada lielus ūdens zudumus. Saskaņā ar AS “Grindeks” pieredzi, augsto attīrītajam ūdenim noteikto tīrības prasību dēļ līdz pat 50 % no ieplūdes ūdens attīrītā ūdens sistēmas reversās osmozes moduļos tiek novadīti kanalizācijā kā notekūdeņi. Tomēr šis ūdens joprojām ir pietiekami tīrs, lai to atkārtoti izmantotu ražošanas tehnoloģiskajos procesos, kur ūdens nenonāk tiešā kontaktā ar farmaceitisko produktu, piemēram, dzesēšanas sistēmās, kas identificēts kā ūdens ietilpīgākais tehnoloģiskais process farmaceitisko produktu ražošanā. Konstatēts, ka, īstenojot šādu pieeju, uzņēmums varētu samazināt gada vidējo ūdens patēriņu par 7000 m^3, kas atbilst 12 % no kopējā ražošanā izmantotā dzeramā ūdens apjoma.

Izvērtējot bioloģiski attīrītu farmācijas notekūdeņu atkārtotas izmantošanas iespējas tehnoloģiskiem mērķiem, secināts, ka bez papildu trešējās attīrīšanas to otrreizēja izmantošana, piemēram, dzesēšanas sistēmās, nav iespējama šādu iemeslu dēļ:

1) augsta ūdenī izšķīdušo neorganisko sāļu satura dēļ šādu notekūdeņu izmantošana var veicināt cauruļvadu aizaugšanu un koroziju;

2) notekūdeņos atlikušās barības vielas un aktīvo dūņu daļiņas var veicināt bioplēves attīstību un nogulumu veidošanos ūdens sistēmās, tādējādi mazinot siltuma pārejas procesa efektivitāti;

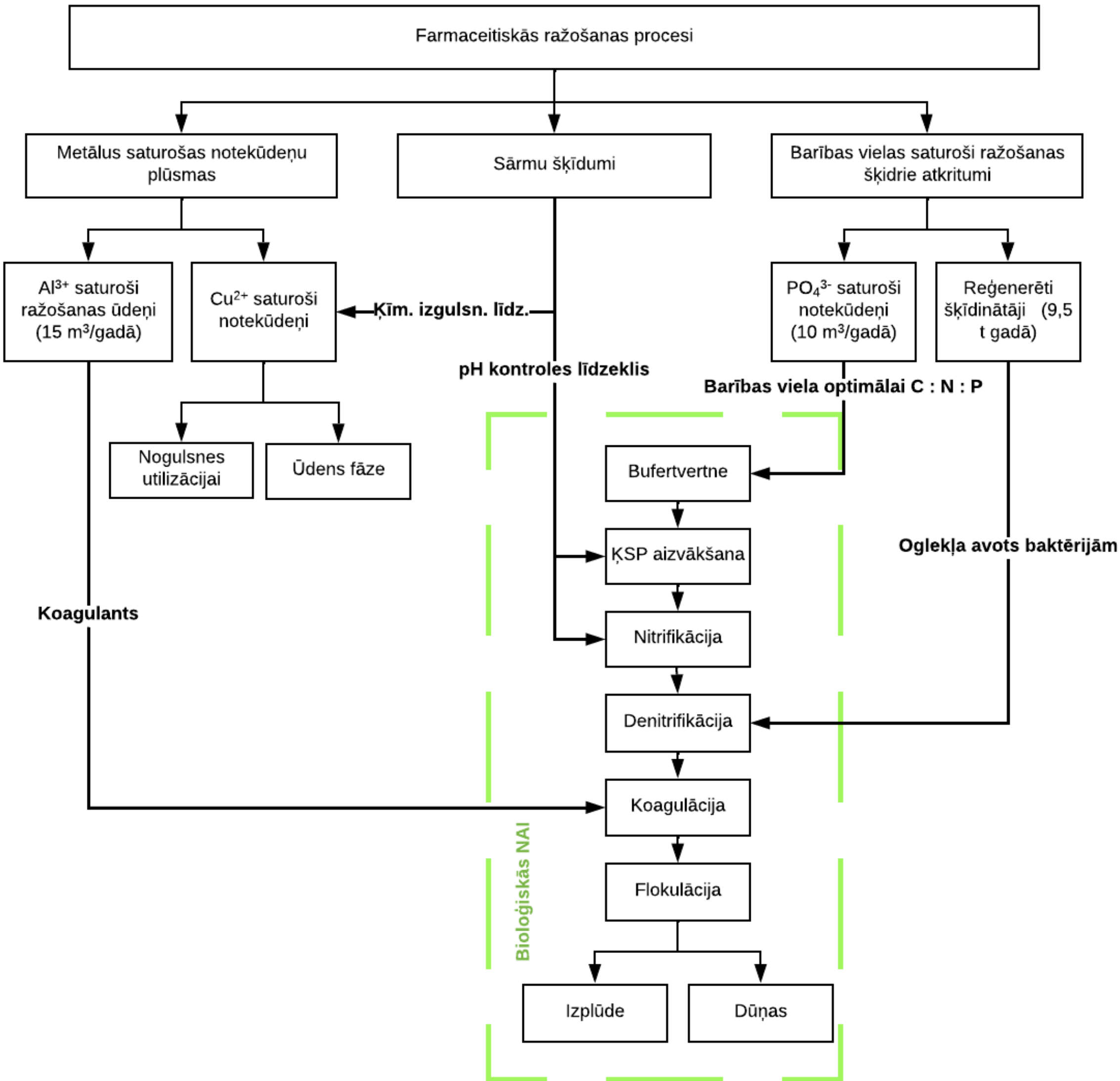
3) notekūdeņu temperatūra pēc bioloģiskās attīrīšanas var sasniegt pat 42 ºC, kas ir pārāk augsta, lai tos izmantotu dzesēšanas mērķim;

4) notekūdeņos palikušie ķīmiskie savienojumi var nebūt savietojami ar dzesēšanas sistēmās izmantotajiem materiāliem un var izraisīt neprognozējamas ūdens noplūdes un farmaceitisko produktu ķīmiskās un mikrobioloģiskās kontaminācijas riskus.

Fizikāli ķīmisko un termisko notekūdeņu attīrīšanas tehnoloģiju vai to kombināciju lietošana ļauj attīrīt farmaceitiskos notekūdeņus līdz tādai pakāpei, kas ļauj tos atkārtoti izmantot tehnoloģiskajos procesos, kur ūdens nenonāk kontaktā ar AFV vai GZF, tajā skaitā dzesēšanas sistēmās un tvaika ģeneratoros [59], [60]. Taču šādu tehnoloģiju ieviešanas pamatotība jāvērtē kontekstā ar lokālo ūdens pieejamību un izmaksām. Ņemot vērā to, ka, lai nodrošinātu attīrīto notekūdeņu kvalitātes atbilstību atkārtotas izmantošanas specifikācijai, ir jālieto ķīmiski un enerģētiski intensīvi notekūdeņu attīrīšanas procesi, atsevišķos gadījumos notekūdeņu atkārtota izmantošana var radīt lielāku ietekmi uz vidi nekā tīra ūdens izmantošana [61].

Neraugoties uz šiem ierobežojumiem, praksē ieviestie un pārbaudītie risinājumi, kas atspoguļoti **5. publikācijā**, parāda, ka aprites ekonomikas elementus var ieviest farmācijas notekūdeņu pārvaldības praksē, otrreizēji izmantojot specifiskas ražošanas ūdeņu plūsmas un ražošanas šķidros atkritumus kā resursus dažādās notekūdeņu bioloģiskajās attīrīšanas stadijās pat bez iepriekšējas attīrīšanas (6. att.), ja tiek nodrošināta efektīva sadarbība starp ražošanas nodaļu un NAI.

Kā uzsvērts iepriekšējā nodaļā, PO_4^{3-} saturošie notekūdeņi no AFV ipidakrīna ražošanas NAI tiek izmantoti kā P avots optimālās barības vielu attiecības nodrošināšanai aktīvo dūņu mikroorganismiem periodos, kad PO_4^{3-} saturs ieplūdes notekūdeņos ir zems. Al^{3+} saturoši ūdens šķīdumi no AFV milnaciprāna hidrogēnhlorīda sintēzes, kur kā Luisa skābi izmanto $AlCl_3$, tika identificēti kā izteikti toksiski aktīvo dūņu biocenozei (skat. nodaļu "Farmaceitiskās ražošanas ūdeņu toksicitātes novērtēšana"), tādēļ būtu jānodod utilizācijai kā bīstamie atkritumi. Atsevišķā pētījumā noteikts, ka šos ūdeņus var atkārtoti izmantot kā koagulantu liekā fosfora izgulsnēšanā un dūņu atdalīšanai bioloģiskajās NAI. Ņemot vērā šī brīža ražošanas jaudas, Al^{3+} saturošo ražošanas ūdeņu apjoms veido 15 m^3/gadā, un tie pilnā apjomā tiek izmantoti koagulācijas procesā, aizstājot komerciālo $FeCl_3$ koagulantu un tādējādi samazinot ķīmisko vielu patēriņu un notekūdeņu attīrīšanas izmaksas. Praksē pārbaudīts, ka organiskos šķīdinātājus, kas pēc vairākiem reģenerācijas cikliem vairs nav derīgi izmantošanai farmaceitiskās ražošanas procesos, var izmantot kā oglekļa avotu denitrifikācijas procesā, lai nodrošinātu NO_3^- un NO_2^- redukciju līdz N_2 gāzei. Gada laikā AS "Grindeks" šim mērķim izlieto ~ 9,5 t organisko šķīdinātāju (galvenokārt, etilspirta), kas veido ~ 3,5 % no rūpnīcā reģenerēto šķīdinātāju apjoma. Turklāt uzņēmuma pieredze liecina, ka koncentrētus sārmu šķīdumus, kas rodas ražošanas procesos, var savākt atsevišķi un izmantot pH regulācijai NAI bioreaktoros vai smago metālu, piemēram, vara izgulsnēšanai no citām ražotnes notekūdeņu plūsmām. Kopumā uzrādītie piemēri ļauj secināt, ka ražošanas šķidro atkritumu otrreizēja izmantošana atbilstoši aprites ekonomikas principiem ir izdevīga gan apkārtējās vides piesārņojuma mazināšanas, gan ekonomisko apsvērumu dēļ, jo ļauj samazināt gan utilizācijai nododamo bīstamo atkritumu apjomu un izmaksas, gan uzlabot notekūdeņu attīrīšanas efektivitāti un ķīmiski piesārņotu ūdens plūsmu pārvaldību farmācijas ražotnē.



6. att. Specifisku farmaceitiskās ražošanas ūdeņu atkārtotas izmantošanas iespējas.

SECINĀJUMI

1. Veicot GNAI darbības analīzi, kā galvenie farmaceitiskās ražošanas ūdeņu bioloģisko attīrīšanu ietekmējoši stresa faktori tika identificēti:
   1) toksicitāte, kas saistīta ar ražošanas ūdeņu ķīmiskā sastāva specifiku;
   2) nesabalansēts barības vielu saturs (> 93 % dienu gadā attiecība ĶSP : N_{kop} neatbilst optimālajam līmenim 100 : 5; analizētajā trīs gadu periodā 58–74 % dienu gadā PO_4-P saturs NAI ieplūdes ūdeņos < 5 mg/L);
   3) paaugstināta notekūdeņu temperatūra bioreaktoros (analizētajā trīs gadu periodā 2–3 % dienu gadā t > 39 ºC);
   4) plūsmas un koncentrācijas fluktuācijas (notekūdeņu plūsmas fluktuācijas: Q = 6–21 m^3/h, koncentrācijas fluktuācijas: ĶSP koncentrācija GNAI ieplūdes ūdeņos pārsniedz NAI projektēto slodzi 15–21 % dienu gadā, N_{kop} koncentrācija 26–28 % dienu gadā);
   5) pH svārstības NAI ieplūdes ūdeņos (pH = 4,3–12).
2. Izstrādāta jauna metode ķīmiski piesārņotu notekūdeņu toksicitātes uz aktīvo dūņu biocenozi novērtēšanai. Metode nodrošina to, ka, veicot ražošanas ūdeņu BSP mērījumus plašā sākumkoncentrāciju diapazonā, var iegūt eksperimentālās raksturlīknes, kas ļauj:
   1) identificēt aktīvo dūņu biocenozei toksiskās ūdens plūsmas;
   2) salīdzināt atšķirīgu ķīmiski piesārņotu ražošanas ūdeņu toksicitāti;
   3) prognozēt bioloģiskā notekūdeņu attīrīšanas procesa efektivitāti, ja tiek intensificēta ražošana un palielinās notekūdeņu apjoms vai specifisku piesārņojošo vielu koncentrācija notekūdeņos.
3. No promocijas darbā pētītajām mikroorganismu kultūrām kā piemērotākie kandidāti ĶSP un AFV degradācijas efektivitātes paaugstināšanai bioaugmentācijas ceļā ir identificēta baktērija *Sphingobacterium thalpophilum* un mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum*.
4. Papildu oglekļa avota (vājpiena pulvera) pievienošana būtiski uzlabo AFV meldonija dihidrāta degradāciju ar baktēriju *Sphingobacterium thalpophilum* un mikroskopiskajām sēnēm *Fusarium solani* un *Fusarium udum*.
5. Izstrādāti un GNAI ieviesti šādi aprites ekonomikas elementi:
   1) aktīvo dūņu biocenozei toksisko Al^{3+} saturošu ūdeņu no AFV milnaciprāna hidrogēnhlorīda ražošanas (V = 15 m^3/gadā) otrreizēja izmantošana aktīvo dūņu koagulācijas procesā un liekā P ķīmiskā izgulsnēšanā, aizstājot komerciālo $FeCl_3$;
   2) koncentrētu sārmu šķīdumu no farmaceitiskās ražošanas procesiem otrreizēja izmantošana optimālā pH nodrošināšanai dažādās notekūdeņu attīrīšanas procesa stadijās;
   3) fosfātus saturošu AFV ipidakrīna ķīmiskās sintēzes ūdeņu (C = 10 g/L PO_4-P) otrreizēja izmantošana optimālā P satura nodrošināšanai GNAI bioreaktoros, kas ļauj samazināt komerciālās H_3PO_4 patēriņu par 30 % gada laikā;

4) reģenerēto šķīdinātāju kā oglekļa avota otrreizēja izmantošana denitrifikācijas procesā, kas ļauj samazināt utilizācijai nododamo organisko šķīdinātāju apjomu par ~ 3,5 % gada laikā.

LITERATŪRAS SARAKSTS

[1] "Annex 2 WHO good manufacturing practices: water for pharmaceutical use 1." Accessed: May 18, 2023. [Online]. Available: https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/production/trs970-annex2-gmp-wate-pharmaceutical-use.pdf?sfvrsn=39eb16b8_0.

[2] C. Gadipelly *et al.*, "Pharmaceutical industry wastewater: Review of the technologies for water treatment and reuse," *Ind. Eng. Chem. Res.*, vol. 53, no. 29. American Chemical Society, pp. 11571–11592, Jul. 23, 2014. doi: 10.1021/ie501210j.

[3] A. M. Deegan *et al.*, "Treatment options for wastewater effluents from pharmaceutical companies," *Int. J. Environ. Sci. Tech*, vol. 8, no. 3, pp. 649–666, 2011.

[4] P. Loganathan, S. Vigneswaran, J. Kandasamy, A. K. Cuprys, Z. Maletskyi, and H. Ratnaweera, "Treatment Trends and Combined Methods in Removing Pharmaceuticals and Personal Care Products from Wastewater – A Review," *Membranes (Basel)*, vol. 13, no. 2, Feb. 2023, doi: 10.3390/membranes13020158.

[5] R. Changotra, H. Rajput, and A. Dhir, "Treatment of real pharmaceutical wastewater using combined approach of Fenton applications and aerobic biological treatment," *J. Photochem. Photobiol., A*, vol. 376, pp. 175–184, May 2019, doi: 10.1016/j.jphotochem.2019.02.029.

[6] A. khalidi-idrissi *et al.*, "Recent advances in the biological treatment of wastewater rich in emerging pollutants produced by pharmaceutical industrial discharges," *Int. J. Environ. Sci. Technol.* Institute for Ionics, 2023. doi: 10.1007/s13762-023-04867-z.

[7] Q. Y. Wu, Y. T. Zhou, W. Li, X. Zhang, Y. Du, and H. Y. Hu, "Underestimated risk from ozonation of wastewater containing bromide: Both organic byproducts and bromate contributed to the toxicity increase," *Water Res.*, vol. 162, pp. 43–52, Oct. 2019, doi: 10.1016/J.WATRES.2019.06.054.

[8] E. Raper, T. Stephenson, D. R. Anderson, R. Fisher, and A. Soares, "Industrial wastewater treatment through bioaugmentation," *Process Saf. Environ. Prot.*, vol. 118. Institution of Chemical Engineers, pp. 178–187, Aug. 01, 2018. doi: 10.1016/j.psep.2018.06.035.

[9] S. Saini, S. Tewari, J. Dwivedi, and V. Sharma, "Biofilm-mediated wastewater treatment: a comprehensive review," *Mater. Adv.* Royal Society of Chemistry, Feb. 13, 2023. doi: 10.1039/d2ma00945e.

[10] M. Smol, C. Adam, and M. Preisner, "Circular economy model framework in the European water and wastewater sector," *J. Mater. Cycles Waste Manage.*, vol. 22, no. 3, pp. 682–697, May 2020, doi: 10.1007/s10163-019-00960-z.

[11] K. Obaideen, N. Shehata, E. T. Sayed, M. A. Abdelkareem, M. S. Mahmoud, and A. G. Olabi, "The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline," *Energy Nexus*, vol. 7, p. 100112, Sep. 2022, doi: 10.1016/j.nexus.2022.100112.

[12] V. Lazarova, T. Asano, A. Bahri, and J. Anderson, *Milestones in water reuse : the best success stories*. IWA Publishing, 2013.

[13] European Commission, "European Union Strategic Approach to Pharmaceuticals in the Environment," 2019. Accessed: May 22, 2023. [Online]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52019DC0128&qid=1605854880622.

[14] European Commission, "Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL concerning urban wastewater treatment (recast)," 2022. Accessed: May 22, 2023. [Online]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52022PC0541.

[15] L.-E. Olsson, E. Strade, Ekenber Maria, E. Torresi, L. Quadri, and F. Morgan-Sagastume, "Il Sistema MBBR per il trattamento degli scarichi da industrie farmaceutiche: aspetti tecnici ed esperienze gestionali," in *La gestione degli impianti di depurazione MBBR*, M. Vaccari and G. Favali, Eds., Santarcangelo di Romagna: Maggioli Editore, 2021, pp. 172–183.

[16] T. Brinkmann, G. Giner Santonja, H. Yükseler, S. Roudier, and L. Delgado Sancho, "Best Available Techniques (BAT) Reference Document for Common Waste Water and Waste Gas Treatment/Management Systems in the Chemical Sector – Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control)," 2016.

[17] B. Cebere, E. Faltina, N. Zelcäns, and D. Kalnina, "Toxicity Tests For Ensuring Succesful Industrial Wastewater Treatment Plant Operation," *Environ. Clim. Technol.*, vol. 3, no. 3, pp. 41–47, 2009, doi: 10.2478/v10145-009-0005-8.

[18] M. Gutiérrez, J. Etxebarria, and L. de las Fuentes, "Evaluation of wastewater toxicity: comparative study between Microtox® and activated sludge oxygen uptake inhibition," *Water Res.*, vol. 36, no. 4, pp. 919–924, 2002, doi: https://doi.org/10.1016/S0043-1354(01)00299-8.

[19] G. Ricco, M. C. Tomei, R. Ramadori, and G. Laera, "Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox®," *Water Res.*, vol. 38, no. 8, pp. 2103–2110, 2004, doi: https://doi.org/10.1016/j.watres.2004.01.020.

[20] K. Ma, Z. Qin, Z. Zhao, C. Zhao, and S. Liang, "Toxicity evaluation of wastewater collected at different treatment stages from a pharmaceutical industrial park wastewater treatment plant," *Chemosphere*, vol. 158, pp. 163–170, 2016, doi: 10.1016/j.chemosphere.2016.05.052.

[21] K. Froehner, T. Backhaus, and L. H. Grimme, "Bioassays with Vibrio fischeri for the assessment of delayed toxicity," *Chemosphere*, vol. 40, no. 8, pp. 821–828, 2000, doi: https://doi.org/10.1016/S0045-6535(99)00274-X.

[22] E. Strade and D. Kalnina, "Cost Effective Method for Toxicity Screening of Pharmaceutical Wastewater Containing Inorganic Salts and Harmful Organic Compounds," *Environ. Clim. Technol.*, vol. 23, no. 1, pp. 52–63, Jan. 2019, doi: 10.2478/rtuect-2019-0004.

[23] S. A. Mirbagheri, M. Bagheri, S. Boudaghpour, M. Ehteshami, and Z. Bagheri, "Performance evaluation and modeling of a submerged membrane bioreactor treating combined municipal and industrial wastewater using radial basis function artificial

neural networks," *J. Environ. Health Sci. Eng*, vol. 13, no. 1, Mar. 2015, doi: 10.1186/s40201-015-0172-4.

[24] A. C. Bader, H. J. Hussein, and M. T. Jabar, "BOD: COD Ratio as Indicator for Wastewater and Industrial Water Pollution," *Int. J. Spec. Educ.*, vol. 37, no. 3, pp. 2164–2171, 2022.

[25] D. G. J. Larsson, "Pollution from drug manufacturing: Review and perspectives," *Philos. Trans. R. Soc. B: Biol. Sci.*, vol. 369, no. 1656. Royal Society of London, Nov. 19, 2014. doi: 10.1098/rstb.2013.0571.

[26] D. G. J. Larsson, C. de Pedro, and N. Paxeus, "Effluent from drug manufactures contains extremely high levels of pharmaceuticals," *J. Hazard. Mater.*, vol. 148, no. 3, pp. 751–755, Sep. 2007, doi: 10.1016/j.jhazmat.2007.07.008.

[27] M. Patel, R. Kumar, K. Kishor, T. Mlsna, C. U. Pittman, and D. Mohan, "Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods," *Chem. Rev.*, vol. 119, no. 6. American Chemical Society, pp. 3510–3673, Mar. 27, 2019. doi: 10.1021/acs.chemrev.8b00299.

[28] B. Dalecka, M. Strods, P. Cacivkins, E. Ziverte, G. K. Rajarao, and T. Juhna, "Removal of pharmaceutical compounds from municipal wastewater by bioaugmentation with fungi: An emerging strategy using fluidized bed pelleted bioreactor," *Environ. Adv.*, vol. 5, Oct. 2021, doi: 10.1016/j.envadv.2021.100086.

[29] H. Fang, Y. Jia, S. Zhou, L. Lu, L. Sun, and H. Lu, "A novel biotechnology for enhanced ciprofloxacin removal via bioaugmentation of Paraclostridium sp.," *Bioresour. Technol. Rep.*, vol. 20, Dec. 2022, doi: 10.1016/j.biteb.2022.101246.

[30] M. Herrero and D. C. Stuckey, "Bioaugmentation and its application in wastewater treatment: A review," *Chemosphere*, vol. 140. Elsevier Ltd, pp. 119–128, Dec. 01, 2015. doi: 10.1016/j.chemosphere.2014.10.033.

[31] M. Vuković Domanovac, M. Šabić Runjavec, and E. Meštrović, "Bioaugmentation effect of Aeromonas hydrophila and Pseudomonas putida on kinetics of activated sludge process in treating pharmaceutical industrial wastewater," *J. Chem. Technol. Biotechnol.*, vol. 94, no. 8, pp. 2721–2728, Aug. 2019, doi: 10.1002/jctb.6085.

[32] M. Neibergs, E. Strade, V. Nikolajeva, I. Susinskis, D. Rozitis, and D. Kalnina, "Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency," in *Key Eng. Mater.*, Trans Tech Publications Ltd, 2019, pp. 122–131. doi: 10.4028/www.scientific.net/KEM.800.122.

[33] J. B. Quintana, S. Weiss, and T. Reemtsma, "Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor," *Water Res*, vol. 39, no. 12, pp. 2654–2664, 2005, doi: 10.1016/j.watres.2005.04.068.

[34] A. Sellier, S. Khaska, and C. Le Gal La Salle, "Assessment of the occurrence of 455 pharmaceutical compounds in sludge according to their physical and chemical properties: A review," *J. Hazard. Mater.*, vol. 426. Elsevier B.V., Mar. 15, 2022. doi: 10.1016/j.jhazmat.2021.128104.

[35] C. Avanti *et al.*, "A new strategy to stabilize oxytocin in aqueous solutions: II. Suppression of cysteine-mediated intermolecular reactions by a combination of divalent metal ions and citrate," *Mol. Pharmaceutics*, vol. 9, no. 3, pp. 554–562, Mar. 2012, doi: 10.1021/mp200622z.

[36] K. Wiśniewski, J. Finnman, M. Flipo, R. Galyean, and C. D. Schteingart, "On the mechanism of degradation of oxytocin and its analogues in aqueous solution," *Biopolymers*, vol. 100, no. 4, pp. 408–421, Jul. 2013, doi: 10.1002/bip.22260.

[37] Grundfos BioBooster A/S, "Full scale advanced wastewater treatment at Herlev Hospital. Treatment performance and evaluation," 2016. Accessed: May 22, 2023. [Online]. Available: https://www.dhigroup.com/ global/news/2016/08/hospital-wastewater-from-a-pollution-problem-to-new-water-resources.

[38] D. G. Fujimori, "A novel enzymatic rearrangement," *Chem. Biol.*, vol. 17, no. 12. Elsevier Ltd, pp. 1269–1270, Dec. 22, 2010. doi: 10.1016/j.chembiol.2010.12.003.

[39] L. Henry, I. K. H. Leung, T. D. W. Claridge, and C. J. Schofield, "γ-Butyrobetaine hydroxylase catalyses a Stevens type rearrangement," *Bioorg. Med. Chem. Lett.*, vol. 22, no. 15, pp. 4975–4978, Aug. 2012, doi: 10.1016/j.bmcl.2012.06.024.

[40] I. K. H. Leung *et al.*, "Structural and mechanistic studies on γ-butyrobetaine hydroxylase," *Chem. Biol.*, vol. 17, no. 12, pp. 1316–1324, Dec. 2010, doi: 10.1016/j.chembiol.2010.09.016.

[41] K. Yoshisue *et al.*, "Pharmacokinetics and Biological Fate of 3-(2,2,2-Trimethylhydrazinium)Propionate Dihydrate (MET-88), a Novel Cardioprotective Agent, in Rats," *Drug Metab. Dispos.*, vol. 28, no. 6, p. 687, Jun. 2000.

[42] B. Dalecka, T. Juhna, and G. K. Rajarao, "Constructive use of filamentous fungi to remove pharmaceutical substances from wastewater," *J. Water Process. Eng.*, vol. 33, Feb. 2020, doi: 10.1016/j.jwpe.2019.100992.

[43] A. Pacholak, W. Smułek, A. Zgoła-Grześkowiak, and E. Kaczorek, "Nitrofurantoin—microbial degradation and interactions with environmental bacterial strains," *Int. J. Environ. Res. Public Health*, vol. 16, no. 9, May 2019, doi: 10.3390/ijerph16091526.

[44] D. Rozitis and E. Strade, "COD reduction ability of microorganisms isolated from highly loaded pharmaceutical wastewater pre-treatment process," *J. Mater. Environ. Sci.*, vol. 6, no. 2, pp. 507–512, 2015.

[45] I. Turtin, A. Vatansever, and F. Dilek Sanin, "Phosphorus deficiency and sludge bulking," *Environ. Technol.*, vol. 27, no. 6, pp. 613–621, 2006, doi: 10.1080/09593332708618677.

[46] J. Guo, Y. Peng, S. Wang, X. Yang, and Z. Yuan, "Filamentous and non-filamentous bulking of activated sludge encountered under nutrients limitation or deficiency conditions," *Chem. Eng. J.*, vol. 255, pp. 453–461, Nov. 2014, doi: 10.1016/j.cej.2014.06.075.

[47] F. Yang *et al.*, "Phosphorus deficiency leads to the loosening of activated sludge: The role of exopolysaccharides in aggregation," *Chemosphere*, vol. 290, Mar. 2022, doi: 10.1016/j.chemosphere.2021.133385.

[48] J. Bhathena, B. T. Driscoll, T. C. Charles, and F. S. Archibald, “Effects of Nitrogen and Phosphorus Limitation on the Activated Sludge Biomass in a Kraft Mill Biotreatment System,” *Water Environ. Res.*, vol. 78, no. 12, pp. 2303–2310, Nov. 2006, doi: 10.2175/106143006x95401.

[49] D. Cordell, J. O. Drangert, and S. White, “The story of phosphorus: Global food security and food for thought,” *Glob. Environ. Change*, vol. 19, no. 2, pp. 292–305, May 2009, doi: 10.1016/j.gloenvcha.2008.10.009.

[50] “European Commission, Study on the EU’s list of Critical Raw Materials – Final Report (2020)”, doi: 10.2873/11619.

[51] M. Smol *et al.*, “Strategies for sustainable and circular management of phosphorus in the baltic sea region: The holistic approach of the inPhos project,” *Sustainability (Switzerland)*, vol. 12, no. 6, Mar. 2020, doi: 10.3390/su12062567.

[52] E. Strade, D. Kalnina, and J. Kulczycka, “Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry,” *Water Resour. Ind.*, vol. 24, 2020, doi: 10.1016/j.wri.2020.100132.

[53] G. Qiu, Y. Song, P. Zeng, S. Xiao, and L. Duan, “Phosphorus recovery from fosfomycin pharmaceutical wastewater by wet air oxidation and phosphate crystallization,” *Chemosphere*, vol. 84, no. 2, pp. 241–246, 2011, doi: 10.1016/j.chemosphere.2011.04.011.

[54] W. Sun *et al.*, “Effective treatment of high phosphorus pharmaceutical wastewater by chemical precipitation,” *Can. J. Chem. Eng.*, vol. 95, no. 8, pp. 1585–1593, Aug. 2017, doi: 10.1002/cjce.22799.

[55] “EudraLex Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Annex 15: Qualification and Validation.,” 2015. Accessed: May 24, 2023. [Online]. Available: https://health.ec.europa.eu/system/files/2016-11/2015-10_annex15_0.pdf.

[56] “EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Part II: Basic Requirements for Active Substances used as Starting Materials,” 2014. Accessed: May 24, 2023. [Online]. Available: https://health.ec.europa.eu/system/files/2016-11/2014-08_gmp_part1_0.pdf.

[57] E. V. Sargent *et al.*, “The regulatory framework for preventing cross-contamination of pharmaceutical products: History and considerations for the future,” *Regul. Toxicol. Pharmacol.*, vol. 79, pp. S3–S10, Aug. 2016, doi: 10.1016/j.yrtph.2016.05.029.

[58] E. Medicines Agency, “Committee for Medicinal Products for Human Use (CHMP) Committee for Medicinal Products for Veterinary Use (CVMP) Guideline on the quality of water for pharmaceutical use,” 2020. Accessed: May 02, 2023. [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-water-pharmaceutical-use_en.pdf.

[59] A. J. Tóth, D. Fózer, P. Mizsey, P. S. Varbanov, and J. J. Klemeš, “Physicochemical methods for process wastewater treatment: powerful tools for circular economy in the chemical industry,” *Rev. Chem. Eng.*, 2022, doi: doi:10.1515/revce-2021-0094.

[60] K. Patel, R. Aghera, D. Mistry, and E. Jasrotia, “A Zero Liquid Discharge in Pharmaceutical Industry,” *Int. Res. J. Eng. Technol.*, vol. 4, no. 5, pp. 1110–1114, 2017.

[61] C. Baresel, L. Dalgren, M. Almemark, and A. Lazic, “Environmental performance of wastewater reuse systems: Impact of system boundaries and external conditions,” *Water Sci. Technol.*, vol. 73, no. 6, pp. 1387–1394, 2016, doi: 10.2166/wst.2015.624.

DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council on 17 October 2023 14:00 at the Faculty of Materials Science and Applied Chemistry of Riga Technical University, 3/7 Paula Valdena Street, Room 272.

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for review to Riga Technical University for promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis has not been submitted to any other university for promotion to a scientific degree.

Elīna Strade ……………………………. (signature)
Date: ………………………

The Doctoral Thesis has been prepared as a collection of thematically related scientific publications complemented by summaries in Latvian and English. The Doctoral Thesis unites five scientific publications. The scientific publications have been written in English, with a total volume of 65 pages.

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Appendix 5: Strade, E., Kalnina, D., Kulczycka, J. Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry. *Water Resour. Ind.* **2020**, 24, 100132.

Appendix 6: Characterization of pharmaceutical WWTP studied in the thesis (available under request). A: latvian, B: english.

ABBREVIATIONS

API	active pharmaceutical ingredient
BOD	biochemical oxygen demand
CFU	colony-forming unit
COD	chemical oxygen demand
EU	European Union
FDF	finished dosage form
GWWTP	wastewater treatment plant of JSC “Grindeks”
H	Shannon index
HPLC-MS	high-performance liquid chromatography-mass spectrometry
I.U.	International Unit
log K_{ow}	logarithm of the octanol-water partition coefficient
MP	skim milk powder
N_{tot}	total nitrogen
P_{tot}	total phosphorus
Q	flow, m^3/h
TOC	total organic carbon
WHO	World Health Organization
WWTP	wastewater treatment plant

GENERAL OVERVIEW OF THE THESIS

Topicality

According to the World Health Organization (WHO) data, water is the most used resource in the pharmaceutical industry [1]. It is used both as a raw material and solvent in the synthesis of active pharmaceutical ingredients (API) and in the production of finished dosage forms (FDF), as well as in the washing of equipment and for technical purposes-cooling and steam generation. The diverse use of water forces pharmaceutical factories to effectively manage both incoming and outgoing water flows in the production facility and to apply different treatment solutions in water preparation to guarantee that its quality meets regulatory requirements.

In the technological processes of pharmaceutical production, chemically polluted processing water flows occur, which differ significantly in terms of chemical composition, volume, and toxicity [2]–[4]. Depending on the specific API synthesis process, they may be polluted with various organic solvents, API residues, nitrogen-containing heterocyclic compounds, and various inorganic salts [2].

The high pollution concentration and flow fluctuations and the diverse composition of pollution of processing waters create multi-stress conditions. They can cause malfunctions in biological wastewater treatment plants (WWTP), negatively affecting their ability to provide a degree of wastewater treatment that meets the legislative requirements. Although biological treatment is one of the most commonly used technologies in pharmaceutical wastewater treatment [3], an increasing number of studies emphasize that biological WWTPs do not sufficiently treat wastewater from API residues and harmful compounds and, therefore, should be combined with physicochemical treatment technologies [4]–[6]. But this approach also has its limitations, as, for example, ozonation of processing water containing halogens can increase toxicity [7]. An alternative solution for increasing the efficiency of the biological treatment of processing waters may be the bioaugmentation of activated sludge systems with microorganism cultures [8], which, in turn, requires conducting a feasibility study, to determine the most suitable cultures for bioaugmentation. Additional challenges in the pharmaceutical industry are introduced by the changing demands for different APIs, which require a rapid reorientation of production and predicting how changes in production will affect the WWTP's ability to treat new types of pollution. Consequently, the development and implementation of new solutions that can be used to predict and preventively avoid or reduce the influence of various stress factors on the biological treatment process and improve the degree of wastewater treatment are highly topical.

The different range of raw materials used in API syntheses and the specificity of technological processes determine that the degree of pollution of pharmaceutical processing waters, the content of biogenic elements and salts can also differ significantly between production plants. However, the different engineering solutions applied in biological WWTPs can have different effects on the resilience of the system to various stress factors and changes in the composition of wastewater [9]. This requires the use of an individual approach also in evaluating the stress factors affecting the biological treatment and in developing the most suitable process optimization solutions.

The transition to a circular economy model in the field of wastewater treatment requires a focus not only on the reduction and treatment of industrial pollution but also on the recovery and reuse of water and chemicals [10]. Despite the fact that water reuse has been recognized as a key action to reduce water consumption in production plants and contribute to the achievement of sustainable development goals [11], [12], there is a lack of studies analyzing aspects of the implementation of the circular economy in a pharmaceutical plant using a holistic approach, evaluating them in the context of the specific water quality requirements set for the pharmaceutical industry; therefore it has been chosen as a topical research direction.

Aims and objectives

The aims of the Doctoral Thesis are:

1) develop solutions for increasing the efficiency of biological treatment of pharmaceutical processing waters under multi-stress conditions;

2) identify the possibilities and limitations of the reuse of processing waters in the pharmaceutical industry after evaluating the use of water in the pharmaceutical production plant and the quality requirements of the water used in pharmaceutical production according to Good Manufacturing Practice guidelines, Pharmacopoeia monographs, and the guidelines from European Medicines Agency and the World Health Organisation.

To achieve the aims, the following objectives have been set:

1) based on the analysis of the operation of WWTP of JSC “Grindeks” (GWWTP) over a period of 3 years, identify the main stress factors affecting the biological treatment of pharmaceutical processing waters;

2) develop a method for assessing the toxicity of chemically polluted processing water to activated sludge microorganisms;

3) in laboratory conditions, determine the most suitable microorganism cultures for bioaugmentation for increasing the efficiency of API and chemical oxygen demand (COD) degradation in biological WWTPs exposed to multi-stress conditions;

4) recommend alternative sources of P for ensuring the optimal level of nutrients in the process of biological treatment of pharmaceutical wastewater to reduce the dependence on commercial H_3PO_4.

Scientific novelty and main results

As part of the Doctoral Thesis, solutions for mitigating the effects of multi-stress in pharmaceutical WWTPs have been developed, which are based on the toxicity analysis of individual flows, the application of bioaugmentation strategy to increase the efficiency of API and COD degradation, and include proposals for the reuse of chemically polluted pharmaceutical processing water flows in accordance with the principles of the circular economy.

Within the framework of the Doctoral Thesis, a new method for evaluating the toxicity of chemically polluted wastewater to the biocenosis of activated sludge has been developed, which has been introduced at GWWTP and can also be adapted to the work of laboratories of other biological WWTPs treating chemically polluted industrial wastewater, especially in production plants that generate wastewater flows of variable chemical composition containing potentially toxic chemicals.

As a result of the conducted study, for the first time, the limitations of the reuse of pharmaceutical processing water in the pharmaceutical production plant were identified, highlighting the specific role of the pharmaceutical industry among the water-intensive industrial sectors. The solutions proposed and implemented in practice for the reuse of processing waters as chemicals in various stages of the biological treatment of pharmaceutical wastewater show a new approach to promote the circular flow of materials in the production plant, thus reducing the consumption of chemicals and the costs of wastewater treatment.

The selection of microorganism cultures suitable for bioaugmentation provides an opportunity for further optimization of WWTP operation through bioaugmentation in order to improve the efficiency of degradation of chemical pollution, especially API, which may be particularly relevant when introducing new regulatory requirements for the control of API emissions from pharmaceutical production plants based on the European Union (EU) strategic approach to pharmaceuticals in the environment [13], and increasing the responsibility of pharmaceutical manufacturers for the treatment of API pollution arising from the use of their products, in the context of the planned changes in the Urban Wastewater Treatment Directive 91/271/EEC [14].

Structure and volume of the Thesis

The Doctoral Thesis was prepared as a collection of thematically related scientific publications on a) the development of the method for assessing the toxicity of chemically polluted pharmaceutical processing waters on activated sludge biocenosis (**Publication 1**); b) the application of bioaugmentation strategy for increasing API treatment efficiency in biological WWTPs (**Publication 2**); c) possibilities of increasing COD removal efficiency using bioaugmentation with selective microorganism cultures (**Publication 3**); (d) circular economy-based phosphorus management (**Publication 4**); e) possibilities and limitations of reuse of processing waters in the pharmaceutical industry (**Publication 5**).

Publications and approbation of the Thesis

The main results of the Doctoral Thesis are presented in 5 scientific publications. The research results were presented at 4 scientific conferences.

Scientific publications:

1. **Strade, E.,** Kalnina, D. Cost Effective Method for Toxicity Screening of Pharmaceutical Wastewater Containing Inorganic Salts and Harmful Organic Compounds. *Environ. Clim. Technol.* **2019**, *23*, 52–63.
2. Neibergs, M., **Strade, E.**, Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *Key Eng. Mater.* **2019**, *800*, 122–131.
3. Rozitis, D., **Strade, E.** COD Reduction Ability of Microorganisms Isolated from Highly Loaded Pharmaceutical Wastewater Pre-Treatment Process. *J. Mater. Environ. Sci.* **2015**, 6, 507–512.
4. Smol, M., Preisner, M., Bianchini, A., Rossi, J., Hermann, L., Schaaf, T., Kruopienė, J., Pamakštys, K., Klavins, M., Ozola-Davidane, R., Kalnina, D., **Strade, E.**, Voronova, V., Pachel, K., Yang, X., Steenari, B.-M., Svanström, M. Strategies for Sustainable and Circular Management of Phosphorus in the Baltic Sea Region: The Holistic Approach of the InPhos Project. *Sustainability* **2020**, *12*, 2567.
5. **Strade, E.**, Kalnina, D., Kulczycka, J. Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry. *Water Resour. Ind.* **2020**, *24*, 100132.

Publication in a collective monograph:

Olsson, L. E., **Strade, E**., Ekenberg, E., Torresi, E, Quadri, L., Morgan-Sagastume F. Il Sistema MBBR per il trattamento degli scarichi da industrie farmaceutiche: aspetti tecnici ed esperienze gestionali. In: *La gestione degli impianti di depurazione MBBR*; Vaccari, M., Favali, G., Eds.; Maggioli Editore: Santarcangelo di Romagna, **2021**, pp. 172–183 (Italian).

Participation in scientific conferences:

1. Neibergs, M., **Strade, E.**, Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *59th International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry*. Riga, Latvia, 26 October **2018**.
2. **Strade, E**. The pollution of water with pharmaceutical residues: a growing environmental concern. *60th International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry.* Riga, Latvia, 24 October **2019**.
3. **Strade, E**. Biological nitrogen removal from pharmaceutical wastewater. *61st International Scientific Conference of Riga Technical University Section of Materials Science and Applied Chemistry*. Riga, Latvia, 23 October **2020**.
4. **Strade, E**., Kalnina, D., Kulczycka, J. Water Diversity and Problems in Water Re-use in Pharmaceutical Enterprises. *12th Eastern European Young Water Professionals Conference*. Riga, Latvia, 1–2 April, **2021**.

MAIN RESULTS OF THE THESIS

1. Identification of stress factors affecting the biological treatment of pharmaceutical processing waters

By conducting a study of the technological process of GWWTP and evaluating the monitoring data on the characteristics of the chemical and physical parameters of wastewater and the microbiological parameters of activated sludge, five main factors influencing the biological treatment of pharmaceutical wastewater were identified: toxicity, unbalanced nutrient content, flow and concentration fluctuations, elevated wastewater temperature, and pH fluctuations (Fig. 1). Each of these factors cause stress to activated sludge microorganisms and negatively affects the operation of the biological treatment process, but most often the effects of these factors occur simultaneously, creating multi-stress conditions.

The impact of certain stress factors, such as pH, flow, and concentration fluctuations, on the efficiency of the biological wastewater treatment process can be reduced, technologically regulated, or completely eliminated by providing appropriate technological solutions already during the design of WWTPs [15], [16]. However, wastewater characteristics such as toxicity and nutrient composition cannot be predicted in the long term, as the composition of processing water can change dramatically depending on changes in the range of manufactured products and global demand for specific pharmaceutical products.

The solutions developed in the Doctoral Thesis for optimizing the biological treatment process of pharmaceutical processing waters under multi-stress conditions are reflected in the scientific publications, the connection of which with the set aims and objectives of the Doctoral Thesis is shown in Fig. 1.

Data on the technological process of GWWTP, characteristics of the chemical and physical parameters of influent wastewater of GWWTP, as well as activated sludge microbiological parameters used for the identification of stress factors, are summarized in an appendix available under request.

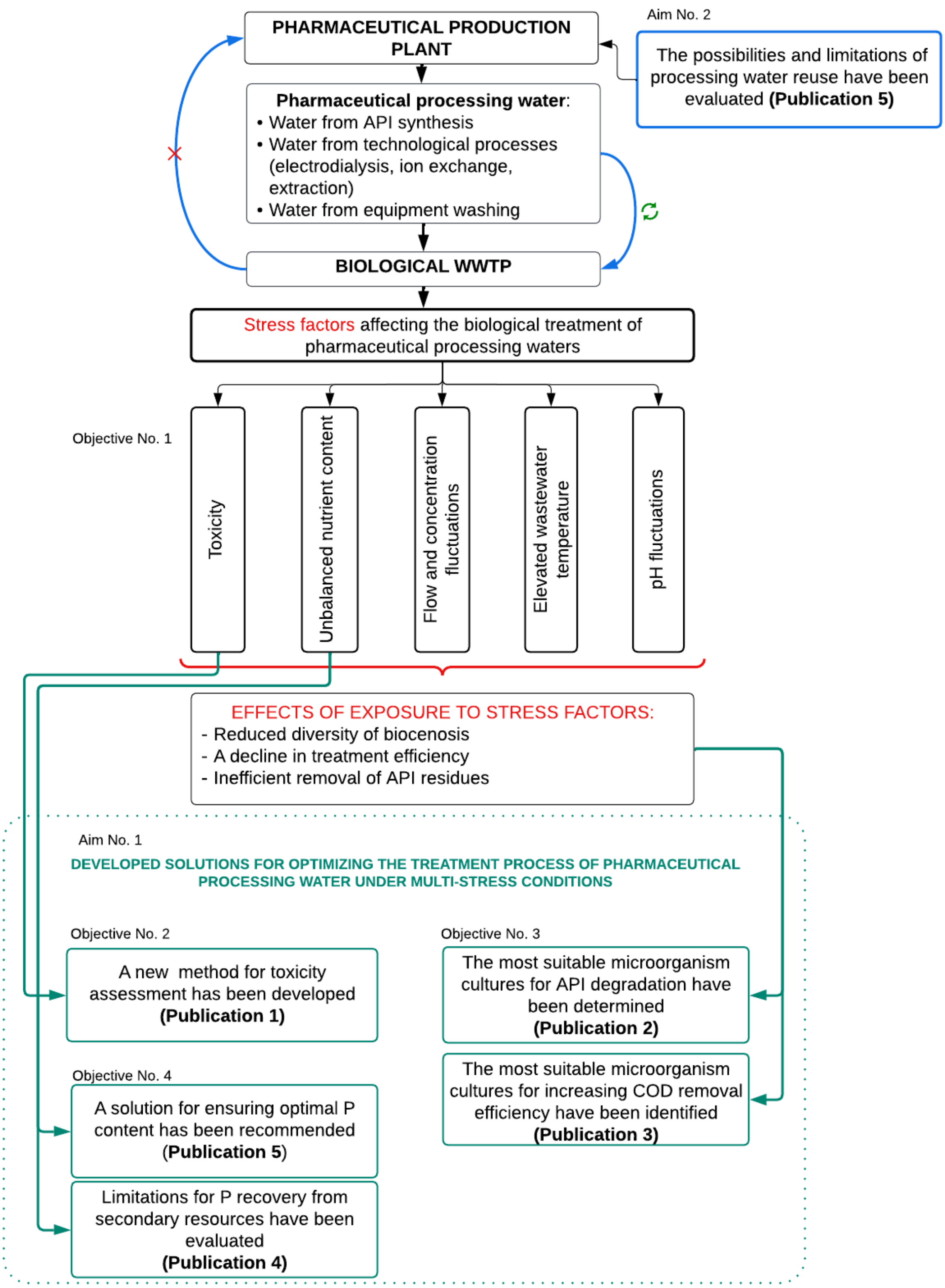


Fig. 1. Stress factors affecting the biological treatment of pharmaceutical processing waters and the structure of the Doctoral Thesis.

2. Assessment of the toxicity of pharmaceutical processing waters

The chemical characteristics of wastewater do not provide direct information about the toxicity of wastewater. Therefore, to prevent the death of activated sludge biocenosis under the influence of toxic substances and to avoid cases when the level of wastewater treatment does not comply with the requirements of the legislation, it is important, in addition to chemical analyses, to carry out toxicity tests and take preventive measures if toxicity is detected.

To objectively assess the toxicity of the influent water of WWTP, it is important to use activated sludge from a given treatment plant as test organisms since the result of the toxicity test depends on the test organism chosen and the reference function [17]. Studies have shown that test organisms not specific to the WWTP system, such as the luminescent bacteria *Vibrio qinghaiensis* and *Vibrio fischeri*, may show increased sensitivity, not reflecting the actual toxicity of the wastewater to the WWTP biocenosis [18]–[20], while in other cases, the short incubation time may lead to reduced toxicity results [21], resulting in an inadequate assessment of potential harm and toxicity risk.

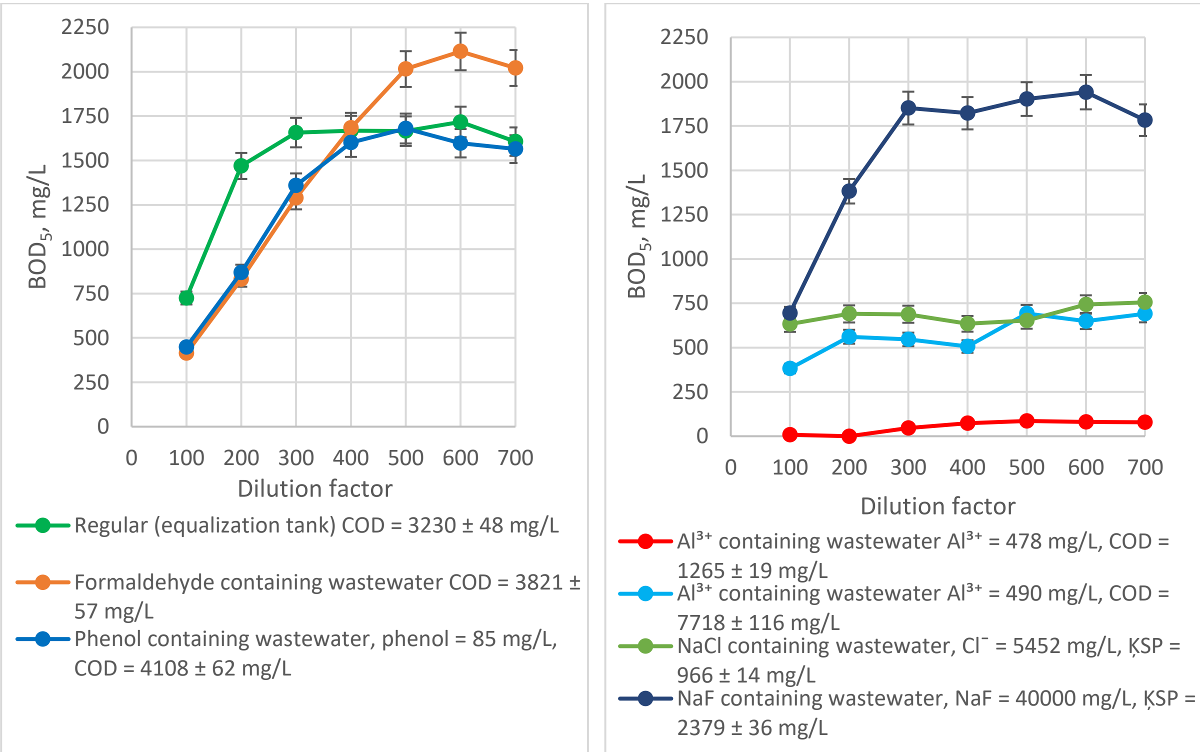
Publication 1 [22] presents the developed method for the systematic assessment of the toxicity and biodegradation potential of chemically polluted processing waters based on BOD measurements made in an extended range of initial concentrations. By applying this method, experimental characteristic curves are obtained that allow us to evaluate how the biodegradability and toxicity of wastewater to activated sludge microorganisms changes with dilution. If the BOD values increase markedly with increasing dilution, it indicates that the water contains substances that, in high concentrations, have an inhibitory effect on the activated sludge biocenosis and may cause disturbances in the treatment process. If the curve quickly reaches a plateau and the ratio BOD/COD is high (≥50 %), this indicates good biodegradability in the activated sludge system and a low level of toxicity [23]. Whereas, if the ratio BOD/COD remains low (<10 %) and does not change with dilution, then this indicates that the tested wastewater is toxic and the organic compounds in its composition are not biodegradable [24]; therefore, they cannot be discharged to biological WWTPs to avoid the toxic shock of microorganisms.

The application of the method is clearly demonstrated by analyzing the influent wastewater of GWWTP (from the buffer tank) and specific pharmaceutical processing water streams polluted with organic compounds and inorganic salts. The samples and the information on the main chemical pollutant and its concentration in the wastewater were received from the production department, and it was used to identify samples. Therefore, the COD analysis was used as a suitable indicator for the characterization of the total organic content of the wastewater.

Dilutions in the range from 100–700 were tested, which corresponds to the actual amount of wastewater generated per day in the manufacturing processes and their possible dilution in the buffer tank (equalization tank) of GWWTP. Seeded dilution water was used as a blank sample. The oxygen consumed over a 5-day incubation period of the blank sample shall not exceed 1.5 mg/L of oxygen. Tests were performed in duplicate.

Figure 2 a) shows that the BOD values of the analyzed buffer tank wastewater sample change little with dilution. An inhibitory effect on activated sludge microorganisms is observed at the dilution factor of 100, however the curve reaches the plateau phase already at the dilution factor of 200. The character of the curve shows that the wastewater is relatively well biodegradable, and no strong toxic effect on activated sludge microorganisms is expected. Depending on the degree of dilution, the ratio of BOD/COD varies from 22–53 % (Table 1).

The obtained experimental curves show that activated sludge microorganisms are strongly inhibited by pharmaceutical processing waters containing phenol and formaldehyde. As can be seen in Fig. 2 a), the character of the toxicity curves obtained during the testing of both wastewater flows is similar – the biodegradability strongly depends on the degree of dilution of the sample, and the curve reaches the plateau phase only at the dilution degree of 500 that corresponds to the degree of dilution of the wastewater sample in the test solution. To prevent the risks of intoxication of microorganisms, this type of wastewater, which is biodegradable but in high concentrations toxic to the activated sludge biocenosis, can be collected in separate reservoirs and dosed in a controlled manner in the total wastewater flow.



a) b)

Fig. 2. Toxicity characteristic curves for a) buffer tank wastewater, processing waters containing phenol and formaldehyde; b) processing waters containing inorganic salts.

When evaluating the toxicity of pharmaceutical wastewater containing inorganic salts, it was concluded that aqueous solutions containing Al^{3+} ions, which are produced in the synthesis of API milnacipran hydrochloride, where $AlCl_3$ is used as a Lewis acid, have a highly toxic effect on the biocenosis of activated sludge. Toxicity tests were performed on wastewater samples with different organic matter content (COD concentration) and practically the same

Al^{3+} concentration. As can be seen in Table 1, the ratio BOD/COD for both samples remains low even at a high degree of dilution of the sample and does not exceed 10 %, indicating that these wastewaters are practically non-degradable. Moreover, the toxicity does not change depending on the COD, which confirms that the toxic effect is caused by Al^{3+}. The obtained results allow us to conclude that wastewater containing Al^{3+} should not be let into biological treatment plants, as it can cause a toxic shock to microorganisms and destroy the activated sludge biocenosis. Such toxic and non-biodegradable wastewater streams must be separately collected at production sites and pre-treated prior to entering biological WWTPs or disposed of as hazardous waste.

Analysing NaCl-containing wastewater flow, no toxic effect on activated sludge microorganisms was detected when comparing at equal dilutions (Fig. 2 b)). This can be explained by the fact that the NaCl content in the tested wastewater sample corresponds to the salt concentration in the physiological solution and the BOD measurements were performed in a high dilution. The experimental results allow us to conclude that the organic matter in the wastewater has a good biodegradability – the BOD/COD ratio is greater than 65 % (Table 1). When performing a toxicity test for NaF containing wastewater flow, the inhibitory effect on activated sludge microorganisms was observed only at the dilution factor of 100 (Fig. 2 b)), but at the dilution factor of 200, the BOD/COD ratio already reached 58 % (Table 1), indicating that at this dilution, fluorides no longer affect the biodegradation of organic matter present in wastewater.

Table 1

BOD/COD ratio (%) depending on the degree of dilution of wastewater

Type of wastewater	COD, mg/L	BOD/COD ratio, %						
		Dilution factor						
		100	200	300	400	500	600	700
Regular wastewater (equalization tank)	3230 ± 48	22.4	45.4	51.3	51.6	51.5	53.2	49.7
Formaldehyde containing wastewater	3821 ± 57	10.8	21.7	33.7	44.1	52.7	55.3	52.9
Phenol containing wastewater Phenol = 85 mg/L	4108 ± 62	10.9	21.2	33.1	38.9	40.9	38.9	38.1
Al^{3+} containing wastewater Al^{3+} = 478 mg/L	1265 ± 19	0.6	0.5	3.6	5.8	6.8	6.3	6.3
Al^{3+} containing wastewater Al^{3+} = 490 mg/L	7718 ± 116	5.0	7.3	7.1	6.6	9.0	8.4	9.0
NaCl containing wastewater Cl^- = 5452 mg/L	966 ± 14	65.5	71.4	71.3	65.6	67.5	76.9	78.2
NaF containing wastewater NaF = 40000 mg/L	2379 ± 36	29.2	58.1	77.8	76.6	79.9	81.6	75.0

The data obtained during the experiment demonstrate that the ratio of BOD/COD for pharmaceutical processing waters can vary up to ten times at the same dilutions (Table 1). This confirms that waters from various technological processes differ significantly in their biodegradability and toxicity to activated sludge microorganisms, which is directly influenced by the different compositions of pollutants and the ecotoxicity of compounds.

The results show that the developed toxicity method is sufficiently sensitive and allows comparing different processing water flows to each other and identifying compounds toxic to WWTP biocenosis. The extended range of initial concentrations used in the testing shows at what dilution the toxicity of the wastewater changes, which can be used to predict the impact on the treatment process of increasing product production volumes. A significant advantage of the method is the relatively low cost and the ability to determine the effect of toxic substances on the biocenosis of specific treatment plants, using it as seed material.

3. Selection of microorganism cultures suitable for bioaugmentation to increase target API and COD degradation in pharmaceutical WWTPs

API residues that enter the environment with insufficiently treated pharmaceutical wastewater have received increased attention in recent decades, as studies have shown that pharmaceutical production plants can be an important point source of API emissions [25], [26]., Still these substances are biologically active even at very low concentrations. Several studies confirm that their release into the aquatic ecosystem leads to negative consequences for aquatic organisms and human health [27].

Bioaugmentation of activated sludge systems with selective cultures of microorganisms is one of the technologies that can be applied to increase the efficiency of API degradation in biological WWTPs of both municipal and pharmaceutical wastewaters [28], [29]. The selection of suitable cultures for bioaugmentation is one of the key aspects that determines the effectiveness of a bioaugmentation strategy. Cultures must not only provide a high ability to degrade target compounds but also be resistant to wastewater environmental conditions, toxicity, and be able to survive in competition with other activated sludge microorganisms [30].

The range of pollutants in pharmaceutical wastewater is very wide. Therefore, in order to ensure that bioaugmentation is effective and that the total water pollution indicators in the effluent of WWTP comply with the requirements of the legislation, the microorganism cultures used should not only provide a high degree of API treatment but also be able to effectively degrade other chemical compounds present in water. Studies by other authors confirm that the bioaugmentation strategy allows for intensifying the degree of treatment of pharmaceutical wastewater and improving the efficiency of COD degradation [31], therefore, within the framework of the Doctoral Thesis, the most suitable microorganisms for bioaugmentation were searched, which can provide both a high degree of degradation of the target API and effectively reduce COD, and would be suitable candidates for increasing the efficiency of wastewater treatment in GWWTP.

Publication 2 [32] of the Doctoral Thesis presents the screening results obtained by testing the ability of ten bacteria, ten yeasts and three filamentous fungi isolated from the GWWTP-activated sludge to degrade three APIs with different chemical structures and therapeutic effects – the cyclic nonapeptide oxytocin, the heterocyclic nitrogen compound zopiclone and a hydrazine derivative meldonium dihydrate (Table 2). These APIs were chosen as model compounds for the study because their microbiological degradation has not been studied so far, but according to the volume of production, they occupy a significant place in the product range of JSC “Grindeks”, and during their production, a large amount of chemically polluted processing water is generated. Table 2 clearly shows that the selected compounds differ significantly in terms of their chemical properties, which determine their behavior in water, i.e. water solubility and logarithmic values of octanol-water partition coefficient log K_{ow}.

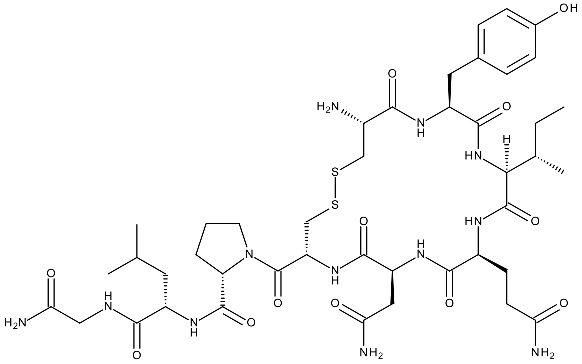
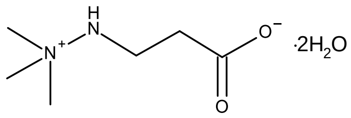
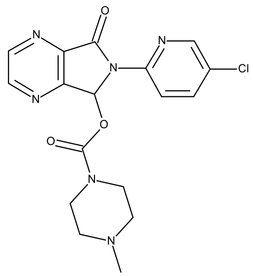
The research was conducted in laboratory-scale experiments, testing the ability of microorganisms to eliminate API as a sole carbon source and by cometabolism with the addition of skim milk powder (MP) as an additional nutrient. MP was chosen as an additional nutrient source based on the results published by Quintana et al., which demonstrate that the addition of powdered milk allows co-metabolic degradation of those APIs that could not be utilized as the sole source of carbon and energy [33]. API removal efficiency % during the 168 h incubation period determined by HPLC-MS analysis is shown in Tables 3 and 4. The chosen incubation period corresponds to the longest possible hydraulic retention time in GWWTP. The elimination of API from the solution is a total result of sorption and biodegradation. In our experiments these two processes were not differentiated; however, logarithmic values of octanol-water partition coefficient log K_{ow} for selected API are low (Table 2), indicating that sorption onto activated sludge is of minor importance in the removal process [34].

Most of the tested microorganism cultures, incl. bacteria – *Acinetobacter schindleri, Bacillus cereus, Chryseobacterium balustinum, Myroides odoratus, Sphingobacterium thalpophilum*; yeasts – *Apiotrichum montevideense, Cutaneotrichosporon arboriforme, Trichosporon asahii* and the filamentous fungus *Fusarium udum* within 168 h completely (>99 %) utilized all oxytocin in a solution where API was the sole carbon source. The addition of skim milk powder in a ratio of 1:10 to the starting concentration of API significantly improved the removal efficiency of oxytocin in biodegradation tests with the yeasts *Candida inconspicua 1, Cutaneotrichosporon cutaneum, Farysia acheniorum*, and the filamentous fungus *Talaromyces radicus.*

Studies show that the oxytocin molecule is not stable in aqueous solutions, and at elevated temperatures its degradation occurs, and biological activity is lost [35], [36]. During the bioaugmentation experiments, oxytocin degradation in the control sample without the addition of microorganisms was 12 % within 168 h, which confirms that microbiological degradation was still the primary form of oxytocin degradation in the conducted studies.

Table 2

APIs used in bioaugmentation studies

API	Chemical formula and structure	Molecular weight, g/mol	CAS No.	Solubility in water, g/L	log K_{ow}	Therapeutic class
Oxytocin	$C_{43}H_{66}N_{12}O_{12}S_2$ 	1007.19	50-56-6	12	–6.27	Exogenous hormone
Meldonium dihydrate	$C_6H_{14}O_2N_2 \cdot 2\ H_2O$ 	182.26	86426-17-7	20.2	0.45	Cardiovascular
Zopiclone	$C_{17}H_{17}ClN_6O_3$ 	388.81	43200-80-2	0.151	1.54	Sedative/ hypnotic nonbarbiturate

Limited information is available in the literature on the biodegradability of the heterocyclic nitrogen compound zopiclone. When treating hospital wastewater in a membrane bioreactor, its removal efficiency was less than 10 % [37]. Zopiclone removal efficiency varied significantly between different groups of microorganisms in our studies. The yeast *Apiotrichum domesticum* removed >99 % of zopiclone from the test solution in the presence of skim milk powder, while the other yeasts showed a limited ability to degrade zopiclone. *Pseudomonas putida* and *Moraxella osloensis* were identified as the most effective bacterial cultures for the selective degradation of zopiclone and showed 85 % and 89 % removal efficiency, respectively, over a 168 h incubation period. Whereas the filamentous fungi *Fusarium solani* and *Fusarium udum* removed more than 90 % of zopiclone both in tests where this API was the sole carbon source and in the presence of skim milk powder, indicating the high potential for further application of these cultures for the purpose of API degradation.

Meldonium dihydrate was identified as the most persistent compound against biodegradation. Most of the microorganism cultures tested could not degrade meldonium dihydrate as the sole carbon source. Yeast *Apiotrichum domesticum* degraded 65 % of meldonium dihydrate in 168 h in the presence of MP, while other yeast cultures showed low removal efficiency (<20 %). Out of all ten tested bacterial species, only *Sphingobacterium thalpophilum* showed a high removal efficiency of meldonium dihydrate in the presence of skim milk powder – 91 % within 168 h, while the other bacteria utilized meldonium dihydrate in the range of 1.3–40 % during the 168 h incubation period, both in test solutions where meldonium dihydrate was the sole carbon source, both in the presence of MP. Filamentous fungi *Fusarium solani* and *Fusarium udum* in test solutions, where meldonium dihydrate was the sole carbon source, showed 21 % and 46 % removal efficiency, respectively, but in the presence of skim milk powder, the removal efficiency increased significantly, reaching 91 % and 94 %, respectively.

Table 3

API removal efficiency (%) after 168 h incubation period in test solutions where API is the sole carbon source (initial concentration of API 20 mg/L) and in the presence of MP (initial concentration of API 5 mg/L) using bioaugmentation with bacterial cultures

	Oxy-tocin	Oxy-tocin + MP	Zopi-clone	Zopi-clone + MP	Meldonium dihydrate	Meldonium dihydrate + MP
Acinetobacter schindleri	>99	>99	54	82	4.7	35
Aeromonas caviae	99	99	59	71	1.3	1.8
Bacillus cereus	>99	>99	63	60	3.0	12
Chryseobacterium balustinum	>99	>99	68	81	5.2	13
Comamonas testosteroni	99	>99	63	67	12	23
Moraxella osloensis	69	57	89	91	14	40
Myroides odoratus	>99	>99	64	60	24	27
Pseudomonas aeruginosa	58	57	50	79	10	18
Pseudomonas putida	73	68	85	87	19	34
Sphingobacterium thalpophilum	>99	>99	75	84	17	91
Control sample without microorganisms	12	12	25	17	17	10

Although the degradation processes and metabolites of meldonium in the human and animal body have been relatively extensively studied [38]–[41], there are no studies in the literature that analyze the mechanisms of microbial degradation of this API in activated sludge systems or with selective microbial cultures and evaluate the role of cometabolism in the degradation process. The low results of meldonium elimination show that most of the studied microorganisms do not have the appropriate enzymes to degrade this API, but the microorganisms *Sphingobacterium thalpophilum, Fusarium solani,* and *Fusarium udum* can efficiently degrade meldonium only in the presence of an additional carbon source.

Table 4

API removal efficiency (%) after 168 h incubation period in test solutions where API is the sole carbon source (initial concentration of API 20 mg/L) and in the presence of MP (initial concentration of API 5 mg/L) using bioaugmentation with yeast and filamentous fungal cultures

	Oxy-tocin	Oxy-tocin + MP	Zopi-clone	Zopi-clone + MP	Meldonium dihydrate	Meldonium dihydrate + MP
Yeasts						
Apiotrichum domesticum	98	>99	42	>99	17	65
Apiotrichum montevideense	>99	>99	21	44	14	11
Candida inconspicua 1	13	51	29	35	6.7	8.5
Candida inconspicua 2	98	>99	56	26	12	3.5
Cutaneotrichosporon arboriforme	>99	>99	0.7	32	6.0	8.0
Cutaneotrichosporon cutaneum	1.0	7.1	16	29	1.6	15
Farysia acheniorum	11	>99	14	28	13	25
Rhodotorula mucilaginosa	91	61	16	47	13	14
Saprochaete gigas	45	81	64	59	7.6	16
Trichosporon asahii	>99	>99	7.7	24	4.4	2.8
Filamentous fungi						
Fusarium solani	99	>99	98	>99	21	91
Fusarium udum	>99	>99	>99	91	46	94
Talaromyces radicus	12	98	2.3	26	5.8	11
Control sample without microorganisms	12	12	25	17	17	10

Summarizing the obtained API degradation screening results, it was concluded that the most effective cultures for target API degradation are the bacterium *Sphingobacterium thalpophilum* and the filamentous fungi *Fusarium solani* and *Fusarium udum*. These microorganism cultures were able to eliminate all three tested APIs with an efficiency above 84 % over a 168 h incubation period.

In other API degradation studies, the fungus *Fusarium solani* has been identified as a suitable culture for the removal of API diclofenac and ketoprofen from wastewater [42], while the bacterium *Sphingobacterium thalpophilum* has shown the ability to degrade the antibiotic nitrofurantoin [43]. To the best of our knowledge, the role of *Fusarium udum* in increasing the efficiency of API degradation has not been investigated so far.

The experimental data summarized in **Publication 3** [44] show that among the 65 microorganism isolates tested, the bacterium *Sphingobacterium thalpophilum* and the filamentous fungi *Fusarium solani* and *Fusarium udum* are among those cultures that have also shown the highest COD reduction when treating pharmaceutical wastewater. Pharmaceutical wastewater samples for the tests were taken from the buffer tank of the GWWTP, into which processing waters from all production sites enter and mix before being discharged for treatment in the first bioreactor. Using such a complex sample, it is possible to investigate the ability of microorganisms to degrade a wide range of chemical compounds, which is an essential condition for bioaugmentation to be effective under changing production conditions.

As can be seen in the results summarized in Figs. 3 and 4, during 120 h of incubation, the bacterium *Sphingobacterium thalpophilum* and the filamentous fungi *Fusarium solani* and *Fusarium udum* reduced COD by 75.9 %, 89.4 %, and 88.7 % respectively. Since COD is a combined indicator of the level of organic pollution in wastewater and is also included in the regulatory requirements governing the maximum allowable pollutant concentrations in the discharge of WWTP, the high COD reduction rates obtained in microorganism screening tests confirm that the cultures of *Sphingobacterium thalpophilum*, *Fusarium solani* and *Fusarium udum* could be suitable candidates for increasing the treatment efficiency of pharmaceutical wastewater by bioaugmentation.

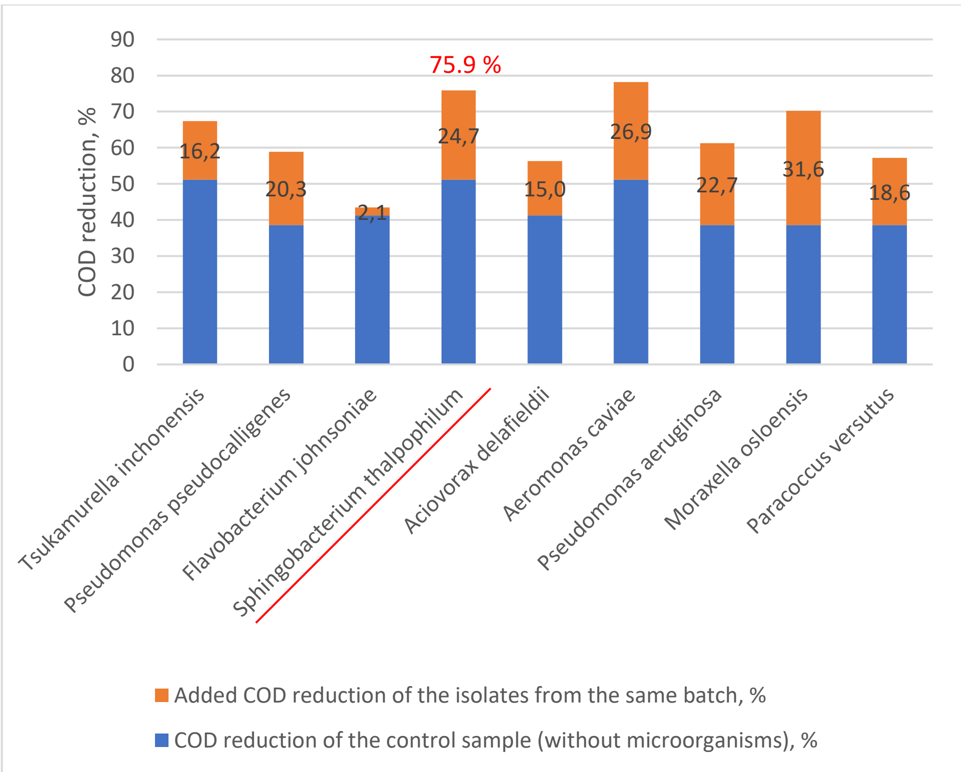


Fig. 3. COD reduction efficiency using bioaugmentation with bacterial cultures.

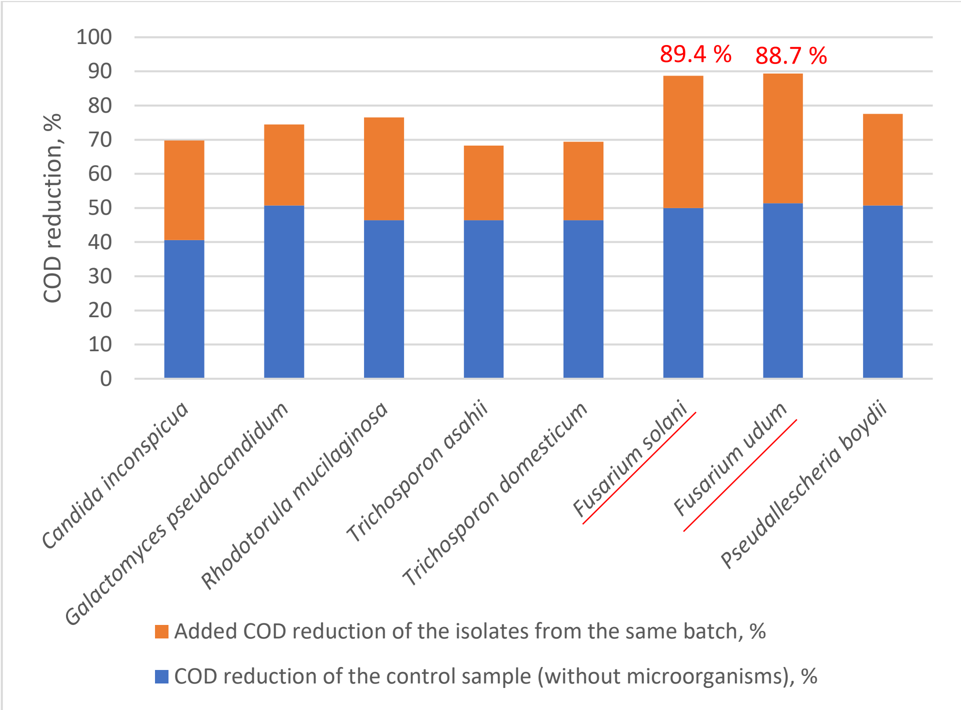


Fig. 4. Efficiency of COD reduction using bioaugmentation with yeast and filamentous fungal cultures.

4. Solutions to the problem of phosphorus deficiency in pharmaceutical wastewater biological treatment systems

According to the wastewater pollution monitoring data from GWWTP, the median COD and N_{tot} concentration of the influent water is 5500 mg/L and 160 mg/L, respectively, while the average PO_4-P content is only 3 mg/L, which is insufficient to ensure the optimum ratio COD : N : P of 100 : 5 : 1 for the treatment process. The lack of phosphorus in wastewater impairs the efficiency of biological treatment, promotes the development of filamentous bacteria (Fig. 5), and leads to an increase in the volume and structural change of sludge, which negatively affects its settleability and dewaterability [45]–[48]. To ensure an optimal P level for the biological treatment process, H_3PO_4 is usually used as an additional P source.

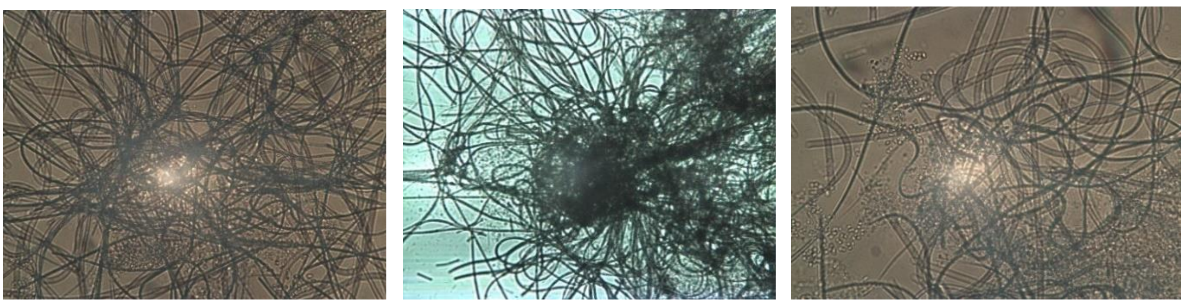


Fig. 5. P deficiency-induced dominance of filamentous bacteria in GWWTP activated sludge (magnification 100 x, light microscope Optika B-600 Tiph).

However, the availability of phosphoric acid has significantly decreased in recent years. One of the main reasons for this situation is the rapid depletion of natural P resources [49] and the fact that phosphate rock deposits in the world are located only in a few regions outside the European Union (EU), mainly in China, Morocco, the USA, and Russia, which makes the EU countries completely dependent on P compound imports and rising prices. Due to these circumstances, the phosphate rock and phosphorus used in the production of H_3PO_4 are included in the list of critical raw materials for the EU economy [50], and the need to recover P compounds from various waste streams and to search for the most optimal solutions for the transition to circular P management not only at the national but also at the regional level is particularly emphasized.

In **Publication 4** [51], analyzing various P flows and their current management practices in the countries of the Baltic Sea region, globally available technological solutions for the recovery of P compounds from secondary resources and the region-specific conditions, it was concluded that, despite the fact that several P-containing secondary resources have been identified, including wastewater, sewage sludge, and sewage sludge ash (Table 5), P recovery from them is not widely used in the countries of the Baltic Sea region, mainly due to the complex processing and high costs of installation and operation of P recovery technologies, as well as the lack of appropriate legislative framework [51].

Publication 5 [52] presents an alternative solution to the problem of P deficiency in GWWTP. It has been determined that the only technological process in which orthophosphate-rich production waters are generated (C = 10 g/L PO_4-P) in the production plant of JSC “Grindeks” is the synthesis of API ipidacrine. In order to avoid extreme peak loadings of phosphates and overdose of phosphorus during the production stage of this API, which lasts only several weeks a year, according to the recommendations provided within the work, these waters (~ 10 m^3 or ~ 50 % of the annual volume) are collected separately in the production site and reused as a P source during periods when the orthophosphate content in the GWWTP influent is low or insufficient. This practice allows to reduce the annual average consumption of H_3PO_4 by ~ 30 %. Currently, the use of the entire volume of ipidacrine synthesis waters is constrained by the limited area of the GWWTP, and the limited possibility of long-term storage of processing waters collected in separate containers for further use.

Table 5

Waste streams identified as possible secondary P sources

Sector	Secondary P sources
Industrial	Industrial wastewater, industrial sewage sludge, industrial sewage sludge ash, phosphogypsum, biomass ash
Agriculture	Manure, meat and bone meal, fish sludge
Municipal	Municipal wastewater, municipal sewage sludge, municipal sewage sludge ash, food waste

According to literature data, in the synthesis of APIs waters polluted with organic phosphorus compounds may also occur, which cannot be directly used as a bio-available source of phosphorus for activated sludge microorganisms. However, the research conducted with the waters of the synthesis of the antibiotic Fosfomycin shows that a suitable solution for the recovery of P from such streams of pharmaceutical processing waters is wet air oxidation to transform organic phosphorus compounds into inorganic phosphates, followed by precipitation in the form of hydroxyapatite and struvite [53].

Despite this, it has been concluded that P deficiency cannot be attributed to all pharmaceutical WWTPs. In contrast to the insufficient P content in processing waters characteristic of GWWTP, other studies have shown that the opposite situation may also occur, and the orthophosphate content in pharmaceutical processing waters can reach up to 380 mg/L (at COD and N_{tot} concentrations of 4200 mg/L and 410 mg/L, respectively), which eliminates the need to dose an additional P source in biological treatment reactors [54]. But the use of phosphate-rich processing water from one production plant in the WWTP of another production plant is not economically justified due to the high water transportation costs and complicated logistics.

5. Possibilities and limitations of reuse of processing waters in the pharmaceutical industry

The transition to a circular economy model in the field of wastewater treatment requires evaluating the possibility of recovering not only P but also other chemicals from wastewater and at the same time obtaining reusable water to reduce the consumption of resources and the impact of production processes on the environment.

Multi-stage batch production processes, the diversity of processing water flows, and the fragmentation of technological processes do not allow the application of a universal solution for water treatment and reuse in the pharmaceutical industry. However, the application of individual solutions for each water flow is technically difficult and expensive, therefore limiting the implementation of circular economy-based technologies on a large scale.

The specificity of the pharmaceutical industry also demands that any changes in production processes must be validated and accepted by the relevant regulatory authorities, as well as approved by industrial customers [55], which is time-consuming and costly and does not motivate pharmaceutical production plants to introduce changes in the technological processes of manufacturing pharmaceutical products.

As a result of the research carried out in **Publication 5** it is concluded that due to the high chemical and microbiological purity requirements set for water, the reuse of treated processing water is not possible in production processes where the water comes into direct contact with the pharmaceutical product. Depending on the stage in the manufacturing process at which water is used, the formulation, and the route of administration, the water must meet the quality requirements of drinking or pharmaceutical-grade water defined in the Pharmacopoeia (Table 6). The available wastewater treatment technologies cannot guarantee such a high degree of water treatment, but the contamination of pharmaceutical products can pose serious risks to the health of patients and is not allowed according to the good manufacturing practice requirements set for the industry [56]. It should be considered that in multi-product pharmaceutical factories, the residues of pharmaceutical substances also enter the processing water, which, when insufficiently treated water is reused, can pose risks of API cross-contamination. Since APIs are biologically active even at very low concentrations, product cross-contamination can critically impact the quality and safety of pharmaceutical products [57].

The study concluded that pharmaceutical-grade water preparation systems themselves cause significant water losses. According to the experience of JSC "Grindeks", due to the very high purity requirements of purified water, up to 50 % of the feed water in the reverse osmosis modules is discharged into the sewer as wastewater. However, this water is still clean enough to be reused in technological processes where water does not come into direct contact with pharmaceutical products, for example, in the cooling system, which is identified as the most water-intensive technological process in the production of pharmaceutical products. It was established that by implementing such an approach, the company could reduce the annual average water consumption by 7000 m^3, which corresponds to 12 % of the total volume of drinking water used in production.

Table 6

The quality requirements set for water used in pharmaceutical production and the relevant type of use [58]

Water grade	Quality requirements according to the European Pharmacopoeia	Applied treatment technologies	Usage in production
Potable water	Compliance with WHO guidelines for drinking water quality	Not specified	Chemical synthesis of intermediates of APIs;rinsing/cleaning of manufacturing equipment and facilities; production of pharmaceutical-grade water
Purified water	TOC < 500 µg/L Conductivity ≤ 4.3 µS/cm (20 ºC) Nitrate ≤ 0.2 mg/L Aerobic bacteria ≤ 100 CFU/mL	Ion exchange, ultrafiltration, reverse osmosis, distillation, activated carbon filtration	Final isolation and purification of APIs; preparation of nonsterile FDFs; a final rinse of equipment, containers, and closures in the manufacture of non-parenteral FDFs
Water for injections	TOC < 500 µg/L Conductivity ≤ 1.1 µS/cm (20 ºC) Nitrate ≤ 0.2 mg/L Aerobic bacteria ≤ 10 CFU/mL Bacterial endotoxins ≤ *I.U.*/mL	Reverse osmosis, electro-deionization, ultrafiltration, nanofiltration	Manufacturing of sterile pharmaceutical products

Evaluating the possibilities of reuse of biologically treated pharmaceutical wastewater for technological purposes, it was concluded that without additional tertiary treatment, their reuse, for example, in cooling systems, is not possible for the following reasons:

1) due to the high content of inorganic salts dissolved in water, the use of such wastewater can contribute to the scaling and corrosion of pipelines;

2) nutrients and activated sludge particles remaining in wastewater can contribute to the development of biofilm and the formation of sediments in water systems, thereby reducing the efficiency of the heat transfer process;

3) the temperature of wastewater after biological treatment can reach up to 42 °C, which is too high to be used for cooling purposes;

4) the chemical compounds remaining in the wastewater may not be compatible with the materials used in the cooling systems and may cause unpredictable risks of water leakage and chemical and microbiological contamination of pharmaceutical products.

The application of physico-chemical and thermal wastewater treatment technologies or their combinations allows to treat pharmaceutical wastewater to such a degree that it can be reused in technological processes where water does not come into contact with API or FDF, including cooling systems and steam generators [59], [60]. However, the feasibility of implementing such technologies should be evaluated in the context of local water availability and costs. Given that chemical and energy-intensive wastewater treatment processes need to be used to ensure that the quality of the treated wastewater meets the reuse specification, in some cases the reuse of wastewater can have a higher impact on the environment than the use of clean water [61].

Despite these limitations, the implemented and tested solutions presented in **Publication 5** show that elements of circular economy can be implemented in pharmaceutical wastewater management practice by reusing specific processing water streams and liquid wastes as resources in different stages of biological treatment of wastewater even without prior treatment (see Figure 6) if effective cooperation between the production department and the WWTP is ensured.

As highlighted in the previous section, the PO_4^{3-} containing processing water from the API ipidacrine production is used as a P source in WWTP to ensure the optimal nutrient ratio for activated sludge microorganisms during periods when the PO_4^{3-} content of the influent wastewater is low. Aqueous solutions containing Al^{3+} from the synthesis of API milnacipran hydrochloride, where $AlCl_3$ is used as a Lewis acid, have been identified as highly toxic to the biocenosis of activated sludge (see section "Assessment of the toxicity of pharmaceutical processing waters"), and should therefore be disposed of as hazardous waste. A separate study determined that these waters can be reused as a coagulant for the precipitation of excess phosphorus and the separation of sludge in biological WWTP. At the current production capacity, the volume of processing waters containing Al^{3+} is 15 m^3/year, and they are used in full in the coagulation process, replacing the commercial $FeCl_3$ coagulant and thus reducing the consumption of chemicals and the costs of wastewater treatment. It has been verified in practice that organic solvents, which after several regeneration cycles are no longer suitable for use in pharmaceutical production processes, can be used as a carbon source in the denitrification process to ensure the reduction of NO_3^- and NO_2^- to N_2 gas. During the year, JSC "Grindeks" uses ~ 9.5 t of organic solvents (mainly ethanol) for this purpose, which makes up ~ 3.5 % of the volume of solvents recovered at the production plant. The company's experience shows that concentrated alkaline solutions, resulting from production processes, can be collected separately and subsequently used for pH regulation in bioreactors of WWTP or for precipitation of heavy metals such as copper from other wastewater streams of the plant. In general, the presented examples allow us to conclude that the reuse of liquid waste from production in accordance with the principles of the circular economy is beneficial both for the reduction of environmental pollution and for economic reasons, as it allows to reduce both the volume and costs of hazardous waste to be disposed of, as well as to improve the efficiency of wastewater treatment and the management of chemically polluted water flows at the pharmaceutical production plant.

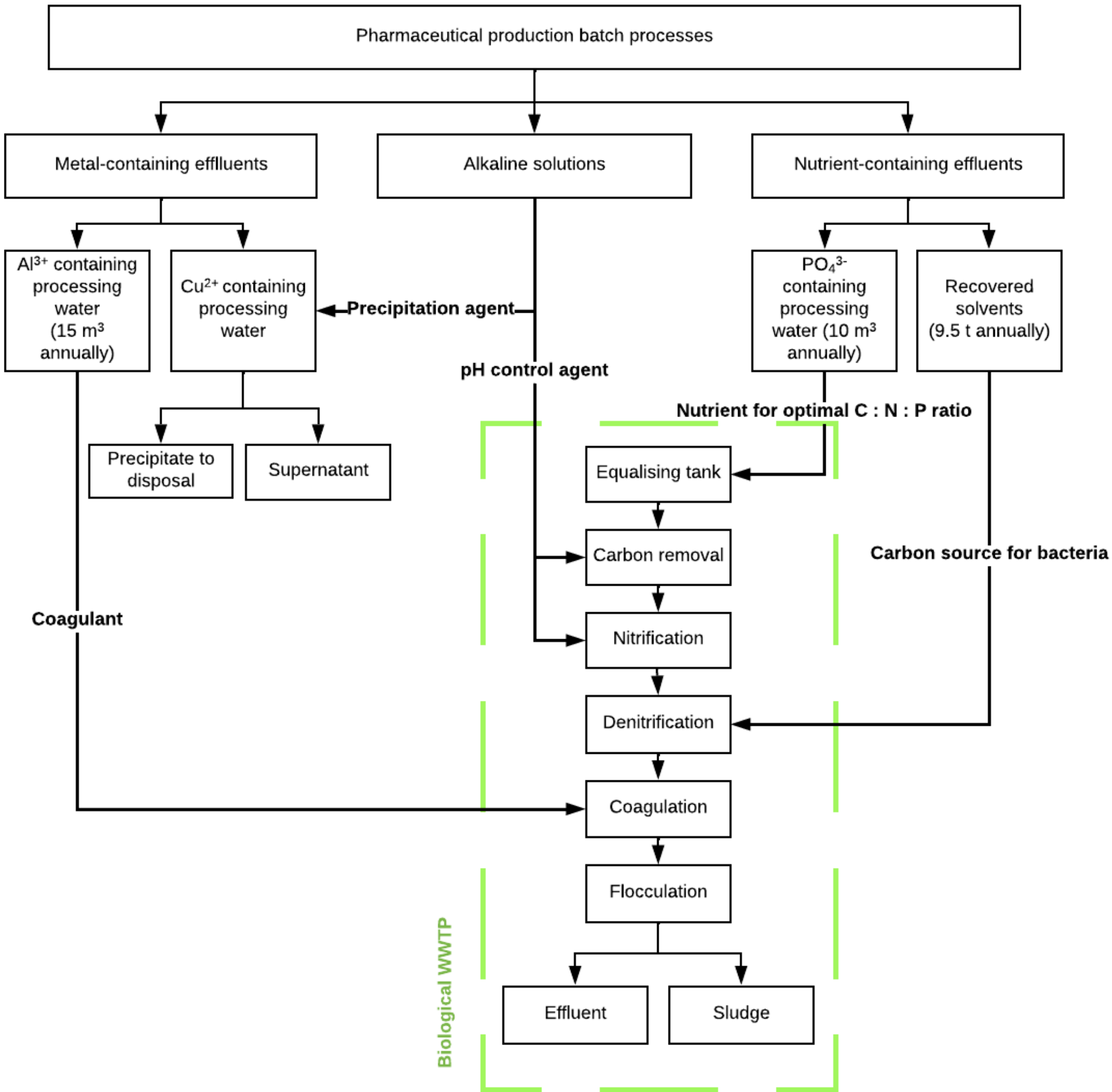


Fig. 6. Possibilities of reuse of specific pharmaceutical processing waters.

CONCLUSIONS

1. Analyzing the operation of GWWTP, the following main stress factors affecting the biological treatment of pharmaceutical production waters were identified:

1) toxicity associated with the specifics of the chemical composition of processing waters;
2) unbalanced nutrient content (>93 % of days per year the ratio COD : N_{tot} does not meet the optimal level of 100 : 5; 58–74 % of days per year PO_4-P concentration in the influent waters of WWTP < 5 mg/L in the analyzed 3-year period);
3) elevated wastewater temperature in bioreactors (2–3 % of days per year t > 39 °C in the analyzed 3-year period);
4) flow and concentration fluctuations (wastewater flow fluctuations: Q = 6–21 m^3/h, concentration fluctuations: COD concentration in GWWTP influent waters exceeds the designed load of GWWTP 15–21 % days per year, N_{tot} concentration 26–28 % of days per year);
5) pH fluctuations in the influent waters of WWTP (pH = 4.3–12).

2. A new method for assessing the toxicity of chemically polluted processing waters to the biocenosis of activated sludge has been developed. The method provides that by performing BOD measurements of processing waters in an extended range of initial concentrations, experimental characteristic curves can be obtained, which allow:

1) to identify toxic water flows to activated sludge biocenosis;
2) to compare different chemically polluted processing waters in terms of toxicity;
3) to predict the performance of the biological wastewater treatment process if production is intensified and the volume of wastewater or the concentration of specific pollutants increases.

3. It was found that among the microorganism cultures studied in the Doctoral Thesis, the bacterium *Sphingobacterium thalpophilum* and the filamentous fungi *Fusarium solani* and *Fusarium udum* are the most suitable candidates for increasing the efficiency of degradation of COD and API by bioaugmentation.

4. It was established that the addition of an additional carbon source (skim milk powder) significantly improves the degradation of API meldonium dihydrate by the bacteria *Sphingobacterium thalpophilum* and the filamentous fungi *Fusarium solani* and *Fusarium udum*.

5. The following circular economy elements have been developed and implemented in GWWTP:

1) reuse of Al^{3+} containing waters from the production of API milnacipran hydrochloride (V = 15 m^3/year), which is toxic to activated sludge biocenosis in the process of activated sludge coagulation and chemical precipitation of excess P, replacing commercial $FeCl_3$;
2) reuse of concentrated alkaline solutions from pharmaceutical production processes to ensure optimal pH in various stages of the wastewater treatment process, reducing the overall consumption of chemicals;

3) reuse of phosphate-containing waters from chemical synthesis of API ipidacrine (C = 10 g/L PO_4-P) to ensure optimal P content in bioreactors of GWWTP, which allows to reduce the consumption of commercial H_3PO_4 by 30 % annually;
4) reuse of the recovered solvents in the denitrification process as a carbon source, which allows for a reduction in the amount of organic solvents to be disposed of by ~ 3.5 % annually.

REFERENCES

[1] "Annex 2. WHO good manufacturing practices: water for pharmaceutical use 1." Accessed: May 18, 2023. [Online]. Available: https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/production/trs970-annex2-gmp-wate-pharmaceutical-use.pdf?sfvrsn=39eb16b8_0

[2] C. Gadipelly *et al.*, "Pharmaceutical industry wastewater: Review of the technologies for water treatment and reuse," *Ind. Eng. Chem. Res.*, vol. 53, no. 29. American Chemical Society, pp. 11571–11592, Jul. 23, 2014. doi: 10.1021/ie501210j.

[3] A. M. Deegan *et al.*, "Treatment options for wastewater effluents from pharmaceutical companies," *Int. J. Environ. Sci. Tech*, vol. 8, no. 3, pp. 649–666, 2011.

[4] P. Loganathan, S. Vigneswaran, J. Kandasamy, A. K. Cuprys, Z. Maletskyi, and H. Ratnaweera, "Treatment Trends and Combined Methods in Removing Pharmaceuticals and Personal Care Products from Wastewater –A Review," *Membranes (Basel)*, vol. 13, no. 2, Feb. 2023, doi: 10.3390/membranes13020158.

[5] R. Changotra, H. Rajput, and A. Dhir, "Treatment of real pharmaceutical wastewater using combined approach of Fenton applications and aerobic biological treatment," *J. Photochem. Photobiol., A*, vol. 376, pp. 175–184, May 2019, doi: 10.1016/j.jphotochem.2019.02.029.

[6] A. Khalidi-Idrissi *et al.*, "Recent advances in the biological treatment of wastewater rich in emerging pollutants produced by pharmaceutical industrial discharges," *Int. J. Environ. Sci. Technol.* Institute for Ionics, 2023. doi: 10.1007/s13762-023-04867-z.

[7] Q. Y. Wu, Y. T. Zhou, W. Li, X. Zhang, Y. Du, and H. Y. Hu, "Underestimated risk from ozonation of wastewater containing bromide: Both organic byproducts and bromate contributed to the toxicity increase," *Water Res.*, vol. 162, pp. 43–52, Oct. 2019, doi: 10.1016/J.WATRES.2019.06.054.

[8] E. Raper, T. Stephenson, D. R. Anderson, R. Fisher, and A. Soares, "Industrial wastewater treatment through bioaugmentation," *Process Saf. Environ. Prot.*, vol. 118. Institution of Chemical Engineers, pp. 178–187, Aug. 01, 2018. doi: 10.1016/j.psep.2018.06.035.

[9] S. Saini, S. Tewari, J. Dwivedi, and V. Sharma, "Biofilm-mediated wastewater treatment: a comprehensive review," *Mater. Adv.* Royal Society of Chemistry, Feb. 13, 2023. doi: 10.1039/d2ma00945e.

[10] M. Smol, C. Adam, and M. Preisner, "Circular economy model framework in the European water and wastewater sector," *J. Mater. Cycles Waste Manage.*, vol. 22, no. 3, pp. 682–697, May 2020, doi: 10.1007/s10163-019-00960-z.

[11] K. Obaideen, N. Shehata, E. T. Sayed, M. A. Abdelkareem, M. S. Mahmoud, and A. G. Olabi, "The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline," *Energy Nexus*, vol. 7, p. 100112, Sep. 2022, doi: 10.1016/j.nexus.2022.100112.

[12] V. Lazarova, T. Asano, A. Bahri, and J. Anderson, *Milestones in water reuse : the best success stories*. IWA Publishing, 2013.

[13] European Commission, “European Union Strategic Approach to Pharmaceuticals in the Environment,” 2019. Accessed: May 22, 2023. [Online]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52019DC0128&qid=1605854880622
[14] European Commission, “Proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL concerning urban wastewater treatment (recast),” 2022. Accessed: May 22, 2023. [Online]. Available: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52022PC0541
[15] L.-E. Olsson, E. Strade, Ekenber Maria, E. Torresi, L. Quadri, and F. Morgan-Sagastume, “Il Sistema MBBR per il trattamento degli scarichi da industrie farmaceutiche: aspetti tecnici ed esperienze gestionali,” in *La gestione degli impianti di depurazione MBBR*, M. Vaccari and G. Favali, Eds., Santarcangelo di Romagna: Maggioli Editore, 2021, pp. 172–183.
[16] T. Brinkmann, G. Giner Santonja, H. Yükseler, S. Roudier, and L. Delgado Sancho, “Best Available Techniques (BAT) Reference Document for Common Waste Water and Waste Gas Treatment/Management Systems in the Chemical Sector – Industrial Emissions Directive 2010/75/EU (Integrated Pollution Prevention and Control),” 2016.
[17] B. Cebere, E. Faltina, N. Zelcäns, and D. Kalnina, “Toxicity Tests for Ensuring Successful Industrial Wastewater Treatment Plant Operation,” *Environ. Clim. Technol.*, vol. 3, no. 3, pp. 41–47, 2009, doi: 10.2478/v10145-009-0005-8.
[18] M. Gutiérrez, J. Etxebarria, and L. de las Fuentes, “Evaluation of wastewater toxicity: comparative study between Microtox® and activated sludge oxygen uptake inhibition,” *Water Res.*, vol. 36, no. 4, pp. 919–924, 2002, doi: https://doi.org/10.1016/S0043-1354(01)00299-8.
[19] G. Ricco, M. C. Tomei, R. Ramadori, and G. Laera, “Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox®,” *Water Res.*, vol. 38, no. 8, pp. 2103–2110, 2004, doi: https://doi.org/10.1016/j.watres.2004.01.020.
[20] K. Ma, Z. Qin, Z. Zhao, C. Zhao, and S. Liang, “Toxicity evaluation of wastewater collected at different treatment stages from a pharmaceutical industrial park wastewater treatment plant,” *Chemosphere*, vol. 158, pp. 163–170, 2016, doi: 10.1016/j.chemosphere.2016.05.052.
[21] K. Froehner, T. Backhaus, and L. H. Grimme, “Bioassays with Vibrio fischeri for the assessment of delayed toxicity,” *Chemosphere*, vol. 40, no. 8, pp. 821–828, 2000, doi: https://doi.org/10.1016/S0045-6535(99)00274-X.
[22] E. Strade and D. Kalnina, “Cost Effective Method for Toxicity Screening of Pharmaceutical Wastewater Containing Inorganic Salts and Harmful Organic Compounds,” *Environ. Clim. Technol.*, vol. 23, no. 1, 2019, doi: 10.2478/rtuect-2019-0004.
[23] S. A. Mirbagheri, M. Bagheri, S. Boudaghpour, M. Ehteshami, and Z. Bagheri, “Performance evaluation and modeling of a submerged membrane bioreactor treating combined municipal and industrial wastewater using radial basis function artificial

neural networks," *J. Environ. Health Sci. Eng*, vol. 13, no. 1, Mar. 2015, doi: 10.1186/s40201-015-0172-4.

[24] A. C. Bader, H. J. Hussein, and M. T. Jabar, "BOD: COD Ratio as Indicator for Wastewater and Industrial Water Pollution," *Int. J. Spec. Educ.*, vol. 37, no. 3, pp. 2164–2171, 2022.

[25] D. G. J. Larsson, "Pollution from drug manufacturing: Review and perspectives," *Philos. Trans. R. Soc. B: Biol. Sci.*, vol. 369, no. 1656. Royal Society of London, Nov. 19, 2014. doi: 10.1098/rstb.2013.0571.

[26] D. G. J. Larsson, C. de Pedro, and N. Paxeus, "Effluent from drug manufactures contains extremely high levels of pharmaceuticals," *J. Hazard. Mater.*, vol. 148, no. 3, pp. 751–755, Sep. 2007, doi: 10.1016/j.jhazmat.2007.07.008.

[27] M. Patel, R. Kumar, K. Kishor, T. Mlsna, C. U. Pittman, and D. Mohan, "Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods," *Chem. Rev.*, vol. 119, no. 6. American Chemical Society, pp. 3510–3673, Mar. 27, 2019. doi: 10.1021/acs.chemrev.8b00299.

[28] B. Dalecka, M. Strods, P. Cacivkins, E. Ziverte, G. K. Rajarao, and T. Juhna, "Removal of pharmaceutical compounds from municipal wastewater by bioaugmentation with fungi: An emerging strategy using fluidized bed pelleted bioreactor," *Environ. Adv.*, vol. 5, Oct. 2021, doi: 10.1016/j.envadv.2021.100086.

[29] H. Fang, Y. Jia, S. Zhou, L. Lu, L. Sun, and H. Lu, "A novel biotechnology for enhanced ciprofloxacin removal via bioaugmentation of Paraclostridium sp.," *Bioresour. Technol. Rep.*, vol. 20, Dec. 2022, doi: 10.1016/j.biteb.2022.101246.

[30] M. Herrero and D. C. Stuckey, "Bioaugmentation and its application in wastewater treatment: A review," *Chemosphere*, vol. 140. Elsevier Ltd, pp. 119–128, Dec. 01, 2015. doi: 10.1016/j.chemosphere.2014.10.033.

[31] M. Vuković Domanovac, M. Šabić Runjavec, and E. Meštrović, "Bioaugmentation effect of Aeromonas hydrophila and Pseudomonas putida on kinetics of activated sludge process in treating pharmaceutical industrial wastewater," *J. Chem. Technol. Biotechnol.*, vol. 94, no. 8, pp. 2721–2728, Aug. 2019, doi: 10.1002/jctb.6085.

[32] M. Neibergs, E. Strade, V. Nikolajeva, I. Susinskis, D. Rozitis, and D. Kalnina, "Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency," in *Key Eng. Mater.*, 2019. doi: 10.4028/www.scientific.net/KEM.800.122.

[33] J. B. Quintana, S. Weiss, and T. Reemtsma, "Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor," *Water Res*, vol. 39, no. 12, pp. 2654–2664, 2005, doi: 10.1016/j.watres.2005.04.068.

[34] A. Sellier, S. Khaska, and C. Le Gal La Salle, "Assessment of the occurrence of 455 pharmaceutical compounds in sludge according to their physical and chemical properties: A review," *J. Hazard. Mater.*, vol. 426. Elsevier B.V., Mar. 15, 2022. doi: 10.1016/j.jhazmat.2021.128104.

[35] C. Avanti *et al.*, "A new strategy to stabilize oxytocin in aqueous solutions: II. Suppression of cysteine-mediated intermolecular reactions by a combination of divalent

metal ions and citrate," *Mol. Pharmaceutics*, vol. 9, no. 3, pp. 554–562, Mar. 2012, doi: 10.1021/mp200622z.

[36] K. Wiśniewski, J. Finnman, M. Flipo, R. Galyean, and C. D. Schteingart, "On the mechanism of degradation of oxytocin and its analogues in aqueous solution," *Biopolymers*, vol. 100, no. 4, pp. 408–421, Jul. 2013, doi: 10.1002/bip.22260.

[37] Grundfos BioBooster A/S, "Full scale advanced wastewater treatment at Herlev Hospital. Treatment performance and evaluation," 2016. Accessed: May 22, 2023. [Online]. Available: https://www.dhigroup.com/ global/news/2016/08/hospital-wastewater-from-a-pollution-problem-to-new-water-resources

[38] D. G. Fujimori, "A novel enzymatic rearrangement," *Chem. Biol.*, vol. 17, no. 12. Elsevier Ltd, pp. 1269–1270, Dec. 22, 2010. doi: 10.1016/j.chembiol.2010.12.003.

[39] L. Henry, I. K. H. Leung, T. D. W. Claridge, and C. J. Schofield, "γ-Butyrobetaine hydroxylase catalyses a Stevens type rearrangement," *Bioorg. Med. Chem. Lett.*, vol. 22, no. 15, pp. 4975–4978, Aug. 2012, doi: 10.1016/j.bmcl.2012.06.024.

[40] I. K. H. Leung *et al.*, "Structural and mechanistic studies on γ-butyrobetaine hydroxylase," *Chem. Biol.*, vol. 17, no. 12, pp. 1316–1324, Dec. 2010, doi: 10.1016/j.chembiol.2010.09.016.

[41] K. Yoshisue *et al.*, "Pharmacokinetics and Biological Fate of 3-(2,2,2-Trimethylhydrazinium)Propionate Dihydrate (MET-88), a Novel Cardioprotective Agent, in Rats," *Drug Metab. Dispos.*, vol. 28, no. 6, p. 687, Jun. 2000.

[42] B. Dalecka, T. Juhna, and G. K. Rajarao, "Constructive use of filamentous fungi to remove pharmaceutical substances from wastewater," *J. Water Process. Eng.*, vol. 33, Feb. 2020, doi: 10.1016/j.jwpe.2019.100992.

[43] A. Pacholak, W. Smułek, A. Zgoła-Grześkowiak, and E. Kaczorek, "Nitrofurantoin – microbial degradation and interactions with environmental bacterial strains," *Int. J. Environ. Res. Public Health*, vol. 16, no. 9, May 2019, doi: 10.3390/ijerph16091526.

[44] D. Rozitis and E. Strade, "COD reduction ability of microorganisms isolated from highly loaded pharmaceutical wastewater pre-treatment process," *J. Mater. Environ. Sci.*, vol. 6, no. 2, pp. 507–512, 2015.

[45] I. Turtin, A. Vatansever, and F. Dilek Sanin, "Phosphorus deficiency and sludge bulking," *Environ. Technol.*, vol. 27, no. 6, pp. 613–621, 2006, doi: 10.1080/09593332708618677.

[46] J. Guo, Y. Peng, S. Wang, X. Yang, and Z. Yuan, "Filamentous and non-filamentous bulking of activated sludge encountered under nutrients limitation or deficiency conditions," *Chem. Eng. J.*, vol. 255, pp. 453–461, Nov. 2014, doi: 10.1016/j.cej.2014.06.075.

[47] F. Yang *et al.*, "Phosphorus deficiency leads to the loosening of activated sludge: The role of exopolysaccharides in aggregation," *Chemosphere*, vol. 290, Mar. 2022, doi: 10.1016/j.chemosphere.2021.133385.

[48] J. Bhathena, B. T. Driscoll, T. C. Charles, and F. S. Archibald, "Effects of Nitrogen and Phosphorus Limitation on the Activated Sludge Biomass in a Kraft Mill Biotreatment

System," *Water Environ. Res.*, vol. 78, no. 12, pp. 2303–2310, Nov. 2006, doi: 10.2175/106143006x95401.

[49] D. Cordell, J. O. Drangert, and S. White, "The story of phosphorus: Global food security and food for thought," *Glob. Environ. Change*, vol. 19, no. 2, pp. 292–305, May 2009, doi: 10.1016/j.gloenvcha.2008.10.009.

[50] "European Commission, Study on the EU's list of Critical Raw Materials – Final Report (2020)", doi: 10.2873/11619.

[51] M. Smol *et al.*, "Strategies for sustainable and circular management of phosphorus in the baltic sea region: The holistic approach of the inPhos project," *Sustainability (Switzerland)*, vol. 12, no. 6, Mar. 2020, doi: 10.3390/su12062567.

[52] E. Strade, D. Kalnina, and J. Kulczycka, "Water efficiency and safe re-use of different grades of water-Topical issues for the pharmaceutical industry," *Water Resour. Ind.*, vol. 24, 2020, doi: 10.1016/j.wri.2020.100132.

[53] G. Qiu, Y. Song, P. Zeng, S. Xiao, and L. Duan, "Phosphorus recovery from fosfomycin pharmaceutical wastewater by wet air oxidation and phosphate crystallization," *Chemosphere*, vol. 84, no. 2, pp. 241–246, 2011, doi: 10.1016/j.chemosphere.2011.04.011.

[54] W. Sun *et al.*, "Effective treatment of high phosphorus pharmaceutical wastewater by chemical precipitation," *Can. J. Chem. Eng.*, vol. 95, no. 8, pp. 1585–1593, Aug. 2017, doi: 10.1002/cjce.22799.

[55] "EudraLex, Volume 4, EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Annex 15: Qualification and Validation.," 2015. Accessed: May 24, 2023. [Online]. Available: https://health.ec.europa.eu/system/files/2016-11/2015-10_annex15_0.pdf

[56] "EudraLex – The Rules Governing Medicinal Products in the European Union, Volume 4, Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Part II: Basic Requirements for Active Substances used as Starting Materials," 2014. Accessed: May 24, 2023. [Online]. Available: https://health.ec.europa.eu/system/files/2016-11/2014-08_gmp_part1_0.pdf

[57] E. V. Sargent *et al.*, "The regulatory framework for preventing cross-contamination of pharmaceutical products: History and considerations for the future," *Regul. Toxicol. Pharmacol.*, vol. 79, pp. S3–S10, Aug. 2016, doi: 10.1016/j.yrtph.2016.05.029.

[58] E. Medicines Agency, "Committee for Medicinal Products for Human Use (CHMP) Committee for Medicinal Products for Veterinary Use (CVMP) Guideline on the quality of water for pharmaceutical use," 2020. Accessed: May 02, 2023. [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-water-pharmaceutical-use_en.pdf

[59] A. J. Tóth, D. Fózer, P. Mizsey, P. S. Varbanov, and J. J. Klemeš, "Physicochemical methods for process wastewater treatment: Powerful tools for circular economy in the chemical industry," *Rev. Chem. Eng.* De Gruyter Open Ltd, 2022. doi: 10.1515/revce-2021-0094.

[60] K. Patel, R. Aghera, D. Mistry, and E. Jasrotia, “A Zero Liquid Discharge in Pharmaceutical Industry,” *Int. Res. J. Eng. Technol.*, vol. 4, no. 5, pp. 1110–1114, 2017.

[61] C. Baresel, L. Dalgren, M. Almemark, and A. Lazic, “Environmental performance of wastewater reuse systems: Impact of system boundaries and external conditions,” *Water Sci. Technol.*, vol. 73, no. 6, pp. 1387 – 1394, 2016, doi: 10.2166/wst.2015.624.

PIELIKUMI / APPENDICES

1. Pielikums
Appendix 1

1. Publikācija/ Publication 1

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Cost Effective Method for Toxicity Screening of Pharmaceutical Wastewater Containing Inorganic Salts and Harmful Organic Compounds

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Abstract **– Pharmaceutical wastewater biological treatment plants are stressed with multi-component wastewater and unexpected variations in wastewater flow, composition and toxicity. To avoid operational problems and reduced wastewater treatment efficiency, accurate monitoring of influent toxicity on activated sludge microorganisms is essential. This paper outlines how to predict highly toxic streams, which should be avoided, using measurements of biochemical oxygen demand (BOD), if they are made in a wide range of initial concentration. The results indicated that wastewater containing multivalent Al^{3+} cations showed a strong toxic effect on activated sludge biocenosis irrespectively of dilutions, while toxicity of phenol and formaldehyde containing wastewater decreased considerably with increasing dilution. Activated sludge microorganisms were not sensitive to wastewater containing halogenated sodium salts (NaCl, NaF) and showed high treatment capacity of saline wastewater. Our findings confirm that combined indicators of contamination, such as chemical oxygen demand (COD), alone do not allow evaluating potential toxic influence of wastewater. Obtained results allow identifying key inhibitory substances in pharmaceutical wastewater and evaluating potential impact of new wastewater streams or increased loading on biological treatment system. Proposed method is sensitive and cost effective and has potential for practical implementation in multiproduct pharmaceutical wastewater biological treatment plants.**

Keywords **– Activated sludge; biochemical oxygen demand; pharmaceutical wastewater; toxicity**

Nomenclature		
BOD	Biochemical oxygen demand	mg/L
COD	Chemical oxygen demand	mg/L

1. INTRODUCTION

Biological wastewater treatment systems in industrial facilities often suffer from instability due to shocks of toxic or inhibitory contaminants being released into the wastewater. Pharmaceutical wastewater is highly polluted multi-component mixture of various organic and inorganic constituents, including hardly biodegradable, toxic and bio-persistent xenobiotics and

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antimicrobial agents that may inhibit the biological activity of the activated sludge and cause treatment plant process upsets. Activated sludge microorganisms react to the presence of toxicants in wastewater. Responses displayed include reduced rates of respiration, biomass generation, and BOD degradation patterns [1]. These effects of toxicants can cause failure to reach effluent standards, which also increase treatment costs and cause other operational problems, such as reduction of the efficiency of sludge settling and compacting, because of filamentous bulking and deflocculation [1]–[3]. In extreme cases the bacteria are killed by toxic wastewater, and there is a need for cleanout and reseeding of plant, which is a costly and time-consuming operation [2]. Toxic inflow can cause collapse of nitrification process and lead to significantly exceeded total nitrogen concentration in effluent [4]. Wastewater streams from drug manufacturing also contain residues of active pharmaceutical ingredients (API) and their intermediates, which are classified as micropollutant of emerging concern. They are biologically active compounds that can potentially alter physiology and behavior of non-target organism at low doses [5]. API negatively affect the performance of secondary biological processes in wastewater treatment plants (WWTP), cause shifts in the structure of activated sludge bacterial communities and reduce bacterial diversity in the reactors [6], [7]. Depending on manufacturing processes, the composition and biological treatability as well as salinity of pharmaceutical wastewater fluctuate considerably within a short period of time, which make biological treatment of pharmaceutical wastewater even more troublesome [8]–[12].

To avoid toxic shock to activated sludge microorganisms and ensure the compliance of treated wastewater with regulatory requirements, characterization of wastewater by their degree of biodegradation and potential toxicity to the biocenosis of the specific treatment plant is essential. As toxic influent can partially or completely damage treatment for long periods, protective actions and alternative treatment solutions should be provided when increased toxicity is detected [13], [14]. For companies who treat their own wastewater it is especially important to measure the toxicity of the wastewater before a new process comes on-line to predict their impact on treatment process performance. Mainly inhibitory effect of toxic components depends on their concentration; therefore, potential impact of increased pollution load on biological treatment system should be evaluated in cases when production volumes increase.

Numerous bioassays (toxicity tests) are available for toxicity evaluation [13], [15], [16], but only few are directly relevant to activated sludge microorganisms. For example, the commonly used MicrotoxTM and Biotox TM assays are based on marine luminous bacteria *Vibrio fischeri* that are not representative of activated sludge microbes and does not reflect the status of the microbial community responsible for treatment [1], [7], [13], [17]. Ecotoxicity tests based on the growth inhibition of algae or plants, mortality of crustaceans or fishes and the mobility inhibition of Daphnia magna are suitable to reflect ecological impact of toxic compounds to aquatic environments and, although are sometimes used in the assessment of toxicity of influent water to biological wastewater treatment processes, they are not relevant to WWTP [18]. Furthermore, the use of higher organisms may be ethically undesirable, such test organisms require specialized equipment and operator skills, long acclimatization time, and are labor intensive, expensive and time consuming [19]. For the evaluation of potential toxicity of influents on a wastewater treatment plant, activated sludge microorganisms should be preferred as test species [20], [21]. Many studies on pharmaceutical wastewater treatment plant shows that the microbial population and diversity is vary from one plant to another depending on wastewater characteristics and operating conditions [22]. In some cases, activated sludge from the pharmaceutical industry showed higher resistance against inhibiting toxicants than the sludge from other sources, since biomass is acclimated by the usual presence of toxic compounds in the influent [23]. These aspects highlight the importance of the use of site-specific indicator organisms in toxicity monitoring of influent water, to get accurate results.

For the past two decades, biosensor technologies are undergoing improvements and some biosensors are showing good potential to be used as on-line monitoring tools to provide early warning for WWTP operators to avoid toxic shocks to WWTP's [18]. Enzymes, antibodies, microorganisms or DNA could be used as biological sensing element of biosensor in combination with an appropriate transducer (e.g., electrochemical, optical, colorimetric or piezoelectric) [19]. Wastewater treatment plant can be equipped with early warning system that includes a few toxicity measurement points placed at selected locations [4].

Oxygen demand obtained in respirometric assays represents a direct measure of the activity of microorganisms present in aerobic activated sludge. Furthermore, since respiration of the activated sludge is inhibited in the presence of toxicants, it can be used as an efficient tool for the measurement of acute toxicity on microbial population of biological WWTP sludge [16].

The aim of this study was to evaluate the toxic effect of pharmaceutical wastewater streams containing harmful organic substances and inorganic salts on a mixed culture from activated sludge, which was sampled from operating WWTP treating multiproduct pharmaceutical wastewater. Toxicity was assessed by direct observing the effects of toxicants on the BOD degradation activity of the activated sludge microorganisms by exposing test organisms to various doses of the pollutant. Experiments were performed using chemically polluted wastewater streams from JSC "Grindeks" pharmaceutical production facility.

2. Materials and Methods

2.1. Preparation of Seeded Dilution Water

Determination of BOD was done by using the modified standardized method ISO 5815:1989. Required volume of distilled water for preparation of dilution series was filled in a suitable glass container. 1 mL of each of the salt solutions (Table 1) was added to 1 L of distilled water. Obtained solution was aerated for 1 h by using compressor GAST DOA-P504-BN. The dissolved oxygen concentration in dilution water should be at least 8 mg L^{-1}. The water shall not be supersaturated with oxygen, so it must be allowed to stand 1 h in an opened container before use. Settled activated sludge biomass (2 mL to 1 L dilution water) from JSC „Grindeks" WWTP was added as seed material (inoculum). The oxygen consumed over 5 days, at 20 °C of the seeded dilution water, which is the blank value, shall not exceed 1.5 mg L^{-1} of oxygen.

TABLE 1. PREPARATION OF SALTS SOLUTIONS

Name	Concentration, mg L^{-1} water
Phosphate buffer solution:	
KH2PO4	8 500
K2HPO4	21 750
Na2HPO4·7H20	33 400
NH4Cl	1 700
MgSO4·7H2O solution	22 500
CaCl2 solution	27 500
FeCl3·6H2O solution	250
Allylthiourea solution	1000

2.2. Preparation of Test Solutions (Dilution Series)

The dilution of the test sample was carried out in a 500 mL volumetric flask. Set of 7 different dilutions were prepared for each sample. Known volume of the sample to be analysed and 1 mL of an allylthiourea solution (Table 1) was added in each flask for inhibition of nitrification. Then flasks were filled to the mark with seeded dilution water and mixed gently. Blank sample was prepared in parallel, by using seeded dilution water and allylthiourea solution (Table 1). Organic content of the samples was characterized by chemical oxygen demand (COD) values.

Incubation bottles (V = 350 mL) were filled with each dilution allowing them to overflow slightly. Initial dissolved oxygen concentration at time zero was measured in each bottle. Then bottles were stoppered and put in the incubator at 20 °C in darkness for 5 days. After the incubation, dissolved oxygen concentration was measured in each bottle again.

2.3. BOD_5 Calculation

BOD_5 calculation was done by using Eq. (1):

$$BOD_5 = \left[(c_1 - c_2) - \frac{V_t - V_e}{V_t}(c_3 - c_4)\right]\frac{V_t}{V_e}, \quad (1)$$

where

- c_1 dissolved oxygen concentration of one of the test solution at time zero, mg L^{-1};
- c_2 dissolved oxygen concentration of this same test solution after 5 days, mg L^{-1};
- c_3 dissolved oxygen concentration of the blank solution at time zero, mg L^{-1};
- c_4 dissolved oxygen concentration of the blank solution after 5 days, mg L^{-1};
- V_e volume of sample used for the preparation of the test solution concerned, mL;
- V_t total volume of this test solution, mL.

2.4. Apparatus and Equipment

COD analyses were done by using HACH LANGE cuvette tests LCK 014 and LCK 514. Measurements were done by the spectrophotometer HACH DR 5000. The HACH LANGE thermostat HT 200S was used to heat the samples. Concentration of Cl^- and phenols was determined by using HACH LANGE cuvette tests LCK 311 and LCK 349 respectively. Dissolved oxygen concentration in the respirometric assays was measured with dissolved oxygen meter WTW inolab Oxi Level 2. Incubation of samples at 20 °C was done in thermostat WTW TS 606/2. Analytical grade reagents from Acros Organics were used for preparation of salt solutions.

2.5. Wastewater and Sludge Sampling

JSC "Grindeks" is the leading pharmaceutical company in the Baltic States and produces about 25 different kinds of active pharmaceutical ingredients belonging to heart and cardiovascular, CNS and anticancer medication therapeutic groups. The wastewater from "Grindeks" pharmaceutical production facility is treated in a five-stage moving bed biofilm reactor (MBBR) plant, including COD removal, nitrification and denitrification, after which the wastewater is discharged into the municipal sewer system for the final treatment. The composition and biotreatability of wastewaters are variable and highly stressful for microorganisms. Influent COD load varies from 1 to 2.5 t per day and the total nitrogen load is in the range from 0.015 to 0.045 t per day, while the wastewater flow varies from 160 to 360 m^3 d^{-1}. The hydraulic retention time (HRT) of the plant is in the range of 3–6 days and COD removal is 93–94 % in average. Activated sludge inoculum for BOD measurements was taken from last MBBR where organic biological degradation takes place.

3. Results and Discussion

Pharmaceutical compounds at JSC "Grindeks" are typically produced in multi stage batch processes leading to the presence of a wide variety of products in wastewaters which are generated in different operations – chemical synthesis, washing or extraction of chemical product, washing of equipment, recovery of solvents. To minimize the fluctuation of pollution load and wastewater flow, wastewater from production sites is collected in the equalization tank and dosed to the MBBR system. As shown in the Fig. 1, regular wastewater is relatively well biodegradable. Considerable toxic effect on activated sludge microorganisms is not detected. Depending on the degree of dilution, BOD varies from 22.4 to 53.2 % of COD (Table 2). The ratio BOD/COD is called Biodegradability index and is commonly used as an indicator for biodegradation capacity [24]. COD values of tested (undiluted) samples are used to calculate the ratio BOD/COD. Low ratio BOD/COD indicate the presence either of organic matter that are hard to biodegrade or of toxic substance inhibiting the microbial activities [25].

Fig. 1 and Fig. 2 demonstrate BOD values of chemically polluted pharmaceutical wastewater as a function of dilution factor under two different background conditions. The obtained BOD curves show the response of biocenosis of the biological WWTP to highly organically polluted wastewater streams and presence of inorganic compounds in wastewaters. Dilutions in range from 100–700 were tested, which corresponds to the real amount of wastewater generated per day and their potential dilution in equalization tank of WWTP of "Grindeks".

Toxic and refractory organic compounds are one of major challenges for biological wastewater processes. We used formaldehyde and phenol containing wastewaters as representative contaminants of pharmaceutical wastewaters for our experiments. Phenol and formaldehyde have strong bio-resistance and toxicity to microbes thus limiting the use of biological treatment method [26]. Obtained results show that biodegradability of pharmaceutical wastewater containing such toxic substances depends on concentration of toxicants.

Formaldehyde is highly reactive chemical compound and is widely used in the chemical industry as a raw material and solvent for the production of various products, and as a result it also enters into chemical wastewater. Because of its toxicity [27], formaldehyde is also widely used as a preservative and disinfectant to inhibit the activity of microorganisms; therefore, it is already predictable that its presence may inhibit biological processes in WWTP's [28]. As shown in Fig. 1 biodegradability of formaldehyde containing wastewater is expressly dependent on the degree of dilution of the sample. As formaldehyde concentration increases, it inhibits the biological activity of activated sludge microorganisms, respiration decreases, and biodegradation is disrupted.

Phenol, like formaldehyde, comes into pharmaceutical wastewater of JSC "Grindeks" as a by-product of chemical synthesis and is considered as very toxic compound to living organisms [29]. Although phenol is reported as toxic and inhibitory substrate, however it is also carbon source for an acclimated biomass [30], [31]. Experimental data show that with increasing initial concentration of phenol in the solution, BOD values decrease noticeably. The shape of BOD curve is similar to that of formaldehyde containing wastewater. Obtained results are consistent with studies by other authors that the phenol is recognized as an inhibitory substrate to activated sludge at relatively low concentrations (100 mg L^{-1}) [32].

As shown in Fig. 1, the BOD results are informative only if measurements are made in a wide range of initial concentration. The biodegradability and toxicity of organically contaminated wastewater is strongly dependent on the dilution.

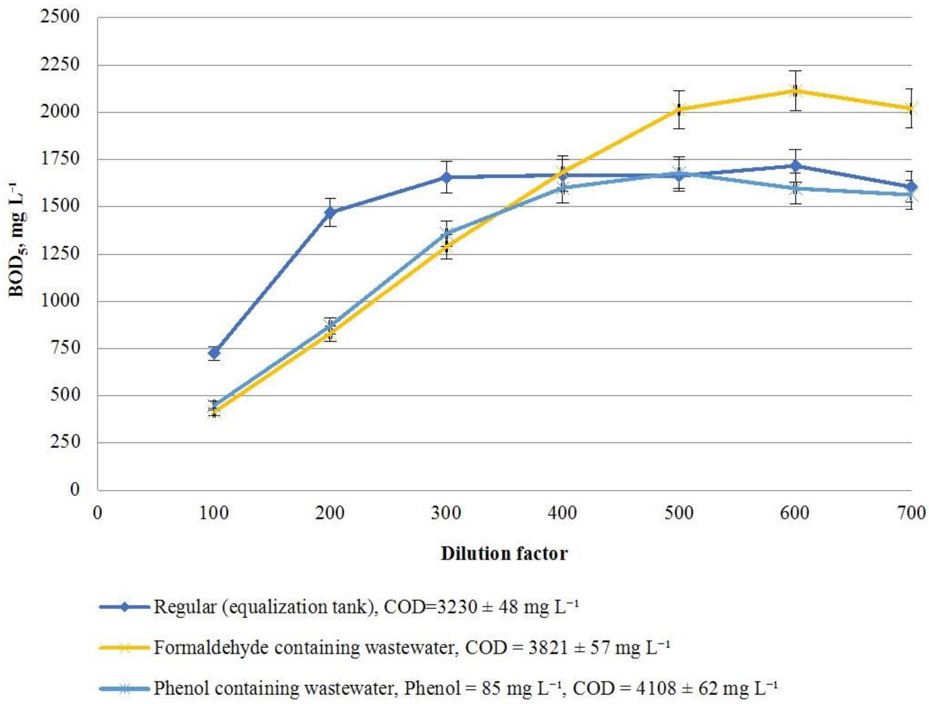

Fig. 1. BOD_5 values of pharmaceutical wastewater's streams containing harmful organic compounds depending on dilution factor.

Results show that the biodegradation curves of wastewater containing phenol and formaldehyde reach the plato phase only at dilution factor of 500. Construction of an equalization tank appears to be of extreme importance in order to ensure dilution of toxic wastewater streams and avoid toxic shock to biological treatment system treating pharmaceutical wastewater. The obtained data can be used to estimate amount of specific wastewater that will not affect the stability of the biological treatment process and can be discharged into WWTP. However, it should be considered, that possible synergistic or antagonistic interactions between pollutants are possible [33].

High salinity in wastewaters is another key issue affecting the performance of biological processes. Inorganic salts, like NaCl, NaF, $MgSO_4$, Na_2CO_3, NH_4Cl, are commonly used and are typical pollutants of pharmaceutical wastewater. Pharmaceutical industry generates saline wastewater, rich in both salts and organic matter. The activity of microorganisms usually is affected by high salt concentration (>2 % w/v), which can lead to the low COD removal efficiency, decreased nitrification and denitrification rate and bulking of the activated sludge as well as increased turbidity [34], [35], therefore it is important to investigate the impact of inorganic salts on activated sludge.

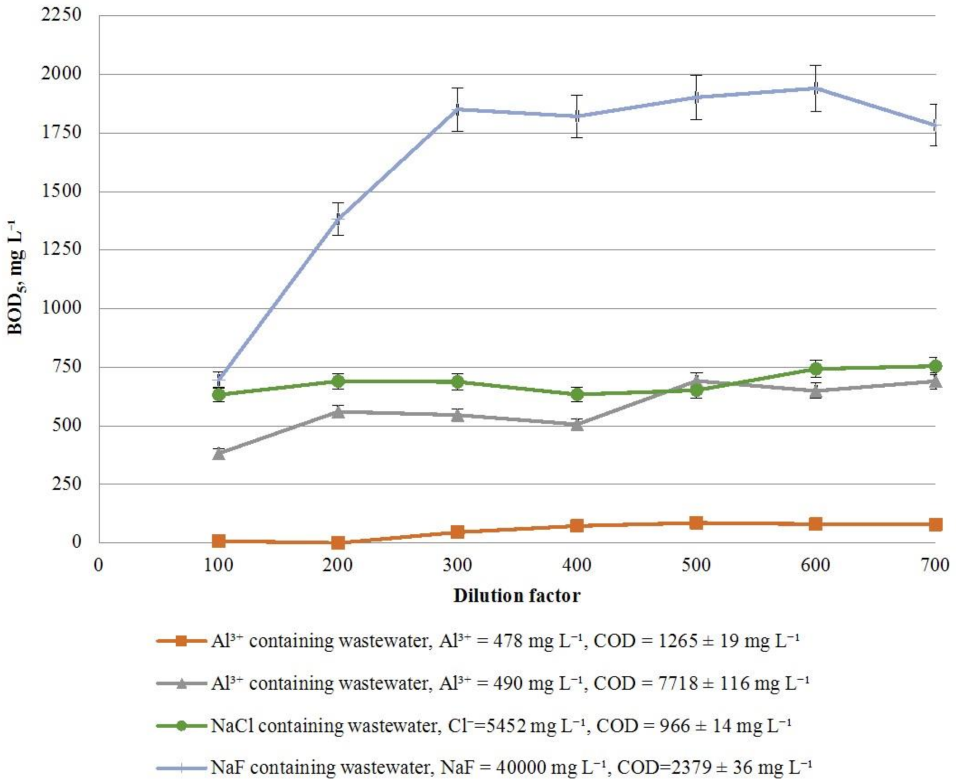

Fig. 2. BOD_5 values of pharmaceutical wastewater's streams containing inorganic salts depending on dilution factor.

The salinity inhibition on biological treatment is mainly caused by osmotic pressure, which may lead to plasmolysis of microbial cells and eventual death of microorganisms eventually leading to the failure of biological treatment systems [36], [37]. Major fluctuation of salinity can cause salinity shock, for this reason, high salinity wastewater must be diluted before biological treatment in most cases, which increases operational costs and water consumption [36]. Each microbial species has its optimum growth salinity, and microorganisms will lose their activity beyond the tolerance limit [38]. In many studies halophilic salt-tolerant microorganisms were considered adaptable to treat saline wastewater [34], [37]. Salt-adapted microorganisms are capable of withstanding high salinities and at the same time of degrading the pollutants that are contained in wastewater [35]. As salt removal from saline wastewater by reverse osmosis or ion exchange is expensive, biological treatment of hyper-saline wastewater with halophilic sludge is recommended [35].

TABLE 2. RATIO BOD_5/COD (%) DEPENDING ON DILUTION FACTOR

Type of wastewater	COD, mg L^{-1}	Ratio BOD_5/COD, %*						
		Dilution factor						
		100	200	300	400	500	600	700
Regular (equalization tank)	3230 ± 48	22.4	45.4	51.3	51.6	51.5	53.2	49.7
Al^{3+} containing wastewater, Al^{3+} = 478 mg L^{-1}	1265 ± 19	0.6	0.5	3.6	5.8	6.8	6.3	6.3
Al^{3+} containing wastewater, Al^{3+} = 490 mg L^{-1}	7718 ± 116	5.0	7.3	7.1	6.6	9.0	8.4	9.0
Formaldehyde containing wastewater	3821 ± 57	10.8	21.7	33.7	44.1	52.7	55.3	52.9
Phenol containing wastewater, Phenol = 85 mg L^{-1}	4108 ± 62	10.9	21.2	33.1	38.9	40.9	38.9	38.1
NaCl containing wastewater, Cl^- = 5452 mg L^{-1}	966 ± 14	65.5	71.4	71.3	65.6	67.5	76.9	78.2
NaF containing wastewater, NaF = 40 000 mg L^{-1}	2379 ± 36	29.2	58.1	77.8	76.6	79.9	81.6	75.0

*100 % ratio BOD_5/COD, % considered as readily biodegradable wastewater.

Saturated solution of NaCl is widely used in the pharmaceutical industry to extract the chemical products. Although according to literature data biological treatment is strongly inhibited by salts (mainly NaCl) [35], respirometric data show, that NaCl concentration in influent wastewater of WWTP of JSC “Grindeks” does not reach inhibitory level on activated sludge microorganisms. This can be explained by the fact that concentration of NaCl in tested wastewater corresponds to the physiological saline and BOD tests were done at high dilution. The results of the respirometry experiment allow concluding that the organic matter of the sample has good biodegradability – the ratio of BOD_5/COD is greater than 65 % and does not essentially changes with dilution (Table 2).

Saturated NaF aqueous solution in the synthesis of pharmaceutical products forms as a by-product of the fluorination reaction. Although fluoride is a common contaminant in a variety of industrial wastewaters, available information on the potential toxicity of fluoride to microorganisms located in biological wastewater treatment plants is very limited. The fluoride concentration in untreated industrial wastewater can vary over a large range from 500–2000 mg L^{-1} [39]. Experimental data show that NaF inhibit activated sludge microorganisms from pharmaceutical WWTP only at dilution factor 100. With increasing dilution, the BOD_5/COD ratio is above 70 %, which indicates that activated sludge microorganisms tolerate fluoride at relatively high concentrations. Activated sludge microorganisms of JSC “Grindeks” are exposed to continuously inflow of halogenated salts and are acclimated to increased salt concentration. The obtained results allow concluding that halogenated sodium salts with tested halogenates concentration do not impact removal of organic matter in wastewater.

$AlCl_3$ is used as a catalyst in chemical synthesis, and therefore multivalent Al^{3+} cations are regularly discharged into pharmaceutical wastewater. Respirometric experiments were performed on samples with different concentrations of organic matter and practically the same content of Al^{3+} ions in water. As shown in the Fig. 2, Al^{3+} ion-containing wastewater is highly toxic to active sludge microorganisms and practically non-biodegradable. The ratio of BOD_5/COD remains low even at high dilution rates of the sample and does not exceed 10 % (Table 2). The obtained results indicate that Al^{3+} containing wastewater should not be let into biological treatment plants, as this can lead to toxic shock to microorganisms and destroy the active sludge biocenosis. The toxicity of both samples is markedly high; it does not change with the COD increase, which confirms toxicity of Al^{3+} ions. Chemical pre-treatment – for example, precipitation of aluminium with lime milk, NaOH or Na_2CO_3 at pH 6.5 prior to biological treatment is necessary to reduce toxicity [40]. Adsorption of aluminium ions on activated carbon could also be used as effective treatment method [41].

Our findings do not conflict with other scientists and confirm the statement that aluminium has no biological role and is a toxic nonessential metal to microorganisms [42], [43].

Based on the literature survey is already known, that aluminium in its ionic form is very toxic to most aquatic organisms such as seaweeds, crawfish, and fish [43] causing osmoregulatory failure by destructing the plasma and hemolymph ions [44]. Aluminium is toxic to fish in acidic (mainly pH 5–5.5), unbuffered waters starting at a concentration of 0.1 mg/L [45], [46].

In experiments were aluminium salts ($AlCl_3$, $Al_2(SO_4)_3$) were using to improve settlement characteristics of the sludge and overcome the bulking problem, it was found that aluminium is toxic to activated sludge rotifer *Lecane inermis* and affected negatively rotifers population size even at low concentrations (4.8 EC and 0.48 EC) [47].

Considering that even trivial amount of aluminium in water causes numerous health problems including Alzheimer and dialysis encephalopathy [41], precautionary principles should be implemented, to avoid toxic reactions and discharge of Al ions containing wastewater in the environment

The obtained data confirm that the pharmaceutical effluents are very different in terms of biodegradability and toxicity to microorganisms. For effluents having the same dilution value, the ratio BOD_5/COD can vary considerably – up to 10 times, indicating the highly complex nature of pharmaceutical wastewater. The magnitude of this effect is shown in Table 2. The described method is very useful to compare different wastewater streams to each other, evaluate their impact on the biocenosis of wastewater treatment plant and to find the optimum treatment solution.

The variations in experimental curves characterize:

1. The ecotoxicological nature of organic matter;
2. The ability of microorganisms present in activated sludge to utilize the pharmaceutical wastewaters;
3. The character of curves gives additional information about impact of dilution factor on biological treatability and toxicity to the activated sludge microorganisms of wastewaters.

Information concerning the BOD values is important information in the process control of the wastewater treatment facilities. It is essential factor not only in a choosing of optimal treatment regime, but also to evaluate the possibility to mix the wastewater streams and optimize treatment process. This method also could be used to predict new production behaviour in wastewater treatment station and determining the safe rate of discharge into the aeration tanks.

4. Conclusions

The obtained results confirm that BOD measurements with local biomass can be used as a screening method for assessing and comparing the biodegradability and the toxicity to activated sludge of different types of chemically polluted wastewaters, if set of dilution series is tested. The extensive initial concentration range used in the experiments extends the use of the method and allows determining at which concentration the toxicity of the sample changes. These data could be use as basis for assessing the risks and predict process performance if production is intensified and the concentration of specific pollutants in wastewater increase or new type of wastewater is generated.

Experiments with phenol and formaldehyde containing wastewater showed that the toxic effect on the activated sludge disappears with increased dilution of the sample, highlighting the extremely import role of equalizing tank in highly polluted pharmaceutical wastewater treatment processes in order to minimize harsh fluctuations and peak loading of toxic contaminants.

Al^{3+} ions containing wastewater showed the highest toxicity against activated sludge microorganisms, clearly indicating need of pretreatment prior to biological wastewater treatment stage. At the same time the results show that the numerical values of the COD cannot be the only parameter for objective pollution characterization, since wastewater with lower COD can be more toxic to activated sludge biocenosis than wastewater with a higher COD. Halogenated sodium salts in tested concentration ranges did not leave negative impact on removal of organic matter from wastewater.

The proposed toxicity evaluation method is directly relevant to activated sludge wastewater treatment process, gives representative results, and is very informative, cost effective and simple. Because of the duration of the test, method could not be used as real time toxicity monitoring tool but is very suitable for systematic screening of various wastewater streams in multiproduct factories, because it allows identifying source of toxicity and choosing a reasonable treatment strategy.

REFERENCES

[1] Neumegen R. A., Fernández-Alba A. R., Chisti Y. Toxicities of Trichlosan, Phenol, and Copper Sulfate in Activated Sludge. *Environmental Toxicology* 2005:20(2):160–164. doi:10.1002/tox.20090

[2] Davies P. S., Murdoch F. The increasing importance of assessing toxicity in determining sludge health and management policy. *Measurement and Control* 2002:35(8):238–242. doi:10.1177/002029400203500804

[3] Katritzky A. R., et al. Estimating the toxicities of organic chemicals in activated sludge process. *Water Research* 2010:44(8):2451–2460. doi:10.1016/j.watres.2010.01.009

[4] Jurga A., Gemza N., Janiak K. A concept development of an early warning system for toxic sewage detection. *E3S Web of Conferences* 2017:17(00036):1–8. doi:10.1051/e3sconf/20171700036

[5] Sanganyado E., Lu Z., Fu Q., Schlenk D., Gan J. Chiral pharmaceuticals: A review on their environmental occurrence and fate processes. *Water Research* 2017:124:527–542. doi:10.1016/j.watres.2017.08.003

[6] Kraigher B., Kosjek T., Heath E., Kompare B., Mandic-Mulec I. Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors. *Water Research* 2008:42(17):4578–4588. doi:10.1016/j.watres.2008.08.006

[7] Vasiliadou I. A., Molina R., Martinez F., Melero J. A., Stathopoulou P. M., Tsiamis G. Toxicity assessment of pharmaceutical compounds on mixed culture from activated sludge using respirometric technique: The role of microbial community structure. *Science of The Total Environment* 2018:630:808–819. doi:10.1016/j.scitotenv.2018.02.095

[8] Rozitis Dz., Strade E. COD reduction ability of microorganisms isolated from highly loaded pharmaceutical wastewater pre-treatment process. *Journal of Materials and Environmental Science* 2015:6(2):507–512.

[9] Tekin H., et al. Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater. *Journal of Hazardous Materials* 2006:136(2):258–265. doi:10.1016/j.jhazmat.2005.12.012

[10] Lefebvre O., et al. Biological treatment of pharmaceutical wastewater from the antibiotics industry. *Water Science and Technology* 2014:69(4):855–861. doi:10.2166/wst.2013.729

[11] Ma K., Qin Z., Zhao Z., Zhao C., Liang S. Toxicity evaluation of wastewater collected at different treatment stages from a pharmaceutical industrial park wastewater treatment plant. *Chemosphere* 2016:158:163–170. doi:10.1016/j.chemosphere.2016.05.052

[12] Shi X., Yeap T. S., Huang S., Chen J., Ng H. Y. Pretreatment of saline antibiotic wastewater using marine microalga. *Bioresource Technology* 2018:258:240–246. doi:10.1016/j.biortech.2018.02.110

[13] Ren S. Assessing wastewater toxicity to activated sludge: recent research and developments. *Environment International* 2004:30(8):1151–1164. doi:10.1016/j.envint.2004.06.003

[14] Sirtori C., et al. Decontamination industrial pharmaceutical wastewater by combining solar photo-Fenton and biological treatment. *Water Research* 2009:43(3):661–668. doi:10.1016/j.watres.2008.11.013

[15] Cēbere B., Faltiņa E., Zelčāns N., Kalniņa D. Toxicity tests for ensuring successful industrial wastewater treatment plant operation. *Environmental and Climate Technologies* 2009:3(3):41–47. doi:10.2478/v10145-009-0005-8

[16] Oller I., Malato S., Sánchez-Pérez J. A. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination – A review. *Science of Total Environment* 2011:409(20):4141–4166. doi:10.1016/j.scitotenv.2010.08.061
[17] Philp J. C., et al. Whole cell immobilised biosensors for toxicity assessment of a wastewater treatment plant treating phenolics-containing waste. *Analytica Chimica Acta* 2003:487(1):61–74. doi:10.1016/S0003-2670(03)00358-1
[18] Xiao Y., De Araujo C., Sze C. C., Stuckey D. C. Toxicity measurement in biological wastewater treatment processes: A review. *Journal of Hazardous Materials* 2015:286:15–29. doi:10.1016/j.jhazmat.2014.12.033
[19] Hassan S. H. A., Van Ginkel S. W., Hussein M. A. M., Abskharon R., Oh S. E. Toxicity assessment using different bioassays and microbial biosensors. *Environment International* 2016:92–93:106–118. doi:10.1016/j.envint.2016.03.003
[20] Kungolos A. Evaluation of toxic properties of industrial wastewater using on-line respirometry. *Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances & Environmental Engineering* 2005:40(4):869–880. doi:10.1081/ESE-200048292
[21] Gutiérrez M., Etxebarria J., de las Fuentes L. Evaluation of wastewater toxicity: comparative study between Microtox® and activated sludge oxygen uptake inhibition. *Water Research* 2002:36(4):919–924. doi:10.1016/S0043-1354(01)00299-8
[22] Meherdad F., et al. Identification of Bacterial Population of Activated Sludge Process and Their Potentials in Pharmaceutical Effluent Treatment. *British Biotechnology Journal* 2014:4(3):317–324. doi:10.9734/BBJ/2014/7913
[23] Surerus V., Giordano G., Teixeira L. A. C. Activated sludge inhibition capacity index. *Brazilian Journal of Chemical Engineering* 2014:31(2):385–392. doi:10.1590/0104-6632.20140312s00002516
[24] Abdalla K. Z., Hammam G. Correlation between Biochemical Oxygen Demand and Chemical Oxygen Demand for Various Wastewater Treatment Plants in Egypt to Obtain the Biodegradability Indices. *International Journal of Sciences: Basic and Applied Research* 2014:13(1):42–48.
[25] Mangkoedihardjo S. Biodegradability Improvement of Industrial Wastewater Using Hyacinth. *Journal of Applied Sciences* 2006:6:1409–1414. doi:10.3923/jas.2006.1409.1414
[26] Cui W., Cui Z., Zhang N., Ma Q., Liu L., Zhang X. A new efficient technology for refractory phenol-formaldehyde resin wastewater treatment. *RSC Advances* 2016:6(23):19078–19088. doi:10.1039/C5RA21502A
[27] Agency for Toxic Substances and Disease Registry (ASTDR). Toxicological profile for Formaldehyde. Atlanta: U.S. Department of Health and Human Services, Public Health Service, 1999.
[28] Eiroa M., Vilar A., Amor L., Kennes C., Veiga M. C. Biodegradation and effect of formaldehyde and phenol on the denitrification process. *Water Research* 2005:39(2–3):449–455. doi:10.1016/j.watres.2004.09.017
[29] Agency for Toxic Substances and Disease Registry (ASTDR). Toxicological profile for Phenol. Atlanta: U.S. Department of Health and Human Services, Public Health Service, 2008.
[30] Yoong E. T., Lant P. A., Greenfield P. F. *In situ* respirometry in an SBR treating wastewater with high phenol concentrations. *Water Research* 2000:34(1):239–245. doi:10.1016/S0043-1354(99)00142-6
[31] Hussain A., Dubey S. K., Kumar V. Kinetic study for aerobic treatment of phenolic wastewater. *Water Resources and Industry* 2015:11:81–90. doi:10.1016/j.wri.2015.05.002
[32] Pradeep N. V., et al. Biological removal of phenol from wastewaters: a mini review. *Applied Water Science* 2015:5(2):105–112. doi:10.1007/s13201-014-0176-8
[33] Heys K. A., Shore R. F., Pereira M. G., Jones K. C., Martin F. L. Risk assessment of environmental mixture effects. *RSC Advances* 2016:6(53):47844–47857. doi:10.1039/C6RA05406D
[34] Kargi F. Enhanced biological treatment of saline wastewater by using halophilic bacteria. *Biotechnology Letters* 2002:24(19):1569–1572. doi:10.1023/A:1020379421917
[35] Lefebvre O., Moletta R. Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research* 2006:40(20):3671–3682. doi:10.1016/j.watres.2006.08.027
[36] Shi X., Lefebvre O., Ng K. K., Ng H.Y. Sequential anaerobic-aerobic treatment of pharmaceutical wastewater with high salinity. *Bioresource Technology* 2014:153:79–86. doi:10.1016/j.biortech.2013.11.045
[37] Zhang X., Gao J., Zhao F., Zhao Y., Li Z. Characterization of a salt-tolerant bacterium Bacillus sp. from a membrane bioreactor for saline wastewater treatment. *Journal of Environmental Sciences* 2014:26(6):1369–1374. doi:10.1016/S1001-0742(13)60613-0
[38] Wang R., et al. Effects of inorganic salts on denitrifying granular sludge: The acute toxicity and working mechanisms. *Bioresource Technology* 2016:204:65–70. doi:10.1016/j.biortech.2015.12.062
[39] Ochoa-Herrera V., et al. Toxicity of fluoride to microorganisms in biological wastewater treatment systems. *Water Research* 2009:43(13):3177–3186. doi:10.1016/j.watres.2009.04.032
[40] Negrea A., et al. Studies Concerning the Aluminium Ions Removal from Waste Water. *Chemical Bulletin of "POLITEHNICA" University of Timişoara* 2005:50:148–51.
[41] Pour P. G., Takassi M. A., Hamoule T. Removal of Aluminum from Water and Industrial Waste Water. *Oriental Journal of Chemistry* 2014:30(3):1365–1369. doi:10.13005/ojc/300356
[42] Olaniran A. O., Balgobind A., Pillay B. Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. *International Journal of Molecular Sciences* 2013:14(5):10197–10228. doi:10.3390/ijms140510197

[43] Jaishankar M., Tseten T., Anbalagan N., Mathew B. B., Beeregowda K. N. Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology* 2014:7(2):60–72. doi:10.2478/intox-2014-0009
[44] Rosseland B. O., Eldhuset T. D., Staurnes M. Environmental effects of aluminium. *Environmental Geochemistry and Health* 1990:12(1–2):17–27. doi:10.1007/BF01734045
[45] Sparling D. W. Ecotoxicology Essentials: Environmental Contaminants and Their Biological Effects on Animals and Plants. London: Academic Press, 2016.
[46] Comber S. D. W., Gardner M. J., Churchley J. Aluminium speciation: implications of wastewater effluent dosing on river water quality. *Chemical Speciation & Bioavailability* 2005:17(3):117–128. doi:10.3184/095422905782774874
[47] Klimek B., et al. The toxicity of aluminium salts to Lecane inermis rotifers: are chemical and biological methods used to overcome activated sludge bulking mutually exclusive? *Archives of Environmental Protection* 2013:39(3):127–138. doi:10.2478/aep-2013-0024

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2. pielikums
Appendix 2

2. Publikācija / Publication 2

Neibergs, M., **Strade, E.**, Nikolajeva, V., Susinskis, I., Rozitis, Dz., Kalnina, D. Application of bioaugmentation to improve pharmaceutical wastewater treatment efficiency. *Key Eng. Mater.* **2019**, *800*, 122-131.
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Kopsavilkums

Ekoloģiskais kaitējums un cilvēku veselības riski, ko rada vides piesārņojums ar aktīvajām farmaceitiskajām vielām (AFV), mūsdienās rada arvien lielākas bažas. Cilvēku un veterināro AFV plašā klātbūtne ūdens vidē skaidri norāda uz šo savienojumu noturību un zemo attīrīšanas efektivitāti farmācijas un sadzīves notekūdeņu attīrīšanas iekārtās (NAI). Aktīvo dūņu sistēmu bioaugmentācija ar specializētiem mikroorganismiem varētu būt efektīgs un videi draudzīgs veids, kā uzlabot noturīgu AFV aizvākšanas efektivitāti. Liela nozīme ir tādu inokulējamo mikroorganismu izvēlei, kuriem ir piemēroti enzīmi, lai metabolizētu sarežģītas AFV molekulas, kas pieder pie dažādām terapeitiskām klasēm. Šajā pētījumā tika izvērtēta 10 baktēriju, 10 raugu un 3 mikroskopisko sēņu kultūru, kas iepriekš izolētas no farmācijas NAI, spēja degradēt līdz šim maz pētītas AFV – oksitocīnu, zopiklonu un meldonija dihidrātu kā vienīgo oglekļa avotu un kometaboliskā veidā, kā papildus barības vielu pievienojot vājpiena pulveri. Baktērija *Sphingobacterium thalpophilum* un mikroskopiskās sēnes *Fusarium solani* un *Fusarium udum* laboratorijas mēroga bioaugmentācijas testos uzrādīja ļoti augstu visu pētīto AFV aizvākšanas efektivitāti un tika atzītas, kā kultūras ar augstu metabolisko potenciālu, lai tās izmantotu AFV mikropiesārņotāju aizvākšanā.

Abstract

Ecological harm and human health risks caused by environmental pollution with active pharmaceutical ingredients (API) nowadays is recognised as issue of growing concern. Widespread presence of human and veterinary API in aquatic environment clearly indicates persistence and low removal efficiency of these compounds at conventional pharmaceutical and municipal wastewater treatment plants (WWTP). Bioaugmentation of activated sludge systems

with specialized microorganisms could be a powerful and environmentally friendly tool to enhance the removal efficiency of recalcitrant API. Selection of inoculum strains, that have appropriate enzymatic pathways to metabolise complex molecules of API, belonging to different therapeutic classes, is of great importance. This study evaluated the potential of pure cultures of 10 bacteria, 10 yeasts and 3 filamentous fungi previously isolated from activated sludge of pharmaceutical WWTP to degrade less investigated API – oxytocin, zopiclone and meldonium dihydrate as sole carbon source and in cometabolic manner with presence of skim milk powder as additional nutrient source. Bacteria *Sphingobacterium thalpophilum* and filamentous fungi *Fusarium solani* and *Fusarium udum* showed very high treatment efficiency of all tested API in laboratory-scale bioaugmentation tests and were recognized as culture with high metabolic potential to be used in bioaugmentation for removal of pharmaceutical micropollutants.

3. pielikums
Appendix 3

3. Publikācija / Publication 3

Rozitis, D., **Strade, E.** COD Reduction Ability of Microorganisms Isolated from Highly Loaded Pharmaceutical Wastewater Pre-Treatment Process. *J. Mater. Environ. Sci.* **2015**, 6, 507-512.

Kā šī raksta autors, es paturu tiesības to iekļaut darbā, ja tas netiek publicēts komerciāli.
As the author of this article, I retain the right to include it in a thesis, provided it is not published commercially.

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COD reduction ability of microorganisms isolated from highly loaded pharmaceutical wastewater pre-treatment process

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Abstract
Efficiency of a biological wastewater treatment process depends on microbial diversity and their ability to degrade specific pollutants. The aim of this study was isolation and identification of predominant microorganisms associated with pharmaceutical wastewater pre-treatment process and evaluation of a chemical oxygen demand (COD) removal efficiency of each individual isolate. There were 65 microorganisms isolated from activated sludge of the JSC "Grindeks" industrial wastewater biological pre-treatment process and subsequently tested for COD reduction in pharmaceutical wastewater containing 2300-3500 mg/l of COD. Out of all isolates 9 bacteria, 5 yeasts and 3 filamentous fungi showed COD reduction. The highest COD reduction levels among all isolates were observed from filamentous fungi *Fusarium udum* (88.7%) and *Fusarium solani* (89.4%). The most effective bacterial strains were *Aeromonas caviae* and *Sphingobacterium thalpophilum*, with 78.1% and 75.9% COD removal, respectively. *Rhodotorula mucilaginosa* was the most effective yeast strain and achieved 76.6% COD reduction.

Keywords: Pharmaceutical wastewater, biodegradation, COD removal, microorganisms

Introduction
Pharmaceutical wastewater is a complex mixture of different organic and inorganic compounds including residues of active pharmaceutical substances, solvents, toxic and biorecalcitrant chemicals that inhibit microbial activity of the activated sludge process and present great challenge for a proper treatment and downstream processing [1]. Furthermore, composition, flow and other characteristics (pH, salinity, etc.) of the wastewater may change considerably from day to day depending on the production stages and intensity, making the biological treatment and downstream processing even more difficult, and the pre-treatment plant of JSC "Grindeks" is no exception [2-3]. Microorganisms within the activated sludge have to face a toxic load and be able to degrade a wide range of chemical compounds in harsh, ever-changing conditions.
JSC "Grindeks" is the leading pharmaceutical company in the Baltic States and produces about 25 different kinds of active pharmaceutical ingredients in heart and cardiovascular, CNS and anticancer medication therapeutic groups. The wastewater from "Grindeks" pharmaceutical production facility is treated in a moving bed biofilm reactor (MBBR) type of pre-treatment plant, after which the wastewater is discharged into the municipal sewer system for the final treatment. Incoming COD load varies from 1 to 2.5 t and the total nitrogen load is in the range from 0.015 to 0.045 t per day, while the wastewater flow varies from 160 to 360 m^3/d. The hydraulic retention time (HRT) of the plant is in the range of 3-6 days and COD removal is 93-94% in average.
This study involved several steps of microbiological examination of the JSC "Grindeks" wastewater treatment process (WWTP) - isolation, identification and characterization of the microorganisms associated with the process. Also, batch experiments on the ability of individual cultures to biodegrade pharmaceutical wastewater containing 2300-3500 mg/l of COD were carried out.

2. Materials and methods
2.1. Isolation of microorganisms
Samples from MBBR process (suspended biomass and biofilm carriers) were collected from JSC "Grindeks" WWTP. The biomass was obtained from biofilm carriers by scraping off a thin layer of biofilm from their plastic surface with the sharp end of a stainless steel spatula, and suspended in sterile distilled water. All

samples were treated in an aseptic manner. Serial dilutions of the samples up to 10^{-8} were prepared for proper isolation of individual colony forming units. The isolation of bacteria involved the use of tryptic glucose yeast extract agar (Biolife) and R2A agar (Conda Laboratorios), and incubation at 28 °C for 72 hours, while the isolation of yeast and filamentous fungi was performed using the Sabouraud dextrose agar with chloramphenicol (Biolife) and incubation at 26 °C for 96 hours (yeast) and at 25 °C for 96 hours (filamentous fungi). Incubation times and temperatures were set to be in the range of optimal growth for mesophilic microorganisms with slightly lower temperatures and increased incubation times for yeasts and filamentous fungi.

2.2. Identification of the microorganisms

2.2.1. Biolog™ Microbial Identification System

As the primary and basic tool for identification of the isolates the GEN III MicroPlate™ and FF MicroPlate™ test panels of the Biolog™ Microbial Identification System (Biolog Inc., USA) were used. The identification was carried out according to the manufacturer's instructions, and the results were processed using the Biolog's identification system software OmniLog® Data Collection.

2.2.2. PCR method

Genomic DNA was extracted from approximately 0.25 g of mycelia or bacterial, or yeast biomass using the method developed by Cenis [4]. Extracted DNA was amplified by PCR with primers ITS4 [5] and ITS1F [6] for fungi or FORB and REVB for bacteria [7-8].

The PCR reactions in Mastercycler Personal (Eppendorf, Germany) were carried out in 50 µl volume. The mixture contained 0.4 µl Hot Start *Taq* DNA Polymerase, 5 µl 10X Hot Start PCR Buffer, 5 µl dNTP Mix, 2 mM each, 4 µl 25 mM $MgCl_2$, 0.75 µl Bovine Serum Albumin 20 mg ml^{-1} (all reagents from Thermo Scientific Fermentas Molecular Biology Solutions, Lithuania), 1 µl of each 25 µM primer (OPERON Biotechnologies, Germany), 30.85 µl sterile distilled water and 1 µl of DNA template. The PCR conditions were as follows: the initial denaturation step of 4 min at 95 °C, 40 s of denaturation at 95 °C, 40 s of annealing at 52 °C (for fungi) and 56 °C (for bacteria), 1 min of primer extension at 72 °C (30 cycles) and final extension for 10 min at 72 °C.

Amplified DNA fragments were treated with FastAP™ Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Scientific Fermentas Molecular Biology Solutions, Lithuania) and sequenced by Macrogen Europe (Amsterdam, the Netherlands). The double stranded sequences of PCR amplicons were assembled using Staden Package 1.6.0. A homology search was performed against the National Centre for Biotechnology Information GenBank nucleotide database using the Basic Local Alignment Search Tool.

2.3. Preparation of the pharmaceutical wastewater

The wastewater samples were collected from JSC "Grindeks" WWTP buffer tank containing an agitated wastewater from the production department, and filtered through 1.6 µm Whatman GF/A glass microfiber filters and afterwards through 0.45 µm Millipore Durapore® membrane filters in order to remove the suspended and particulate matter, followed by filtration through 0.2 µm Sartorius Minisart® high flow polyethersulfone membrane syringe filters for a cold sterilization of the wastewater just before the use in batch experiments. The samples with COD values ranging from 2300 to 3500 mg/l were used. When necessary, dilutions with purified water from Millipore RiOs-DI 3 UV water purification system were made. The pH was adjusted using the 20% H_2SO_4 and 30% NaOH. pH values of the wastewater for bacteria and yeast tests were set to pH 7.0 ± 0.2 and for filamentous fungi to pH 5.6 ± 0.2.

2.4. Batch experiments

COD degradation in batch experiments was performed in 250 ml Erlenmeyer flasks, incubated and shaken in a shaker-incubator Biosan ES-20 with a rotation speed set to 250 rpm and temperature set to 28 °C for bacteria isolates, to 26 °C for yeast isolates and to 25 °C for filamentous fungi isolates. To maintain desired pH value, a Sørensen's phosphate buffer solution (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4) for bacteria and yeasts, and a phosphate-citrate acid buffer solution (0.2 M Na_2HPO_4, 0.1 M citric acid) for filamentous fungi of 1/5 of total volume was added. Turbidity measurements were done on 0 and 5^{th} day with Lovibond turbidimeter TurbiDirect. The initial turbidity measurement was done to determine relative starting concentration of the pure cultures suspended into the wastewater and the turbidity was desired to be not lower than 10 and higher than 70 nephelometric turbidity units (NTU). In case of an exceedingly low initial concentration of the microorganism, more of them were added and suspended from the solid media, and if the concentration was too high a filtered wastewater was added. All experiments were carried out in sterile conditions with sterilized flasks and by

observing aseptic work techniques at all time, and prior to test cultures the pH buffer solutions were added. Flasks were sealed with sterile cotton corks.
COD analyses were done by using HACH LANGE cuvette tests LCK014 and LCK514 and the spectrophotometer Hach DR 5000. During 0, 1st, 3rd and 5th experiment days COD was measured. Samples for COD measurements were filtered through 1.6 µm Whatman GF/A glass microfiber filters.

3. Results and discussion

3.1. Identity of the isolates

Table 1 shows summary of identified species which showed COD reduction. Most of the isolates belong to bacteria but yeasts and filamentous fungi were also present.

Table 1: List of species with COD reduction

Taxonomic groups		
Bacteria	Yeasts	Filamentous fungi
Acidovorax delafieldii	*Candida inconspicua**	*Fusarium solani**
Aeromonas caviae	*Galactomyces pseudocandidum**	*Fusarium udum*
Flavobacterium johnsoniae	*Rhodotorula mucilaginosa**	*Pseudallescheria boydii**
Moraxella osloensis	*Trichosporon asahii**	
*Paracoccus versutus**	*Trichosporon domesticum**	
Pseudomonas aeruginosa		
Pseudomonas pseudoalcaligenes		
*Sphingobacterium thalpophilum**		
Tsukamurella inchonensis		

* Identified by PCR method

There were 65 microorganisms isolated from WWTP in total, 33 of which were bacteria, 22 were yeasts and 10 were filamentous fungi. Activity of bacteria was the highest comparing to yeasts and filamentous fungi in the wastewater samples, however only for nine bacterial isolates that showed COD reduction greater than a negative control, the identification was carried out. Other isolates were considered as irrelevant for the further batch tests. Three out of nine identified isolates *Aeromonas caviae, Moraxella osloensis* and *Sphingobacterium thalpophilum* achieved a total COD degradation higher than 70% during 5 days of contact time (Fig. 1). Other bacteria showed relatively low degradation activity on pharmaceutical wastewater. It could mean that most of bacteria are involved only in the final stage of biodegradation when the complex chemical compounds already are converted to readily biodegradable and non-toxic molecules. Or it could also indicate on their poor utilization of majority of the substances present in the wastewater. Although it was evident that COD degradation curves continue to increase, experiments were finished on the 5th day, due to hydraulic retention time of JSC "Grindeks" WWTP being no longer than 6 days.
Activity of the yeast isolates was lower than that of the bacteria and only five isolates showed higher COD reduction than it was for negative control. Two isolates out of five (*Galactomyces pseudocandidum* and *Rhodotorula mucilaginosa)* were able to reduce COD above 70%. Reason for that is considered to be the same as for the bacterial isolates.
Activity of the filamentous fungi was the weakest among all isolates, while COD reduction for the identified isolates was the highest, exceeding 75% for each isolate, and some like *Fusarium solani* reached its maximum reduction already after 72 hours after coming into contact with the tested water. The comparatively strong reduction of COD for the filamentous fungi isolates is believed to be caused by the specific enzymatic activity of the isolates.

3.2. COD degradation ability of bacterial isolates

As it can be seen in Figure 1, most of the nine bacteria isolates from the batch tests showed similar COD reduction patterns. The specific contact time of 5 days for all batch experiments was chosen to match the HRT of WWTP, therefore, reduction limits could not be determined and even though there is no practical significance, the reduction could potentially be higher if the batch tests were carried out for longer periods of time.

As mentioned before, each batch test was provided with a negative control with no microorganisms added to the wastewater. COD reduction of negative control varied from 38.6% to 51.2% and it can be explained by the high concentration of volatile organic compounds (mainly solvents) present in the tested wastewater. Figure 2 shows individual COD reduction ability of each bacterial isolate, obtained by subtracting COD reduction of negative control from the total COD reduction result. The net COD reduction performance of individual isolates varied from 2.1% to 31.6%. The worst result was provided by *Flavobacterium johnsoniae*.

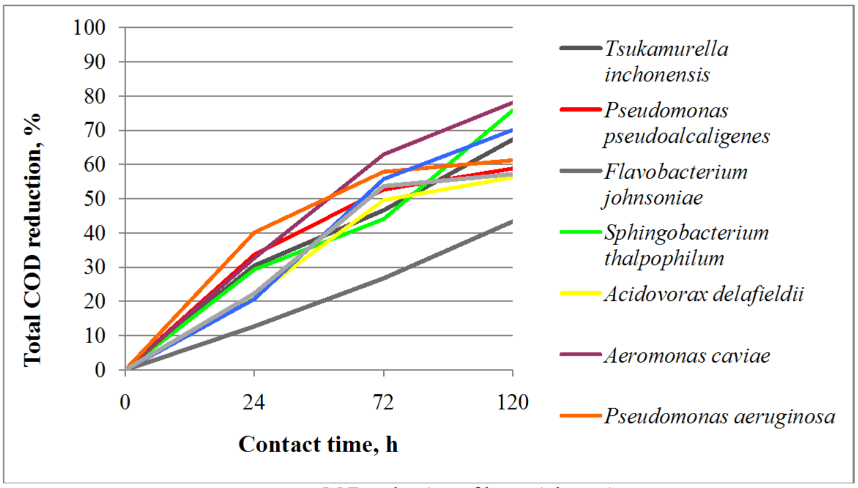

Figure 1: COD reduction of bacterial species

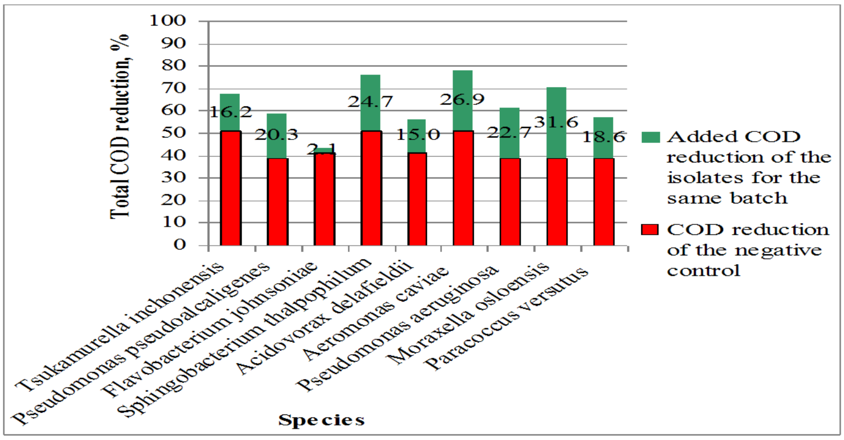

Figure 2: COD reduction performance of the bacterial isolates

Bacteria which showed the highest efficiency in "Grindeks" WWTP are also present in biological treatment of other types of industrial wastewater. *Aeromonas caviae* is known as an efficient biosorbent of toxic heavy metal Cr(VI) from industrial wastewater [9]. *Moraxella osloensis* also has the ability to degrade the acrylamide [10] and synthetic azo dyes in textile industry wastewater [11]. *Sphingobacterium thalpophilum* is known to possess deemulsification properties, and therefore is useful in microbial deemulsification process of oil emulsions in order to recover oil from them [12].

Calculation showed a weak correlation between COD degradation and the increase of turbidity of the batch samples. For example, *Paracoccus versutus* showed only 18.6% of COD reduction, while the turbidity increase for this isolate was the highest, 623.5%. *Pseudomonas pseudoalcaligenes* achieved 20.3% COD degradation, but the turbidity increase was only 165.1%. Increase of turbidity indicates the increase of cell concentration, and from the economical point of view it is not desirable, because of the increased costs for excess sludge removal and recycling.

3.3. COD degradation ability of yeasts and filamentous fungi

Yeasts and filamentous fungi isolates composed almost half of all identified microorganisms with the ability to reduce COD (Table 1). As shown in Figure 3, the potential for the identified yeasts to biodegrade chemical pollution is considerable, and Figure 4 indicates that an average net COD reduction was higher than from bacterial isolates. COD removal efficiency of the five yeast isolates varied from 68.3% to 76.6% with an average net COD reduction of 25.6% compared to bacterial 19.8%. The highest net biodegradation potential of yeasts belonged to *Rhodotorula mucilaginosa*, which is known by its significant ability to absorb heavy metals and to degrade refractory organic pollutants, including petroleum hydrocarbons and polychlorinated biphenyls [15-18].

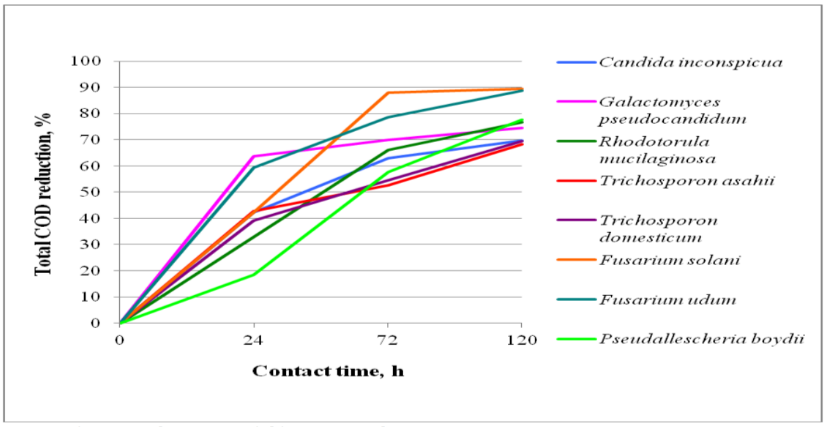

Figure 3: COD reduction of yeasts and filamentous fungi species

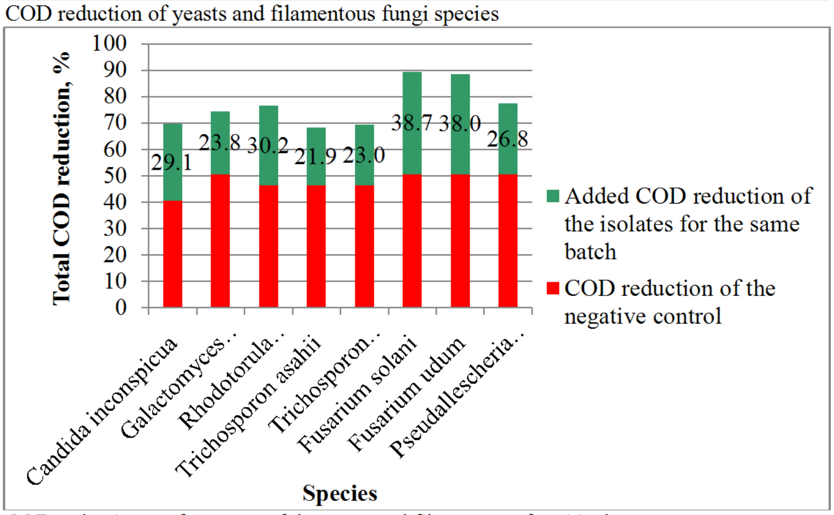

Figure 4: COD reduction performance of the yeast and filamentous fungi isolates

Filamentous fungi showed the highest COD removal efficiency compared to bacteria and yeasts (Fig. 3). The maximum COD reduction of 88.7% and 89.4% was observed from *Fusarium udum* and *Fusarium solani*. Also, the average net COD degradation was 34.5% and the reduction speed was the fastest as well. These filamentous fungi produce laccase enzyme, which can be used for removal/degradation of a number of environmental pollutants, including dyes, phenolic compounds, endocrine disrupting chemicals, polycyclic aromatic hydrocarbons and others xenobiotics [13]. *Fusarium solani* is also useful as a biosorbent for Cr(VI) removal from industrial effluents [14].
As mentioned before the average COD removal efficiency of JSC "Grindeks" wastewater treatment process is 93-94% which is higher than the COD degradation efficiency of individual isolates described in this article, because in real wastewater treatment process activated sludge consist of consortia of microorganisms and in a right combinations they are capable to reach better treatment results.

Conclusions

The highest COD degradation in "Grindeks" pharmaceutical wastewater pre-treatment process was showed by the filamentous fungi species *Fusarium udum* and *Fusarium solani,* which achieved 88.7% and 89.4% COD reduction during the 5-day-long contact time. *Aeromonas caviae* and *Sphingobacterium thalpophilum* showed higher COD degradation efficiency in comparison with other bacterial isolates. The COD degradation achieved by these cultures was 78.1% and 75.9%, respectively. *Rhodotorula mucilaginosa* reduced COD by 76.6% and was determined as the most effective yeast isolate.

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References

1. Sirtori C., Zapata A., Oller I., Gernjak W., Agüera A., Malato S., *Water Res.* 43 (2009) 661.
2. Abu-Safa A., Abu-Salah S., Mosa M., Gharaibeh S., *Int. J. Chem. Biol. Eng.* 6 (2012) 153.
3. Arslan-Alaton I., Akmehmet Balcioglu I., *Arch. Environ. Contam. Toxicol.* 43 (2002) 425.
4. Cenis J.L., *Nucleic Acids Res.* 20 (1992) 2380.
5. Innis M. A., Gelfand D. H., Sninsky J. J., White T. J. PCR Protocols. A Guide to Methods and Applications, Academic Press, (1990).
6. Gardes M., Bruns T. D., *Mol. Ecol.* 2 (1993) 113.
7. Yeates C., Gillings M. R., Davison A. D., Altavilla N., Veal D. A., *Biol. Proced. Online* 1 (1998) 40.
8. Edwards U., Rogall T., Blocker H., Emde M., Bottger E. C., *Nucleic Acids Res.* 17 (1989) 7843.
9. Loukidou M. X., Karapantsios T. D., Zouboulis A. I., Matis K. A., *Ind. Eng. Chem. Res.* 43 (2004) 1748.
10. Jebasingh E. J. S., Lakshmikandan M., Rajesh R. P., Raja P., *Int. Biodeterior. Biodegrad.* 85 (2013) 120.
11. Karunya A., Rose C., Valli Nachiyar C., *World J. Microbiol. Biotechnol.* 30 (2014) 915.
12. Singh A., Van Hamme J. D., Ward O. P., *Biotechnol. Adv.* 25 (2006) 99.
13. Fernández J. L. M. Laccases from new fungal sources and some promising applications, *Doctoral Thesis*, Lund University, Lund, Sweden (2011).
14. Sen M., Ghosh Dastidar M., *Iran. J. Environ. Health. Sci. Eng.* 8 (2001) 153.
15. Jiang B., Wang Q., Jing L., Zhong Y., Liu H., *Adv. Biomed. Eng.* 6 (2012) 185.
16. Ertuğrul S., San N. O., Dönmez G., *Ecol. Eng.* 35 (2009) 128.
17. Suehara K., Kawamoto Y., Fujii E., Kohda J., Nakano Y., Yano T., *J. Biosci. Bioeng.* 100 (2005) 437.
18. Jarboui R., Baati H., Fetoui F., Gargouri A., Gharsallah N., Ammar E., *Environ. Technol.* 33 (2012) 951.

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Strategies for Sustainable and Circular Management of Phosphorus in the Baltic Sea Region: The Holistic Approach of the InPhos Project

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Abstract: Despite the significant reduction of phosphorus (P) discharge in the Baltic Sea in the last decades, obtained through the implementation of some approaches within the Helsinki Convention, eutrophication is still considered the biggest problem for the Baltic Sea environment. Consequently, the reduction of P load is an urgent need to solve, but the complexity of both the environmental and legislative context of the area makes this process difficult (more than in the past). Eutrophication is an intricate issue requiring a proper framework of governance that is not easy to determine in the Baltic Sea Region where the needs of several different countries converge. To identify the most suitable strategy to reduce the eutrophication in the Baltic Sea, the InPhos project (no. 17022, 2018–2019, funded by the European Institute of Innovation & Technology (EIT) Raw Materials) adopted a holistic approach considering technical, political, economic, environmental and social aspects of P management. With the aims to raise awareness about the P challenge, foster the dialogue among all the stakeholders, and find solutions already developed in other countries (such as Germany and Switzerland) to be transferred in the Baltic Sea Region, the InPhos project consortium applied the methodology proposed in this paper, consisting of three main phases: (i) analysis of the available technologies to remove P from waste streams that contribute to eutrophication; (ii) analysis of the main streams involving P in Baltic Sea countries to highlight the potential of more sustainable and circular P management; (iii) study of the current context (e.g., already-existing initiatives and issues). This approach allowed us to identify four categories of recommendations and practical actions proposed to improve P management in the Baltic Sea region. During the project, the consortium mainly addressed social aspects. Following steps beyond the project will be more quantitative to determine the techno-economic feasibility of circular P management in selected demo cases in the region.

Keywords: phosphorus management; circular economy; Baltic Sea; eutrophication

1. Introduction

Phosphorus (P) is a fundamental nutrient for the growth of all living organisms, with properties that cannot be replaced by any other element. In particular, P represents the third major macronutrient (after potash and nitrogen) that is used in industrially produced fertilizers. Consequently, P represents a crucial building block of the food security system. Regrettably, in practice, P is a non-renewable mineral resource since a time misbalance exists between the geological cycle of phosphorous-bearing minerals (million years) and the anthropic use cycle (daily–annual) [1–4]. Moreover, primary P mines are concentrated in a few areas (China, Morocco, USA), mostly not belonging to the EU, which imports more than 90% of its P demand. This context, characterized by quantity and quality scarcity of primary P, increasing demand and high price volatility of P fertilizers, represents a serious "P challenge" for the global economy and for the EU [5–7]. Issues of sustainable P management have been included in European policy since 2013, when the first communication directly related to P management, entitled "Consultative Communication on the Sustainable Use of Phosphorus", was published [8]. The main objective of this document was to start the development of a more rational and sustainable use of phosphorus from both primary and secondary sources. This initiative was also highlighted in the "Roadmap to a Resource-Efficient Europe" [9]. Management of the phosphorus raw materials increased in importance when, in 2014, phosphate rock was identified as a critical raw material (CRM) for the EU economy [10]. On the updated CRMs list, issued in 2017, next to phosphate rock, elementary phosphorus was also indicated [11]. The inclusion of P raw materials on the list indicates their extraordinary importance for the European economy and is intended to stimulate actions that can contribute to better management of phosphorus compounds from policy-makers, food, fertilizer and agricultural industries, farmers and scientists.

The phosphorous that enters the utilization chains in society is not entirely utilized in agriculture, breeding of animals, industrial food production, and neither is it in final human consumption. Only 15% of the total mineral P used in the food production sector is effectively consumed by humans. Even though there are losses of phosphorous in mining and fertilizer manufacturing, the greatest losses are associated with crop cultivation, meat, and dairy production. Along the food value chain, the main "waste" streams, are (in order of importance for the EU) (i) animal manure; (ii) urban wastewater and sewage sludge; and (iii) food processing (involving slaughter and other solid waste and food processing wastewater) [1,5]. These streams constitute a great loss of money and food production capacity. Moreover, when they are discharged into neighboring ecosystems, the effect of the nutrient can cause negative environmental issues, such as water body eutrophication [12–15]. Several coastal areas in the world are affected by eutrophication: in [16], 415 coastal systems were defined as eutrophic and hypoxic worldwide. Among them, the most critical are mainly the following five: the Baltic Sea, the North Sea, The Gulf of Mexico, the Chesapeake Bay, and the Black Sea [17]. Moreover, the eutrophication issue also continues to be pervasive in numerous lakes and rivers in the world. Between the 1970s and the 1990s, the strategies implemented to reduce the problem focused on a drastic decrease of the industrial and domestic P sources, with actions such as the improvement of wastewater treatment and the banning of P in detergents. These measures improved the situation in some areas, such as

Lake Erie (USA) and Lake Geneva (France) [18]. Starting from the beginning of 20th century, different types of strategies were proposed and introduced to address also other sources of P [19]. In [20], the strategies adopted by different areas affected by eutrophication were collected and sorted in clear categories, that can be summarized in (i) legal strategies: regulatory standards for nutrient pollution control, regulatory limits and caps, policies for ecosystem protection and restoration; (ii) technical strategies: research, monitoring and evaluation; (iii) financial strategies: economic and fiscal incentives; and (iv) social strategies: education and outreach, actions to strengthen institution and capacity to reduce nutrient pollution. In [21], a chronological list of laws and legal actions to improve water quality in Japan was provided. In European countries, from a legal point of view, stringent regulations about nutrient emissions to soil and water have been issued to avoid this type of environmental issue. Particularly, specific EU Directives (Nitrates Dir. (91/676/EEC), Sewage Sludge Dir. (86/278/ECC), Urban Waste Water Treatment Dir. (91/271/EEC), Industrial Emissions Dir. (2010/75/EU), and Water Framework Dir. (2000/60/EC) set some specific targets about nutrient contents in "waste" streams to obtain and/or maintain water bodies with a suitable ecological status. Since eutrophication impacts the environment, the economy, and human health (the three pillars of the sustainability), it derives that all these types of strategies and actions are intercorrelated and have to be supported by a suitable legislative framework [19].

The Baltic Sea region was one of the first marine areas to require an urgent solution for eutrophication (in the 1970s), but eutrophication is still considered the biggest problem for the Baltic Sea environment. Despite significant reductions on P discharge since the 1970s (above all from Poland [22]), obtained through the test of some approaches within the Helsinki Commission (HELCOM), signed by all the Baltic Sea coastal States, the P load in the Baltic Sea is still high and needs to be further reduced [23]. Decreasing the actual value of P load in the Baltic Sea, to respect the HELCOM targets, is more difficult than in the past. The greatest sources of P pollution are mainly diffuse-sources, related to agricultural activities and animal production, difficult to reduce and to trace. On the other hand, P point-sources (the second category of P pollution sources, e.g., urban and industrial waste-water) are simpler to divert, since they are concentrated and usually monitored. From the regulatory perspective, the difficulties derive from the complex regulatory settings, characterized by inter-connected, over-lapping levels of regulations and flexible legal approaches [24–27]. From the circular economy perspective, it is recommended to keep this raw material in the value chain (as wastewater and its fractions such as sewage sludge and ash from sewage sludge incineration, and other waste streams) for as long as possible, and to further recover and reuse valuable resources, including P. This could contribute to both eutrophication prevention and raw materials supply security [28]. Unfortunately, due to a lack of legal and economic drivers, nutrient recovery is still not a common solution, while P recovery, as well as energy efficiency, are among the main current challenges in wastewater treatment plant (WWTP) design and operation [29]. To select the most suitable strategy to improve this situation, multi-dimensional and transdisciplinary knowledge is needed. This means that expertise from different disciplines and all the stakeholders need to be integrated to create an understanding of the entire P supply chain, considering both the scientific and technical aspects and the social and economic implications [30,31].

With this holistic approach, the InPhos project no. 17022 (2018–2019), funded by the EIT Raw Materials, aims to develop a P long-term management strategy for the Baltic region, laying out a solid foundation for sustainable management of this critical raw material. The project considers the technical, political, economic, environmental and social aspects of the P cycle to (i) raise awareness about the urgent problem of P sustainability; (ii) foster dialogue among multi-disciplinary practitioners (e.g., policy makers and industries), the scientific and research community, and wider society on the consequences of P scarcity in Europe; and (iii) identify prospective solutions that can be implemented in the Baltic region to effectively reduce eutrophication. These objectives were addressed by a consortium of 12 European partners composed of 3 research Institutes and agencies (MEERI–Poland; GTK–Finland; BAM–Germany); 6 universities (KTU–Lithuania; TTU–Estonia; LU and RTU–Latvia; Chalmers

TU–Sweden; UNIBO–Italy); 3 companies and utilities (OUTOTEC–Germany; BIONOR–Poland; Kauno vandenys–Lithuania). It created an interdisciplinary research team from each part of the knowledge triangle in order to effectively cover a wide range of complementary knowledge, expertise, and experiences of the P life-cycle and to make the project results relevant on the regional, national, and international levels. Recognizing the need for strategic cooperation at the European level, the partners joined forces to not only communicate the complexity of the "P challenge", but also to collect information on opportunities for more effective utilization of P from waste and other P-rich streams in the Baltic region.

2. Materials and Methods

With the aims of defining specific recommendations to find effective solutions to take decisive steps to reduce the eutrophication issue in the Baltic region and, where possible, to initiate well-functioning circular initiatives in P management, the methodology adopted during the InPhos project consisted of three main phases, as shown in Figure 1:

(i) Create an overview of the current P management in the Baltic countries, considering the main P flows (quantity and forms) in terms of P extraction, production, consumption, and losses to the environment (land and water bodies) to highlight the potential of improvement in P recovery;
(ii) Collect knowledge of the available technologies to remove and recover P from waste streams and options for selecting the most suitable solutions;
(iii) With a holistic approach, create an understanding of the main limitations for the implementation of P removal technologies in relation to existing initiatives both on the national and European levels.

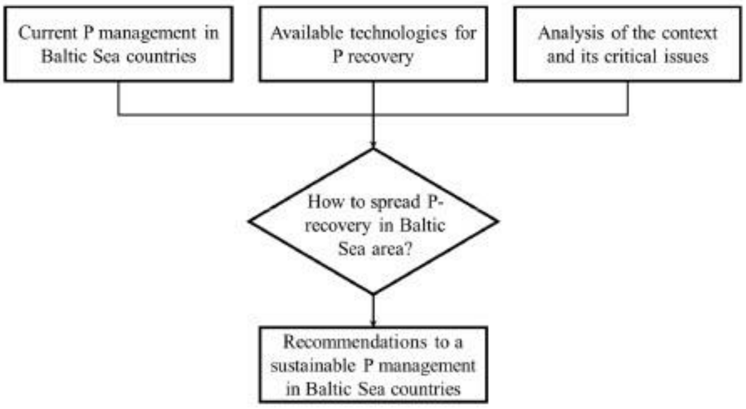

Figure 1. Flowchart of the methodology used in the InPhos project and in this study.

2.1. *P sources, Use and Management in the Baltic Sea Region*

The Baltic Sea in Northern Europe is surrounded by nine countries (Figure 2). Of them, Lithuania, Latvia, and Estonia are entirely within the Baltic Sea drainage area. Nevertheless, the largest Baltic sea drainage area territories belong to Sweden (25.6% of the overall drainage area), Russia (18.3%), Poland (18.1%), and Finland (17.5%). Countries in the Baltic Sea region are classified as developed [32]. The region has a big population (about 85 million inhabitants) and is heavily industrialized, with intensive agriculture [33].

Figure 2. The Baltic Sea drainage area and countries of the Baltic region.

The characteristics of the Baltic sea make it a vulnerable ecosystem. It is a sheltered epicontinental sea with an average depth of only 52 m and a very limited water exchange. The Baltic Sea is one of the largest brackish waters in the world, with salinity in the ranges of 2‰–20‰. Thus, the ecosystem tends to be strongly responsive to the effects of human activity and affected by pollution, including high levels of eutrophication due to the discharge of nutrients.

To boost the installation of P-recovery technologies in the Baltic Sea area and to select the most suitable solution, information about P production, consumption, losses, and dispersion of P were collected for Baltic Sea countries among the EU. This stage of the methodological approach required numerous efforts due to the fragmentation (in formats and in time) of available data for each Baltic Sea country. However, a great effort was applied to find, homogenize, and integrate them in a paper that considered the entire Baltic Sea region.

2.1.1. Primary Sources of Phosphorus

Russia was not within the scope of the in-depth analysis because its situation is different from that of EU member states: EU strategic and legal requirements apply only to member states, and phosphate rock is a critical raw material for EU countries. However, Russian phosphate rock resources, though not all in the Baltic Sea drainage area, are significant enough to be mentioned. Russia had 2% or 1.3 billion tonnes of globally known reserves of phosphate rock in 2015. Production of phosphate rock in Russia constituted 5% of global mine production, the average for 2010–2014 [34].

Finland is unique in Western Europe for its phosphate rock resources, being the only EU member state where these resources are extracted (0.4% of global production [34]). The reported resources amount to 2360 million tonnes, with an average 4.0% phosphorus (P_2O_5) content [35,36]. Finnish phosphorus resources are magmatic. Compared to sedimentary deposits, these have lower heavy metal concentrations. The largest known phosphate rock deposit, in terms of volume, is Siilinjärvi. It was discovered in 1950; quarrying began in 1979. On average, the ore consists of apatite (10%), phlogopite (65%), carbonate (20%, 4/5 calcite), and richterite (5%). Siilinjärvi is Europe's only operating phosphate mine (operated by the Yara company) and processing plant. About 10 million tonnes of apatite ore are mined each year, producing about 1.0 million tonnes of apatite concentrate, which corresponds to about 375,000 tonnes of P_2O_5. It is further used in the production of phosphoric acid and phosphate fertilizers. The other significant deposit is Sokli. Known but not utilized reserves are concentrated in the gabbros of Southern Ostrobothnia. Nevertheless, their phosphorus content is lower than the Finnish average, and there are no plans for their commercial use in the near future. Potentially, the most significant yet unexplored phosphorus resources are included in the Iivaara alkaline massif and in the carbonatitic ring dikes on the southern side of the Sokli complex.

Estonia has approximately 800 Mt P_2O_5 in unused sedimentary phosphate rock reserves containing P_2O_5 in the range of 6% to 20% [37,38]. Currently, Estonian phosphate rock is not mined since there are a lot of technical and environmental problems. Historically, phosphate rock in Estonia has been mined only in one region near Maardu city. The mining started in 1922. A phosphate powder was produced and upgraded to superphosphate fertilizer [39]. During the years of operation (1922–1991), 25 million tonnes of phosphorite ore were extracted [38]. Mining ended in 1991 due to environmental problems and exhaustion of resources at that mine.

Currently, in Poland, there is no mining of phosphate rock. It is worth emphasizing, however, that phosphate rock (on average, 14% P_2O_5) occurs in the sediment strip on the Radom–Iłża–Annopol–Gościeradów–Modliborzyce section in various types of sediments [40]. In the past, they were used to produce phosphate fertilizers. Mining started after World War I. The resources of the 10 deposits identified were 42.4 million tonnes of phosphorite (7.35 million tonnes P_2O_5). At present, however, they are not exploited for economic reasons. The last exploited deposit in Chałupki was closed in 1961, and in Annopol in 1971. Nowadays, reserves do not satisfy the established quality parameters for maximum depth of deposit and for minimum contents of phosphorus. Even more, the deposits are irrigated, which would hinder the potential for exploitation, and various infrastructure objects such as roads, railways, and high-voltage lines run through them, reducing their availability for exploitation by up to 50%–80%. Therefore, all phosphate rock deposits in 2006 were deleted from the national resource balance, and the domestic demand for phosphate rock raw materials is fully covered by imports [41].

In Sweden, there are also no P-containing deposits that are mined for their phosphorus content. However, considerable P amounts are present in apatite-containing ores that are mined for their iron content [42]. The phosphorus ends up in the mining waste. It has been estimated that about 1.5 million tonnes of P is currently stored in these waste deposits, and possibilities for its recovery have been discussed [43].

In Latvia, mineral deposits containing phosphorus are rare [44]. Very small amounts of phosphorus-containing minerals such as phosphorite and vivianite can be found mainly in the east and northeast parts of Latvia [45].

Denmark, Germany and Lithuania do not have primary sources of phosphorus, such as phosphate rocks.

2.1.2. Production of Mineral Phosphorus Fertilizers

Large production capacity of mineral P fertilizers in the Baltic Sea region has been developed in Poland and Lithuania. Their fertilizer industry produced about 4.2% and 1.7% of world phosphate fertilizers in 2017, respectively. Polish fertilizer industry ranked among 5 top producers for monoamonium phosphate, NPK fertilizers, and superphosphate, while Lithuanian was among the top 5 for diamonium phosphate [46]. The demand for phosphorus raw materials both in Poland and in Lithuania is satisfied entirely by imports. Import to Poland is mainly in the form of phosphate concentrates from Morocco (45% in 2016), Senegal (24%), Israel (15%), Algeria (11%), Togo (4%), and other countries [47]. Import to Lithuania is mainly from Russia (about 65%), as well as from South Africa, Morocco, and other countries [48].

Germany had a substantial production of phosphate-fertilizing products in the 1970s and 1980s. Today, only a small capacity remains at ICL Ludwigshafen [49].

2.1.3. Use of Phosphorus and Its Recovery from Secondary Sources

Although there are some industries that need phosphorus for its production, the main user of P, apart from fertilizer production, in the Baltic Sea region is intensive agriculture, for which both mineral and organic fertilizers are used. Depending on regional and national practices and regulations, sewage sludge and other secondary P sources are also exploited in agriculture.

Mineral fertilizers. A comparison of the mineral P fertilizer consumption in countries of the Baltic Sea region is presented in Table 1 (based on [50]). Poland and Germany are the biggest consumers of mineral P fertilizers in the region.

Table 1. Consumption of mineral P fertilizers by agriculture [50].

Baltic Country	P fertilizer Consumption (k tonnes)		Specific P Fertiliser Consumption (tonnes/ha of Utilised Agricultural Area)	
Year	2007	2017	2007	2017
Denmark	14.0	20.8	5.6	8.1
Estonia	3.5	4.1	3.9	4.2
Germany	115.5	100.9	7.1	6.2
Finland	16.0	12.3	8.0	6.1
Latvia	7.3	11.3	5.9	7.7
Lithuania	17.0	23.5	6.6	8.3
Poland	179.9	150.0	12.0	10.7
Sweden	13.7	14.5	4.9	5.2

Organic fertilisers. The practice of using manure, slurry, or other organic fertilizers is common to a certain extent in all the countries. For instance, in Germany, some 200,000–240,000 annual tonnes of P are consumed by using livestock manure [51]. However, livestock manure-borne P is mainly used in regions with high livestock density due to the high water concentration in manure that hampers transportability. Consequently, by recycling P from manure in the form of concentrated, transportable fertilizing products, as demonstrated for instance by the H2020 project "SYSTEMIC" (www.systemicproject.eu), a yet unknown fraction of manure-borne P could be recycled to regions where P is needed and thus replace P from mineral fertilizers. Germany and Denmark have the largest numbers of livestock and, therefore, the largest amounts of manure and slurry to be spread on agricultural land. The livestock intensity (LSU/ha UAA = number of livestock units per hectare of utilised agricultural area) was1.09 LSU/ha UAA in Germany, and 1.58 LSU/ha UAA in Denmark in 2016 [52]. However, the manure in Denmark is currently not recycled in the most efficient way. The utilization of hosphorus from manure is indirectly limited due to the threshold imposed for nitrogen, that is, 140–170 kg N/ha/year for the entire Denmark [53]. Moreover, due to geographical conditions, the relocation of animal manure from farms with manure surplus to farms where it can substitute mineral P fertilizer is critical since the large volumes of the resource reduce possible transport distances, just as in Germany. For improving P use efficiency from manure, current regulations, economic incentives, and technical solutions will be crucial. The lowest livestock intensity is reported in Lithuania (0.29 LSU/ha UAA), Estonia (0.28 LSU/ha UAA), and Latvia (0.26 LSU/ha UAA) [52]. Nevertheless, over-fertilization of fields belonging to intensive livestock farms happens in all the countries of the region [54].

Sewage sludge and other sources. The amount of produced sewage sludge, as one of the most important secondary resources for phosphorus, is indicated in Table 2 [55]. Sewage sludge treatment and utilization practices differ among countries.

Table 2. Sewage sludge production [55].

Country	Denmark	Estonia	Finland	Germany	Latvia	Lithuania	Poland	Sweden
Year	2010	2016	2015	2016	2017	2017	2017	2016
k $tonnes_{dry\ matter}$/year	141,000	18,340	146,000	1,794,443	24,940	42,488	584,454	204,300

In Denmark, sewage sludge contains about 1590 t/year of P. Another important source of P is slaughterhouse waste (accounting for about 3000 tonnes of P for each year in Denmark). There are some success stories in the country regarding the recovery of phosphorus. For instance, the first full-scale demonstration plant, which intended to test the viability of secondary phosphate production, was opened by Aarhus Water treatment company in 2014. This plant is able to extract up to 50 kg P/day, which is 60% of the amount of phosphorus in the wastewater [56]. The largest phosphorus recovery plant was opened in 2015 by the Herning Water treatment company. The same technology was adopted at both plants: struvite is precipitated as a "ready-to-use fertilizer" and sold to a fertilizer company under the name PhosphorCare™. It obtained official approval as commercial fertilizer.

About 20,000 tonnes of dry matter sludge are produced in Estonia annually. In 2016, 84% of the sewage sludge was used in landscaping and land restoration, 15 % was landfilled, and a very small part of the sludge was used in agriculture. There are no obligatory regulations of P recovery from waste. The major alternative source of P in Estonia is municipal and industrial wastewater and sewage sludge. However, a priority is the development and the inclusion of regulations connected with the treatment and recovery of valuable materials from these flows.

Finland produces approximately 140,000 tonnes of dry matter sewage sludge each year. There is no tradition to recycle nutrients from this sludge. Instead, 62% is used for landscaping and reforming of landfills, 33% is used for biogas production plants, 2% for landfill or final disposal, and 2% is used for agricultural soil amendments. One of the major reasons for this little percentage of sludge used as fertilizer is that people do not trust in sludge quality and do not want to cause human health and environmental problems because of organic pollutants and heavy metals that might be present in the sludge. Only 17 of WWTPs in Finland are large (>100,000 PE) [57]. Nevertheless, the Viikinmäki WWTP, located in Helsinki, with a capacity of around 270,000 cubic meters per day, is the largest wastewater treatment plant in Finland and the Nordic countries. Currently, there is no recovery of phosphorus [58,59]. Nevertheless, the construction of a demo plant for phosphorus recovery began at the Viikinmäki WWTP in 2018 [60]. Based on a survey conducted by the Finnish Innovation Fund Sitra on circular economy opportunities for Finland, the total amount of recyclable phosphorus was indicated to be 26,040 tonnes/year, of which 19,300 tonnes (74.1%) came from livestock manure and 2880 tonnes (11.1%) from municipal sewage sludge.

In Germany, about 1.76 million tonnes of dry matter sewage sludge (about 3%–4% P) with approximately 60,000 tonnes of P and 200,000 tonnes of Cat. 1 meat and bone meal (animal by-products, not for consumption, classified within 1 risk category according to Regulation (EC) 1069/2009) [61], with approximately 10,000 tonnes of P, are available for P recycling. In 2017, about 68% of German sewage sludge was converted to energy in so-called mono-incineration plants, power plants, cement plants, and a few municipal solid waste incinerators, 17% applied to agricultural soils, and 13% used for "landscaping", among others, using sewage sludge as cover layer on the top of closed landfills. Due to the amendment of the Fertilising Ordinance in 2017, agricultural sludge use in Germany declined significantly. By this amendment, stricter requirements for nutrient management on cropland have been enforced in response to the high nitrate concentrations in German groundwater and non-compliance with the EU 1991 Nitrates Directive. The maximum annual amount of organic nitrogen has been limited to 170 kg N per hectare, regardless of being applied as manure or sewage sludge. Consequently, the competition of organic fertilizers for available cropland is increasing. Farmers prefer spreading their own manure and digestates to sewage sludge. Due to the significant decrease of sludge use in agriculture, a shortage of disposal capacities occurred. Therefore, there are plans to build up to 15 new incineration plants for sewage sludge [62]. P-recycling from ash is not yet implemented on a large scale, but several projects are underway; the first one will be commissioned in 2020/2021 in Hamburg. Several struvite plants are in operation in Germany; albeit, investment was not motivated by P-recycling but by OPEX (operational expenses) savings: avoidance of struvite scaling in pipes and reactors and lower sludge volumes due to enhanced dewaterability of sludge, the latter in processes applied to sewage sludge. Hence, struvite is considered a byproduct and not much attention is paid to its marketing and sales.

About 25,000 tonnes of dry matter sludge is produced in Latvia annually, 36% of which is produced in Riga. The content of P in sewage sludge obtained in different WWTPs in Latvia varies from 7.8 to 33 g/kg dry sludge [63]. Sludge is mainly utilized in composting (27% in 2016–2018) and in agriculture (16% in 2016–2018) [64]. The annual emission limit value of total P derived from sewage sludge and compost in cultivated agricultural land is 40 kg/ha [65]. In Latvia, sewage sludge is not completely used as a resource, which is confirmed by the fact that approximately 1/3 of the sludge produced is temporarily stored in the areas of WWTPs unused [64]. The dispersed location of small-scale WWTPs limits centralized sludge management. There is no experience with P-recovery pilot plants in Latvia, and any legislation regulates the recovery of phosphorus from alternative phosphorus sources such as sewage sludge. Wastewater sludge is used in the reed canary grass and plantations of willow and fast-growing tree species on empty agriculture lands and to re-cultivate damaged areas (e.g., gravel and peat quarries) in different scales in Latvia [66,67].

More than 40,000 tonnes of dry matter sludge are produced in Lithuania annually. In recent years, the amount of landfilled sludge has decreased. Instead, sludge usage for fertilization and compost production is increasing. In 2017, 48.3% of sewage sludge was used in agriculture, 38.7% for compost production, and 5.2% of sludge was treated by other means (e.g., granulation, drying) [68]. A small part (0.3%) of the sludge was incinerated. There are digestion and drying facilities, also sludge composting sites. However, the development of the current wastewater sludge management infrastructure has not addressed the issue of the use of heat-dried and granular sludge, as there is no (mono)incineration capacity for it. Thus, a small percentage of sludge was incinerated in a municipal waste incinerator and in a cement kiln. In Lithuania, there is no legislation directly requiring recovery of phosphorus. Nevertheless, encouragement of such practices as composting allows the return of some P to the economic cycle.

In Poland, sewage sludge production was 1.035 million tonnes of dry matter in 2017. Of that, 56.5% originated from municipal WWTP and 43.5% from industrial WWTP [69]. The most frequently used methods of treatment were landfilling, use in agriculture, and incineration. However, the use of sewage sludge for applications in agriculture, in land reclamation, and in the cultivation of plants intended for the production of compost has gained no social acceptance, is seasonal, and there are not enough areas suitable for this purpose in the country. In recent years, 11 mono-incinerators for sewage sludge have been built next to large municipal WWTPs. The implemented phosphorus recycling solutions could be a good opportunity for Poland to decrease its dependency on imports of phosphate rock. However, there are no implemented P recycling technologies on the market. This is quite disturbing since a number of research teams have been working on the development and modernization of the technological solutions for efficient phosphorus recovery from waste, such as sewage sludge and sewage sludge ash [70]. Their achievements also include the integrated evaluation of the economic, technological and environmental aspects, in order to identify technologies and methods that are applicable and feasible alternatives to phosphate rock mining and mineral fertilizer production from that rock. Due to economic unprofitability, none of the Polish technologies have been implemented (they require large investment costs). Also, there are no legal requirements for nutrient recovery from waste streams in Poland. In 2018, the first investment in this area was started by the Jarocin Waterworks Company. It invested into the project "Modernisation and Extension of WWTP Jarocin" and is now building a station for the recovery of nutrients from sewage sludge.

Municipal wastewater treatment plants in Sweden receive about 5500 tonnes of phosphorus annually [71]. Most of the phosphorus (96%) ends up in sewage sludge because many of the 1700 treatment plants operate with enhanced phosphorus removal. Looking at the statistics for the more recent years, about 25% of the sludge in Sweden has been used as a fertilizer in agriculture. There has been a debate among many different actors, e.g., food industry, farmers, the concerned public, and NGOs and governmental agencies over several decades in Sweden on potential risks related to sludge use in agriculture. This has led to the development of the REVAQ sludge certification system and to large ongoing efforts to reduce sludge pollution at the wastewater source, but also to several changes in the national sludge management policy over time. The Swedish government has recently commissioned an investigation that was reported in January 2020 [72], which suggested that sludge of high quality could be exempt from a ban on spreading in agriculture. Further, as the investigation report contains a suggestion of recovery of at least 60 percent of the phosphorus from sewage sludge of the public treatment plants of wastewater in excess of 20,000 p.e. (population equivalents), considerable changes to current sludge management could be required for plants with lower quality sludge or with longer distances to suitable agricultural land. It is unclear what consequences this will have in the foreseeable future.

2.2. Technological Background for P Recovery: Field of Applications, Technical Features, and Output

There are several waste streams that are promising sources for P recovery. The three most important waste streams are presented in Table 3.

Table 3. Waste streams identified as possible secondary P sources.

Sector	Secondary P Sources
Agriculture	Manure [73,74]; Meat and bone meal [75]; Fish sludge [76,77]
Municipal	Municipal wastewater [78]; Municipal sewage sludge [79,80]; Municipal sewage sludge ash [81,82]; Food waste [83]
Industrial	Biomass ash [84]; Phosphogypsum [85]; Industrial wastewater [86] Industrial sewage sludge [87]; Industrial sewage sludge ash [88]

The results of most recent research show that sewage sludge, food waste, and manure are the nutrient-rich secondary sources with the highest technical and economic potential for P extraction [28]. However, the greatest development of nutrients recovery methods was observed for sewage sludge and its processed forms. It is related to the fact that 90% of the phosphate load in sewage treatment plant influent is transferred to sewage sludge, as most recovery processes start after wastewater treatment. Currently, most technology providers pursue either integrated phosphate recovery from digested sludge or supernatant liquor or downstream phosphate recovery from sludge ash after mono-incineration. The first group refers to precipitation processes producing struvite or, in some applications, calcium phosphate. P-recycling from ash achieves much higher recovery rates. Technology suppliers offer wet chemical or thermal processes, the former extracting P by acid attack and the latter modifying the P-compounds to enhance the crop availability of P and to remove certain heavy metals. Some of the most advanced phosphate recovery processes have been included in a recent LCA study [89–92].

Essentially, five process approaches have been developed to pilot or full-scale applications: (i) precipitation of struvite (magnesium–ammonium–phosphate) from digested sludge; (ii) precipitation of struvite from the liquid fraction after sludge dewatering; (iii) acid attack of ash leaching phosphates with other compounds from ash after mono-incineration; (iv) thermal solubilization of phosphates in ash after mono-incineration with partial removal of heavy metals, and (v) replacement of phosphate rock by ash in conventional fertilizer manufacturing plants. Struvite precipitation is usually limited to enhanced biological phosphorus removal (EBPR) in wastewater treatment plants because chemical P removal does not leave enough P in solution to meaningful application of precipitation processes. The related business cases are built on improved dewaterability of sludge, reducing its volume and related disposal cost as well as on lower maintenance expenses for pumps and pipes due to avoiding unintended precipitation. P-recovery from ash after mono-incineration of sewage sludge must be re-financed by product sales and savings of ash disposal costs. Refinancing is quite challenging due to high volumes of leaching residues after the acid attack of ash and solid/liquid separation that must be either further treated for use in the building industry or landfilled. The comparatively low cost of ash disposal cannot generate enough savings to justify gate-fees covering a substantial part of the treatment cost, which always exceeds the cost of producing conventional phosphate fertilizers, mainly due to economy of scale effects. Thermal solubilization has the advantage of low waste generation (<3% of ash input) but P concentrations in the product do not exceed P concentrations in the ash and do not allow producing complex fertilizers of high demand in the market. The replacement of phosphate rock by ash in the fertilizer industry is a viable pathway to recycle ash to conventional products not distinguishable from rock-based fertilizers, but replacement is limited by the iron and aluminum concentrations in ash and, consequently, by a maximum amount of ash admixture that has not yet been determined. Mixing 5%–10% of ash to phosphate rock has been successfully tested.

Precipitation processes applied to sludge only recover 5%–15% of P. If similar processes are applied to sludge liquor (the liquid phase after dewatering), the maximum P-recovery rates may achieve 25% without upstream solubilization of P and up to 40% with upstream solubilization by hydrolysis. The so-called Stuttgart process achieves even up to 50% recovery but with excessive chemical use as a downside. Processes recycling P from ash achieve 80%–100% recovery rates [89].

The predominant products of P-recovery methods are struvite or calcium phosphate, which can be obtained using chemical precipitation from the sludge dewatering liquors or raw wastewater with P concentration above 50 mg/L [92,93]. Since a wide spectrum of P-recovery technologies is available, their selection should be guided by byproduct management possibilities and P-recovery efficiency. Moreover, the WWTP efficiency can be improved by P-recovery due to the added value in form of on-site fertilizer production and improved sludge dewatering, especially in plants using enhanced biological nutrient removal (EBPR) [94]. In addition, deliberate struvite precipitation can reduce the failure risk caused by blockage of pipes in the plant by uncontrolled precipitation of struvite crystals.

The appropriate selection of P-recovery technology for a specific situation is a complex task, which needs to include the indirect economic and environmental benefits or drawbacks. However, sewage sludge ash as the input for P-recovery seems to be the preferable pathway in terms of the recovery efficiency and the independence of sludge incineration plant and WWTP location. Japan has the largest concentration of P-recovery technologies: at least 16 full-scale plants were implemented to remove P from waste streams and recover struvite and calcium phosphate [78]. It derives from a robust collaborative program on a national level, that activated an effective partnership between industry, high-level education and research, and government. In Europe, in 2019, there were 37 P-recovery plants based on various technologies. Germany and the Netherlands have the highest number of installed technologies, with 10 plants for each of them, followed by Belgium with 6 plants, and Denmark with 4 plants. France, Spain, and UK have 2 examples of installed P-recovery technologies, and Italy has 1 plant. In North America, 15 full-scale units are operating: these plants mainly produce struvite (Pearl and Airprex are typical installed technologies in this area) [95,96]. Only a limited amount of P-recovery technologies has been applied in the Baltic Sea region, mainly in Germany. In Poland, there is one installation for P recovery under construction in Cielcza [97]. In a few years, a wider implementation of P-recovery technologies is expected in the Baltic Sea area, mainly concerning waste streams from water and wastewater sectors.

2.3. *Analysis of the Context and Its Critical Issues*

Sustainable management of nutrients in the Baltic Sea area has been recognized as an urgent issue to solve for a number of years. Consequently, various initiatives have already taken steps in this direction, both at national and international levels. One of these is the European Union Strategy for the Baltic Sea region (2009). This strategy, which is the first developed on a macro-regional level in Europe, addresses the three key challenges in the region: (i) achievement of a safe sea, (ii) the need to connect the region, and (iii) improved prosperity. The objectives are complementary, and they relate to a wide range of policies. The European countries involved in this action plan are Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, and Sweden (analyzed in this paper), but it is also open for cooperation with EU-neighboring countries, such as Belarus, Iceland, Norway, and Russia. Still, at an international level, the EC periodically analyses and evaluates the methods with which all EU member states manage charging and monitoring systems on the effluents, considering the institutional responsibilities and the conditions to issue permits for effluent discharge directly into natural receivers. The EC also proposed a model calculation of effluent charges. In any case, there are still situations of illegal discharge of sewage, and failures in WWTPs in the Baltic Sea basin, which negatively affect its environment and accelerate eutrophication. Moreover, the obligatory recovery of P from selected waste streams (sewage sludge and sewage sludge ash) generated in the municipal WWTPs is adopted only in Germany and not in the other Baltic countries.

Other existing initiatives at international level aim to prevent the problem of P management in waste streams, incentivizing a sustainable consumption of nutrients. (i) The Farm Sustainability Tool for Nutrients (FaST), a tool of the Good Agricultural and Environmental Conditions (GAECs), aims to provide "elements" and "functionalities" to EU farmers for the sustainable use of fertilizers (in certain member states, direct payments are granted for planting cover crops between the seasons). (ii) The Horizon 2020 LEX4BIO project (www.lex4bio.eu): the expected result of this project is to provide a policy framework for the EU's transition to bio-based fertilizers (BBFs), while minimizing risks to the environment, protecting human health, and ensuring food safety and supply.

At the national level, since the EC asked all member states to develop CE action plans, some countries have already adopted some strategies and practices, such as in Finland, Germany, and Poland, and some of them are already available on the European Circular Economy Stakeholder Platform. However, these plans differ in detailed actions between countries. For example, in the Polish CE roadmap, it is mentioned that the wastewater sector is an important source of nutrients, which should be kept in the economy as long as possible, but there are no specific recommendations regarding the use of P in this document.

From a technical and technological point of view, further expert support for the EC is required in areas of P management. In this direction, the Horizon 2020 created financial support for many eco-innovation projects related to the recovery of raw materials, including phosphorus. In each of the Baltic Sea countries, there are also national funding programs which support the development of new technologies in the field of recovery of CRMs. In the 21st century, research and development (R&D) activities are major driving forces behind higher productivity, quality, and innovation in the services and products offered by many organizations. Expenditures on R&D are the largest in higher-income countries (Denmark, Germany, Sweden), which have the highest levels of the eco-innovation index. National funding programs already exist in Estonia: (i) the environmental investment center supports the implementation of environmental projects at a national level; (ii) the circular economy program supports activities that contribute to the more efficient usage of the resources (P-recovery program). In this context, many outstanding scientists have been working on P-recovery solutions for many years. For example, in Poland, among others, Cracow University of Technology and Wroclaw University of Technology developed innovative technologies to recover phosphorus from sewage sludge and sewage sludge ash. However, the developed technologies have not been implemented due to the lack of funds, and uncertain returns did not allow the commercialization of research results. Moreover, the economic evaluations showed that even with the financial support in the investment phase from the publicly available funds, the operation phase of such installations is not economically viable due to the high costs of recycled product in comparison to the commercial products (as fertilizers) from primary sources.

Consequently, as it was mentioned before, installations for P recovery from various waste streams exist in the Baltic Sea only in Denmark (4), Germany (10), and one under construction in Poland. Moreover, currently, both in the Baltic region and most other countries, the evaluation of environmental impacts of the new or modernized technologies (and of their products) is not mandatory. Providing robust impact information could contribute to the protection of the Baltic Sea from ongoing nutrient-derived pollution and make sure that the best environmental solution can be chosen. The provision of reliable and upgraded data is also fundamental for national and international statistics. Unfortunately, for many raw materials, including P resources, data about mass flows tend to be highly fragmented, lacking entirely, and collected using various methodologies in individual Baltic countries. Moreover, the reference points of data collected in many cases are unclear (provided in words only, not in the numbers), which result in ambiguous meaning and misinterpretation of the data.

3. Results and Discussion

Starting from the analysis of the available technologies for P removal from different waste streams and from the identification of the actual P management in the Baltic Region in relation to the current national and international contexts, the InPhos consortium have investigated the main reasons that prevented the installation of P-recovery technologies in countries in the Baltic Sea area.

The proposed methodological approach is mainly technical since it ensures the construction of a quantitative basis on which to define a common and shared strategy for the Baltic Sea area. In fact, without the identification of the main P flows in each country and without the knowledge of how they can be treated to recover P, it is difficult to highlight what are the priority and most valuable aspects to be addressed. A quantitative overview of the current P management in the Baltic Sea area would be useful for different stakeholders, in particular for (i) legislators and policy makers to estimate and select the most potential initiatives and strategies in this field; (ii) the scientific and technical community to improve technologies and/or widespread their applications; (iii) funding bodies to properly evaluate benefits and risks of an investment in this field; (iv) society to spread the awareness that solutions for the "P challenge" are urgently necessary.

The difficulties and the efforts in the construction of this quantitative basis allowed the identification of some critical factors. As a result, some recommended directions and relative practical actions are proposed to move towards widespread P recovery from waste streams and, where possible, circular phosphorus management in the Baltic Sea region. In particular, for each of the four main areas identified as priorities (legislative, financial, technical, and social), some focal points are depicted, as shown in Table 4.

Summarizing all available information, there are several opportunities, but also barriers, for the implementation of strategies to improve phosphorus management in the Baltic Sea region. This requires systemic changes in people's awareness and their behavior, but also the development of innovation in technologies, financing methods, business models, and policies [98]. Activities of the InPhos project were mainly focused on the recommendations and the actions related to social aspects and to increase the awareness about the "P challenge". In particular, during the InPhos project, a close collaboration with all mentioned stakeholders (policymakers, industrial practitioners, high-education institutions, researchers, farmers, and citizens) was established. The coordinator and participants performed several awareness-raising activities (meetings and consultations), in the form of round-table and panel discussions, video-meetings, workshops and seminars in the countries taking part in the project: Estonia, Finland, Germany, Latvia, Lithuania, Poland, and Sweden (and also in Italy due to the participation of an Italian partner). All these activities helped to promote an interdisciplinary systems-thinking approach among stakeholders in the Baltic Sea region. In addition, several seminars and workshops have been organized as a part of the InPhos project to raise awareness of the importance of phosphorus. This initiative provided education tools about how to avoid food waste (rich in P resources) and how to choose the best regional food and feed supply (in order to support local farmers and minimize the cost of products). An important part of education content was to convince stakeholders to accept a fair price for quality food (role model: C'est qui, le patron; lamarqueduconsommateur.com). Moreover, education games and quizzes were developed and distributed among selected groups of stakeholders such as students, in order to interest future decision-makers in the topic of security of raw materials, including phosphorus. Finally, various dissemination activities were conducted, mainly related to the participation in national and international conferences and presentation of the project results in the form of oral speeches and posters.

Table 4. Recommendations and practical actions for a more sustainable P management in the Baltic region, derived from InPhos project methodology.

Category	Recommendations	Proposed Actions
Legal recommendations	R1. Stricter requirements for more sustainable consumption and production practices (involving agriculture, food industries, water and wastewater sector, phosphate and fertilizer industries).	• Review of good agricultural practices and best available techniques for newly created or modernized enterprises. • Implementation of more effective control of farmers' practices (e.g., excessive spreading of manure). • Revision and further implementation of environmental charges, where the limit values are significantly exceeded, for discharged municipal and industrial wastewater, and illegal discharges to natural receivers. • Alternative incentives/penalties, e.g., tax on effluents with nutrients, landfill tax, emission taxes (methane, N_2O, ammonia).
	R2. Implementation of P-recovery regulations at national level.	Introduction of mandatory recovery of phosphorus from selected wastes to lead to an extension of the life cycle of this element in the economy, and thus reduce the dependence on imports and increasing raw material security for Europe.
	R3. Development of the national action plans for the reuse of recovered P from selected waste streams.	• According to the country's conditions and access to selected P-rich waste streams, it is necessary to take into account all national recyclable P resources and the possibilities of their recovery and reuse in all Baltic countries. • Development of working group at national level in the Baltic Sea countries, who develops the integrated P management system on national level.
	R4. Further work on the development of an integrated management strategy for the Baltic Sea region (including P).	Collaboration among working groups established at national level.
	R5. Deep analysis of P flows on the regional and national levels.	• Calculation, monitoring, and provision of reliable information on phosphorous raw material flows at the NUTS 2 (Nomenclature of territorial units for statistics) minimum level. • Material flow analysis (MFA) on regional and national level for each Baltic country and for the entire Baltic region.
Financial support	R6. Financial tools supporting the sustainable management, consumption and disposal of P in the Baltic Sea countries.	• CAP (Common Agricultural Policy) payments for sustainable farming practices, such as direct payments for implementation of the proposed FaST Tool. • Calculate the cost of pollution of water bodies (and other negative impacts) and create incentive payments for avoidance. • Subsidize nutrient separation from waste flows or penalize nutrient effluents from WWTPs (like in Denmark) by, for instance, charging a fee per kg of P in the WWTP effluent.
	R7. Financial tools supporting research, development, commercialisation, implementation and staying on the market of the P-recovery technologies.	• Conduct further research on the possibility of implementing already developed solutions in all Baltic countries. • Provide programs supporting investment in nutrient recovery technologies. • Financial support for operating installations of P-recovery technologies.

Table 4. *Cont.*

Category	Recommendations	Proposed Actions
Technical and environmental recommendations	R8. Improving soil and farmland management practices.	• Avoiding of erosion and increase soil carbon and microbial status by cover crops. • Avoiding excessive nutrient loads through precision farming (deep knowledge on the soil nutrient status and crop nutrient demand). • Monitoring of farming and fertilizing practices in terms of time of fertilization, respecting river strips (no fertilization zones close to water bodies), and strictly respecting health and safety obligations (no sludge/manure use on crops/vegetables for consumption).
	R9. Identification and environmental assessment of solutions dealing with P-recovery potential of different waste streams and reuse.	• Technical assessment of selected P-rich waste streams (chemical analysis, economic analysis, e.g., reserves, demand, supplies, and other aspects and areas). • Performance analysis of the installations across the Baltic Sea region and evaluation of recycled P availability. • Risk analysis to make a product that is safe for human health according to elaborated national requirements (in WWTPs). • Performing life cycle assessments to better understand and assess the environmental impacts related to the P life cycle, both in linear and circular scenarios.
	R10. Development of new technologies and modernization of the existing P recycling and recovery technologies and solutions.	• Further technical progress in the recovery of nutrients from various waste streams, as wastewater, sewage sludge, sewage sludge ash, manure, biomass, industrial waste, bottom sediments and other. • Elimination of impurities, including heavy metals, presence of which in the waste hampers its recycling and reuse, e.g., for fertilization purposes (due to exceeding regulatory limits, e.g., for cadmium).
Social aspects	R11. Promoting awareness-raising among all stakeholders related to P management.	• Diffusion of knowledge about the typologies of P sources and the need/potential for suitable P management, preferring circular thinking. • Promotion of the alternative management systems and technological practices for a more sustainable phosphorus usage among selected groups of stakeholders.
	R12. Building a "Phosphorus Responsible Society".	• Initiating a multi-disciplinary dialogue involving policymakers, industrial practitioners, high-education institutions, researchers, farmers, and society about the consequences of different P management scenarios on a global and national scale. • Education of selected groups of stakeholders (farmers, companies, individuals, students) on the importance of the sustainable P management. • Preparation of future consumers open to accepting higher prices for products that meet the requirements of a circular economy and are in the line with a "zero or low waste" strategy (e.g., higher costs of the fertilizers produced from recovery and recycling of waste streams).

4. Conclusions

The holistic approach used in the InPhos project to analyze the strategic, technological, legislative, and social context for more sustainable P management in the Baltic Region made it possible to highlight that there are several opportunities to move forwards towards a circular economy in this field, but also numerous barriers. Nevertheless, urgent initiatives are necessary and have to be implemented to reduce the negative environmental impact of P losses to the sea, causing eutrophication. Consequently, several recommendations have been suggested, covering different disciplines related to P management, and practical actions have been conducted in relation to social aspects (R11 and R12). With the same holistic approach, future actions starting from InPhos' results are under development to address several others of the proposed recommendations. In particular, R5-deep analysis of P flows at the regional and national levels, and R9-Identification and environmental assessment of solutions dealing with the P-recovery potential of different waste streams and environmental assessment of engineering solutions dealing with P recovery and reuse will be implemented through an MFA for each involved country and through a techno-economic feasibility study for the implementation of P-recovery technologies in a real industrial setting in the Baltic region.

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References

1. Karunanithi, R.; Szogi, A.A.; Bolan, N.; Naidu, R.; Loganathan, P.; Hunt, P.G.; Vanotti, M.B.; Saint, C.P.; Ok, Y.S.; Krishnamoorthy, S. Phosphorus Recovery and Reuse from Waste Streams. In *Advances in Agronomy*; Elsevier: Amsterdam, The Netherlands, 2015.
2. Van Kernebeek, H.R.J.; Oosting, S.J.; van Ittersum, M.K.; Ripoll-Bosch, R.; de Boer, I.J.M. Closing the phosphorus cycle in a food system: Insights from a modelling exercise. *Animal* **2018**, *12*, 1755–1765. [CrossRef] [PubMed]
3. Cordell, D.; Cordell, D. The Story of Phosphorus: Missing Global Governance of a Critical Resource. In Proceedings of the SENSE Earth Systems Governance, Amsterdam, The Netherlands, 24–31 August 2009.
4. Cieślik, B.; Konieczka, P. A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of "no solid waste generation" and analytical methods. *J. Clean. Prod.* **2017**, *142*, 1728–1740. [CrossRef]
5. Van Dijk, K.C.; Lesschen, J.P.; Oenema, O. Phosphorus flows and balances of the European Union Member States. *Sci. Total Environ.* **2016**, *542*, 1078–1093. [CrossRef] [PubMed]
6. European Commission. EU Agricultural Markets Briefs. Fertilisers in the EU Prices. Available online: https://ec.europa.eu/info/sites/info/files/food-farming-fisheries/farming/documents/market-brief-fertilisers_june2019_en.pdf (accessed on 5 January 2020).
7. Zoboli, O.; Zessner, M.; Rechberger, H. Supporting phosphorus management in Austria: Potential, priorities and limitations. *Sci. Total Environ.* **2016**, *565*, 313–323. [CrossRef]
8. European Commission. Commission of European Communities. Consultative Communication on the Sustainable Use of Phosphorus. Available online: http://www.globaltraps.ch/tl_files/bilder/start/12-01-13_Respose_to_EUSUP.pdf (accessed on 5 January 2020).

9. European Commission. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions Roadmap to a Resource Efficient Europe. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52011DC0571 (accessed on 3 January 2020).
10. European Commission. Commission of European Communities. Communication on the review of the list of critical raw materials for the EU and the implementation of the Raw Materials Initiative. Available online: https://op.europa.eu/en/publication-detail/-/publication/d1be1b43-e18f-11e8-b690-01aa75ed71a1/language-en/format-PDF/source-80004733 (accessed on 23 March 2020).
11. European Commission, Commission of European Communities. Communication on the 2017 list of Critical Raw Materials for the EU. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52017DC0490 (accessed on 23 March 2020).
12. European Environmental Agency (EEA). *Source Apportionment of Nitrogen and Phosphorus Inputs into the Aquatic Environment*; Office for Official Publications of the European Communities: Brussels, Belgium, 2005.
13. Jennings, E. Trinity College. Environmental Research Technological Development and Innovation Programme Eutrophication from Agricultural Sources: Seasonal Patterns & Effects of Phosphorus (2000-LS-2.1.7-M2): Final Report. Available online: https://www.academia.edu/14432774/Eutrophication_from_agricultural_sources_seasonal_patterns_and_effects_of_phosphorus (accessed on 29 December 2019).
14. Stubenrauch, J.; Garske, B.; Ekardt, F. Sustainable Land Use, Soil Protection and Phosphorus Management from a Cross-National Perspective. *Sustainability* **2018**, *10*, 1988. [CrossRef]
15. Meng, C.; Wang, Y.; Li, Y.; Zhou, J.; Li, Y.; Wu, J. Deteriorated Water Quality of Agricultural Catchments in South China by Net Anthropogenic Phosphorus Inputs. *Sustainability* **2017**, *9*, 1480. [CrossRef]
16. Selman, M.; Greenhalgh, S.; Diaz, R.; Sugg, Z. Eutrophication and Hypoxia in Coastal Areas: A Global Assessment of the State of Knowledge. *World Resour. Inst.* **2008**, *284*, 1–6.
17. Ménesguen, A.; Lacroix, G. Modelling the marine eutrophication: A review. *Sci. Total Environ.* **2018**, *636*, 339–354. [CrossRef]
18. Le Moal, M.; Gascuel-Odoux, C.; Ménesguen, A.; Souchon, Y.; Étrillard, C.; Levain, A.; Moatar, F.; Pannard, A.; Souchu, P.; Lefebvre, A.; et al. Eutrophication: A new wine in an old bottle? *Sci. Total Environ.* **2019**, *651*, 1–11. [CrossRef]
19. Ngatia, L.; Taylor, R. Phosphorus Eutrophication and Mitigation Strategies. In *Phosphorus—Recovery and Recycling*; Zhang, T., Ed.; IntechOpen: London, UK, 2019.
20. Selman, M.; Greenhalgh, S. Eutrophication: Policies, Actions, and Strategies to Address Nutrient Pollution. *World Resour. Inst.* **2009**, 1–16.
21. Xu, K.-Q.; Ebie, Y.; Jimbo, Y.; Inamori, Y.; Sudo, R. Measures and Policies against the Eutrophication for Lake Water Quality in Japan. Available online: https://pdfs.semanticscholar.org/ad77/1ab97ced959871570e74a2e3bea424b11a73.pdf (accessed on 12 March 2020).
22. European Court of Auditors. Combating Eutrophication in the Baltic Sea: Further and More Effective Action Needed. Available online: https://www.eca.europa.eu/en/Pages/DocItem.aspx?did=35757 (accessed on 3 January 2020).
23. HELCOM. Eutrophication in the Baltic Sea—An integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. *Balt. Sea Environ. Proc.* **2009**, *115B*, 1–152.
24. Bohman, B. Lessons from the regulatory approaches to combat eutrophication in the Baltic Sea region. *Mar. Policy* **2018**, *98*, 227–236. [CrossRef]
25. Jetoo, S. Multi-level governance innovations of the Baltic Sea and the North American Great Lakes: New actors and their roles in building adaptive capacity for eutrophication governance. *Mar. Policy* **2018**, *98*, 237–245. [CrossRef]
26. Ning, W.; Nielsen, A.B.; Ivarsson, L.N.; Jilbert, T.; Åkesson, C.M.; Slomp, C.P.; Andrén, E.; Broström, A.; Filipsson, H.L. Anthropogenic and climatic impacts on a coastal environment in the Baltic Sea over the last 1000 years. *Anthropocene* **2018**, *21*, 66–79. [CrossRef]
27. Andersen, J.H.; Axe, P.; Backer, H.; Carstensen, J.; Claussen, U.; Fleming-Lehtinen, V.; Järvinen, M.; Kaartokallio, H.; Knuuttila, S.; Korpinen, S.; et al. Getting the measure of eutrophication in the Baltic Sea: Towards improved assessment principles and methods. *Biogeochemistry* **2011**, *106*, 137–156. [CrossRef]
28. Smol, M. The use of membrane processes for the removal of phosphorus from wastewater. *Desalin. Water Treat.* **2018**, *128*, 397–406. [CrossRef]

29. Marazzi, F.; Bellucci, M.; Rossi, S.; Fornaroli, R.; Ficara, E.; Mezzanotte, V. Outdoor pilot trial integrating a sidestream microalgae process for the treatment of centrate under non optimal climate conditions. *Algal Res.* **2019**, *39*, 101430. [CrossRef]
30. Lundberg, C. Eutrophication, risk management and sustainability. The perceptions of different stakeholders in the northern Baltic Sea. *Mar. Pollut. Bull.* **2013**, *66*, 143–150. [CrossRef]
31. Mew, M.; Steiner, G.; Geissler, B. Phosphorus Supply Chain—Scientific, Technical, and Economic Foundations: A Transdisciplinary Orientation. *Sustainability* **2018**, *10*, 1087. [CrossRef]
32. United Nations Development Programme. Human Development Index (HDI). Available online: http://hdr.undp.org/en/indicators/137506 (accessed on 18 February 2020).
33. HELCOM. Our Baltic Sea. Available online: http://stateofthebalticsea.helcom.fi/in-brief/our-baltic-sea/ (accessed on 18 February 2020).
34. European Commission. Study on the Review of the List of Critical Raw Materials. 2017. Available online: https://op.europa.eu/s/dZ7O (accessed on 9 January 2020).
35. Lintinen, P. *Selvitys Suomen Fosforipotentiaalista*; Geologian Tutkimuskeskus: Rovaniemi, Finland, 2015.
36. Ahokas, K. Finland's Phosphorus Resources are More Important than Ever. Available online: http://verkkolehti.geofoorumi.fi/en/2015/10/finlands-phosphorus-resources-are-more-important-than-ever/ (accessed on 26 January 2020).
37. Tamm, K.; Pirr, I.; Kuusik, R.; Tõnsuaadu, K. Benefication of Estonian Phosphate Ore by Flotataion. In "Beneficiation of Phosphates VIII", ECI Symposium Series, (2018). Available online: http://dc.engconfintl.org/phosphates_viii/1 (accessed on 18 February 2020).
38. Põldvere, A.; Bauert, H. Excursions Guidebook. In Proceedings of the 15th Meeting of the Association of European Geological Societies Georesources and Public Policy: Research, Management, Environment, Tallinn, Estonia, 16–20 September 2007.
39. Faure, G.; Mensing, T.M. *The Estonians. The Long Road to Independence*; lulu.com: London, UK, 2012.
40. Polish Geological Institute. *Mineral Resources of Poland*; Przenioslo, S., Ed.; Polish Geological Institute: Warsaw, Poland, 2017.
41. Balance of Mineral Resources in Poland, Polish Geological Institute—National Research Institute, Warsaw 2019. Available online: https://www.bodc.ac.uk/resources/inventories/edmed/org/609/ (accessed on 9 January 2020).
42. Andersson, M.; Carlsson, M.; Ladenberger, A.; Morris, G.; Sadeghi, M.; Uhlbäck, J. *Geochemical Atlas of Sweden*; Geological Survey of Sweden: Uppsala, Sweden, 2014.
43. Sveriges Geologiska Undersokning. Metaller Och Mineral i Gruvavfall. Available online: https://www.sgu.se/mineralnaring/metall--och-mineralatervinning/metaller-och-mineral-i-gruvavfall/ (accessed on 18 February 2020).
44. Sedmalis, U.; Sperberga, I.; Sedmale, G. Classification of Latvian mineral resources. In *Mineral Resources in Latvia and Their Use: With an Introduction to Mineralogy and Petrography*; RTU Press: Riga, Latvia, 2002.
45. Seglins, V.; Stinkule, A.; Stinkulis, G. Phosphorite and vivianite. In *Mineral Deposits of Latvia*; University of Latvia Press: Riga, Latvia, 2013.
46. Knoema. World Data Atlas. Agriculture, Fertilizers, Production Quantity in Nutrients. Available online: https://knoema.com/atlas/topics/Agriculture#Fertilizers-Production-Quantity-in-Nutrients (accessed on 18 February 2020).
47. Polish Central Statistical Office. *Environment 2017 Annual Report*; Statistics Poland: Warsaw, Poland, 2017.
48. Official Statistics Portal. Import of Raw Phosphorus Materials. Available online: https://osp.stat.gov.lt/statistiniu-rodikliu-analize#/ (accessed on 18 February 2020).
49. Food and Agriculture Organization of the United Nations. *World Fertilizer Trends and Outlook to 2018*; FAO: Rome, Italy, 2015.
50. Eurostat. Agri-Environmental indicator—Mineral Fertiliser Consumption. Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agri-environmental_indicator_-_mineral_fertiliser_consumption (accessed on 18 February 2020).
51. Industrieverband Agrar, 2019. Jahresbericht 2018/2019, Frankfurt: Industrieverband Agrar e.V. Available online: https://www.iva.de/publikationen/jahresbericht-20182019 (accessed on 8 January 2020).
52. Eurostat. Livestock Density Index. Available online: https://ec.europa.eu/eurostat/web/products-datasets/-/tai09 (accessed on 18 February 2020).

53. Amery, F.; Schoumans, O.F. Agricultural Phosphorus Legislation in Europe. Available online: https://www.oieau.fr/eaudoc/notice/Agricultural-phosphorus-regulation-Europe-%E2%80%93-Experience-sharing-4-European-countries (accessed on 8 January 2020).
54. Helcom. *Report on Industrial Livestock Farming in the Baltic Sea Region—Environmental Protection Context*; Helcom: Helsinki, Finland, 2014.
55. Eurostat. Sewage Sludge Production and Disposal. Available online: https://ec.europa.eu/eurostat/web/products-datasets/product?code=env_ww_spd (accessed on 18 February 2020).
56. Feature. Efficient recycling of phosphorus from sludge. *Filtr. Separat.* **2015**, *52*, 44–45. [CrossRef]
57. Vilpanen, M. *Phosphorus Recovery in Finland. Case Ravita*; HSY: Helsinki, Finland, 2018.
58. Pihl, T. Recovery and Reuse of Phosphorus from Municipal Wastewater—Applications and Attitudes in Finland. Master's Thesis, Submitted for Examination for the Degree of Master of Science in Technology, Aalto University. 2017. Available online: https://aaltodoc.aalto.fi/handle/123456789/29338 (accessed on 18 January 2020).
59. HSY. Viikinmäki Wastewater Treatment Plant. Available online: https://www.hsy.fi/en/experts/water-services/wastewater-treatment-plants/viikinmaki/Pages/default.aspx (accessed on 18 February 2020).
60. HSY. Ravita Project. Available online: https://www.hsy.fi/ravita/en/Sivut/default.aspx (accessed on 18 February 2020).
61. STN—Servicegesellschaft Tierische Nebenprodukte Mbh. 2016. Verarbeitung Tierischer Nebenprodukte. Available online: https://stn-vvtn.de/index.php (accessed on 18 February 2020).
62. Holm, O.; Thomé-Kozmiensky, E.; Quicker, P.; Kopp-Assenmacher, S. (Eds.) *Verwertung von Klärschlamm 2*; Thomé-Kozmiensky Verlag GmbH: Neuruppin, Germany, 2019.
63. Survey—Quality of Municipal Sewage Sludge in Latvian Water Holdings. *The Plan of Developing the Proposals for Processing and Using Municipal Sewage Sludge (in Latvian)*; Cleantech Latvia: Riga, Latvia, 2015.
64. Report "Water-2" of the Latvian Environment, Geology and Meteorology Centre. Available online: https://www.meteo.lv/lapas/vide/udens/notekudeni/notekudeni?id=1198&nid=428 (accessed on 30 January 2020).
65. Cabinet Regulation No. 362, Republic of Latvia. Regulations Regarding Utilisation, Monitoring and Control of Sewage Sludge and the Compost Thereof, Adopted. Available online: https://likumi.lv/ta/id/134653-noteikumi-par-notekudenu-dunu-un-to-komposta-izmantosanu-monitoringu-un-kontroli (accessed on 30 January 2020).
66. Bardule, A.; Rancane, S.; Gutmane, I.; Berzins, P.; Stesele, V.; Lazdina, D.; Bardulis, A. The effect of fertiliser type on hybrid aspen increment and seed yield of perennial grass cultivated in the agroforestry system. *Agron. Res.* **2013**, *11*, 13–24.
67. Lazdina, D.; Liepiņš, K.; Bardule, A.; Liepiņš, J.; Bardulis, A. Wood ash and wastewater sludge recycling success in fast-growing deciduous tree - Birch and alder plantations. *Agron. Res.* **2013**, *11*, 347–356.
68. Environmental Protection Agency. Wastewater Management Accounting Data. Available online: http://vanduo.gamta.lt/cms/index?rubricId=6c0feeaa-4d89-4a23-9339-19ab0de0adf1 (accessed on 18 February 2020).
69. Polish Central Statistical Office. *Environmental Protection Yearbook*; Statistics Poland: Warsaw, Poland, 2018.
70. Szaja, A. Phosphorus recovery from sewage sludge via pyrolysis. *Ann. Set. Environ. Prot.* **2013**, *15*, 361–370.
71. Statistics Sweden. Discharges to Water and Sewage Sludge Production in 2016. Available online: https://www.scb.se/en/finding-statistics/statistics-by-subject-area/environment/emissions/discharges-to-water-and-sewage-sludge-production--municipal-waste-water-treatment-plants-pulp-and-paper-industry-and-other-industry/pong/statistical-news/discharges-to-water-and-sewage-sludge-production-in-2016/ (accessed on 18 February 2020).
72. SOU Sustainable Sludge Handling. Report SOU 2020:3. Available online: https://www.regeringen.se/rattsliga-dokument/statens-offentliga-utredningar/2020/01/sou-20203/ (accessed on 18 February 2020).
73. Kaikake, K.; Sekito, T.; Dote, Y. Phosphate recovery from phosphorus-rich solution obtained from chicken manure incineration ash. *Waste Manag.* **2009**, *29*, 1084–1088. [CrossRef]
74. Jin, Y.; Hu, Z.; Wen, Z. Enhancing anaerobic digestibility and phosphorus recovery of dairy manure through microwave-based thermochemical pretreatment. *Water Res.* **2009**, *43*, 3493–3502. [CrossRef]
75. Römer, W.; Steingrobe, B. Fertilizer effect of phosphorus recycling products. *Sustainability* **2018**, *10*, 1166. [CrossRef]

76. Brod, E.; Oppen, J.; Kristoffersen, A.Ø.; Haraldsen, T.K.; Krogstad, T. Drying or anaerobic digestion of fish sludge: Nitrogen fertilisation effects and logistics. *Ambio* **2017**, *46*, 852–864. [CrossRef]
77. Hamilton, H.A.; Brod, E.; Hanserud, O.; Müller, D.B.; Brattebø, H.; Haraldsen, T.K. Recycling potential of secondary phosphorus resources as assessed by integrating substance flow analysis and plant-availability. *Sci. Total Environ.* **2017**, *575*, 1546–1555. [CrossRef]
78. Ohura, S.; Harada, H.; Biswas, B.K.; Kondo, M.; Ishikawa, S.; Kawakita, H.; Ohto, K.; Inoue, K. Phosphorus recovery from secondary effluent and side-stream liquid in a sewage treatment plant using zirconium-loaded saponified orange waste. *J. Mater. Cycles Waste Manag.* **2011**, *13*, 293–297. [CrossRef]
79. Shaddel, S.; Bakhtiary-Davijany, H.; Kabbe, C.; Dadgar, F.; Østerhus, S.W. Sustainable sewage sludge management: From current practices to emerging nutrient recovery technologies. *Sustainability* **2019**, *11*, 3435. [CrossRef]
80. Smol, M.; Adam, C.; Preisner, M. Circular economy model framework in the European water and wastewater sector. *J. Mater. Cycles Waste Manag.* **2020**, 1–16. [CrossRef]
81. Herzel, H.; Krüger, O.; Hermann, L.; Adam, C. Sewage sludge ash—A promising secondary phosphorus source for fertilizer production. *Sci. Total Environ.* **2016**, *542*, 1136–1143. [CrossRef] [PubMed]
82. Gorazda, K.; Tarko, B.; Wzorek, Z.; Kominko, H.; Nowak, A.K.; Kulczycka, J.; Henclik, A.; Smol, M. Fertilisers production from ashes after sewage sludge combustion—A strategy towards sustainable development. *Environ. Res.* **2017**, *154*, 171–180. [CrossRef] [PubMed]
83. Schoumans, O.F.; Bouraoui, F.; Kabbe, C.; Oenema, O.; van Dijk, K.C. Phosphorus management in Europe in a changing world. *Ambio* **2015**, *44*, 180–192. [CrossRef]
84. Tan, Z.; Lagerkvist, A. Phosphorus recovery from the biomass ash: A review. *Renew. Sustain. Energy Rev.* **2011**, *15*, 3588–3602. [CrossRef]
85. El Afifi, E.M.; Hilal, M.A.; Attallah, M.F.; EL-Reefy, S.A. Characterization of phosphogypsum wastes associated with phosphoric acid and fertilizers production. *J. Environ. Radioact.* **2009**, *100*, 407–412. [CrossRef]
86. Quintana, M.; Colmenarejo, M.F.; Barrera, J.; García, G.; García, E.; Bustos, A. Use of a Byproduct of Magnesium Oxide Production To Precipitate Phosphorus and Nitrogen as Struvite from Wastewater Treatment Liquors. *J. Agric. Food Chem.* **2004**, *52*, 294–299. [CrossRef]
87. Yang, H.; Liu, J.; Hu, P.; Zou, L.; Li, Y.-Y. Carbon source and phosphorus recovery from iron-enhanced primary sludge via anaerobic fermentation and sulfate reduction: Performance and future application. *Bioresour. Technol.* **2019**, *294*, 122174. [CrossRef]
88. Zhao, Y.; Ren, Q.; Na, Y. Potential utilization of phosphorus in fly ash from industrial sewage sludge incineration with biomass. *Fuel Process. Technol.* **2019**, *188*, 16–21. [CrossRef]
89. Kraus, F.; Zamzow, M.; Conzelmann, L.; Remy, C.; Kleyböcker, A.; Seis, W.; Miehe, U.; Hermann, L.; Hermann, R.; Kabbe, C. Ökobilanzieller Vergleich der P-Einbeziehung Rückgewinnung aus dem Abwasserstrom mit der Düngemittelproduktion aus Rohphosphaten unter von Umweltfolgeschäden und deren Vermeidung, Umweltbundesamt. Texte 13/2019, Dessau-Roßlau, Februar 2019. Available online: https://www.umweltbundesamt.de/publikationen/oekobilanzieller-vergleich-der-p-rueckgewinnung-aus (accessed on 8 January 2020).
90. Chrispim, M.C.; Scholz, M.; Nolasco, M.A. Phosphorus recovery from municipal wastewater treatment: Critical review of challenges and opportunities for developing countries. *J. Environ. Manag.* **2019**, *248*, 109268. [CrossRef]
91. Rossi, L.; Reuna, S.; Fred, T.; Heinonen, M. RAVITA Technology—new innovation for combined phosphorus and nitrogen recovery. *Water Sci. Technol.* **2018**, *78*, 2511–2517. [CrossRef] [PubMed]
92. Egle, L.; Rechberger, H.; Krampe, J.; Zessner, M. Phosphorus recovery from municipal wastewater: An integrated comparative technological, environmental and economic assessment of P recovery technologies. *Sci. Total Environ.* **2016**, *571*, 522–542. [CrossRef] [PubMed]
93. Wong, P.Y.; Cheng, K.Y.; Kaksonen, A.H.; Sutton, D.C.; Ginige, M.P. A novel post denitrification configuration for phosphorus recovery using polyphosphate accumulating organisms. *Water Res.* **2013**, *47*, 6488–6495. [CrossRef] [PubMed]
94. Mehta, C.M.; Khunjar, W.O.; Nguyen, V.; Tait, S.; Batstone, D.J. Technologies to Recover Nutrients from Waste Streams: A Critical Review. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 385–427. [CrossRef]

95. Nättorp, A.; Kabbe, C.; Matsubae, K.; Ohtake, H. *Development of Phosphorus Recycling in Europe and Japan BT—Phosphorus Recovery and Recycling*; Ohtake, H., Tsuneda, S., Eds.; Springer: Singapore, 2019.
96. Oleszkiewicz, J.; Kruk, D.J.; Devlin, T.; Lashkarizadeh, M.; Yuan, Q. Options for Improved Nutrient Removal and Recovery from Municipal Wastewater in the Canadian Context. *Environ. Technol.* **2015**, *20*, 681–695.
97. Kabbe, C. Overview of phosphorus recovery from the wastewater stream facilities operating or under construction. In *Phosphorus Recovery and Recycling*; Springer: Berlin/Heidelberg, Germany, 2017.
98. European Commission. Commission of European Communities. Communication. Towards A Circular Economy: A Zero Waste Programme for Europe (COM no. 398). Available online: https://www.researchgate.net/publication/328682998_Towards_a_Circular_Economy-_a_Zero_Waste_Programme_for_Europe (accessed on 16 February 2020).

5. pielikums
Appendix 5

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Water efficiency and safe re-use of different grades of water - Topical issues for the pharmaceutical industry

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ABSTRACT

The pharmaceutical industry is a highly water-dependent economic sector and requires different degrees of water purity. The study presents an overview of the current water quality requirements, as well as limitations and safety considerations for water re-use in pharmaceutical manufacturing. Several principles for implementing circular economy elements into pharmaceutical wastewater treatment procedures are described: the use of recovered solvents as an easily degradable carbon source for the denitrification process, the use of phosphorus-containing wastewater as a nutrient source for activated sludge microorganisms, the use of inorganic acids and bases for pH control in wastewater treatment processes, and the use of Al^{3+} containing wastewater as a coagulant. The case study indicates that specific wastewater streams can be reused as secondary materials if the chemical composition of wastewater from pharmaceutical batch processing is well constrained and separate collection is ensured.

1. Introduction

According to the European Union (EU) rating, the pharmaceutical and chemical industries, along with agriculture and paper production, provide the greatest gross value sectors of the EU economy, but exhibit the highest dependency on a stable supply of water in sufficient quantity and quality [1]. The OECD Environmental Outlook to 2050 predicts that the global water demand for manufacturing will increase by 400% between 2000 and 2050 [2]. To ensure the sustainability of water resources, the industry should improve water efficiency in a value chain, as well as maximise the opportunities for safe re-use of different grades of water. Moreover, water-related industries need to focus not only on climate change and water scarcity [3], but also on environmental sustainability and the sustainable use of industrial water [4], introduction of instrumentation for real-time water quality monitoring [5], water management and water-energy nexus [6] and strive for zero liquid discharge (ZLD) [7]. According to Toth et al. [8], physicochemical

Abbreviations: EU, European Union; ZLD, Zero liquid discharge; UASB/MBR, up-flow anaerobic sludge blanket/membrane bioreactors; SDG, sustainable development goals; MBBR, moving-bed biofilm reactors; APIs, active pharmaceutical ingredients; FDFs, final dosage forms; GMP, good manufacturing practice; EMA, European Medicines Agency; WHO, World Health Organisation; PW, purified water; RO, reverse osmosis; WFI, water for injections; Ph. Eur., European Pharmacopoeia; USP, United States Pharmacopoeia; HVAC, heating, ventilation and air conditioning; ERA, environmental risk assessment.

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methods are increasingly used for industrial wastewater treatment since they have a smaller footprint compared to that of biological wastewater treatment, although the biological ones have been greatly developed and are successfully used for the treatment of different industrial wastewaters. In the case of the pharmaceutical industry, new eco-innovative proposals in the literature focusing on the application of up-flow anaerobic sludge blanket/membrane bioreactors (UASB/MBR) for wastewater re-use in landscape irrigation while avoiding the hazards of introducing pharmaceutical residues into the environment [9] achieved the removal of organic halogens from process wastewaters [8], performed catalytic oxidation processes for detoxification and improving the biodegradability of pharmaceutical wastewater streams [10], developed nanofiltration [11], and used ionising radiation as the source of both reducing and oxidizing radicals for water treatment [12–14].

Innovative, advanced treatment technologies are currently being developed for highly polluted water. The reviews of biological, chemical, advanced chemical technologies, or advanced oxidation processes, and hybrid technologies for the treatment of industrial wastewaters were presented [15–20].

Although many new technologies have been proposed, the main concept is to use hybrid approaches that combine various treatment mechanisms to remove the full spectrum of chemicals present in pharmaceutical wastewaters.

The rapid development of the pharmaceutical industry presents conflicting challenges to water resources management. The discharge of inadequately treated wastewaters results in the release of pharmaceutical substances into the environment, resulting in risks to water quality and a possible adverse impact on aquatic ecosystems and public health [21].

In recent years, there has been an increasing focus on water use in those several highly water-dependent industries besides the production of pharmaceuticals: the chemical industry [22,23] motor vehicle production [24], paper production [25,26], the steel industry [27,28], the textile industry [29,30], as well as the food and beverage industry [31–33].

The pharmaceutical industry has a special place in this list of highly water-dependent industries. Many pharmaceutical processes require very pure water that meets strict quality standards, simultaneously they can generate heavily polluted and toxic wastewaters that require complex and costly multi-step treatment to meet the regulatory requirements [21]. Pharmaceutical plants typically use a broad range of chemicals and technological processes, presenting major challenges for waste cleanup technologies and procedures. Mineralization represents the most effective treatment, which is the ultimate goal in many areas of pollutant control and destruction.

High-purity water is an indispensable resource for the production of medicines for human and veterinary use, but at the same time the pharmaceutical industry is one of the main polluters of local water resources [21]. Another source of high risk regarding pharmaceuticals is very polluted waste water at hospitals and homes for the elderly; this waste water contains pharmaceuticals together with greatly variable and hazardous microbiological pollutants, where the concentration of pharmaceutical residues could be several times higher than in municipal wastewater [34–37] Futhermore, without any treatment, veterinary pharmaceutical residues from aquaculture and livestock farms can enter into the water as an active parent product or biologically active metabolites [38].

The current trends of sustainable development goals (SDG) include efforts of water re-use, which is recognized as a key option to reduce water consumption at the manufacturing facility level [39]. However, the re-use of water in pharmaceutical production is met with caution from the regulatory agencies concerned with the quality and safety of pharmaceuticals, prompting new strategies that may reconcile the conflicting goals of wastewater re-use and ensuring that contaminants cannot enter pharmaceutical production via reused water. Innovative ZLD technologies make it possible to almost completely eliminate wastewater discharges, maximizing water re-use in the industry [40–43]. Despite the high potential for water saving, the application of ZLD on an industrial scale is currently very limited due to the high costs, energy consumption [40], and the generation of solid hazardous waste [40,41]. Although the data from certain industries point to opportunities for decreasing water consumption by up to 50% [44], implementation of circular water management in the pharmaceutical industry is quite limited due to very strict legislative regulations and safety considerations.

The main barriers for water re-use in the pharmaceutical industry are the unique requirements of water quality and the risks associated with product contamination and human health [15]. With the rapid development of a circular economic strategy within the EU member states in recent years, many incentives have been implemented, providing support for research and innovation through different grant programmes, while promoting new legal and economic regulations. Therefore, pharmaceutical manufacturers are currently interested in practical options for closing the 'water loop' in those technological processes where water does not come into direct contact with pharmaceutical products. Moreover, manufacturers are seeking to minimise the use of water, energy, and hazardous materials, to eliminate waste during the product life cycle, and to use treated wastewater for other purposes. In several countries (Switzerland, the USA, Singapore, Australia), where wastewater after treatment is reused for agricultural irrigation or discharged into aquifers that also serve as sources of drinking water, certain pharmaceuticals are included in the water monitoring programs [45], while these biologically active substances are not systematically regulated at a global level. Despite evidence of the presence of pharmaceutical residues in various aquatic environments of Latvia [46], the state has not yet implemented a long-term strategy to reduce the risk caused by this pollution.

The purpose of this article is to point out water-related challenges facing the pharmaceutical industry. The overall water flow at the pharmaceutical plant of JSC Grindeks is shown. The most water-intensive technological processes are identified and reusable water streams are specified. The second objective is to show and assess the environmental benefit of the best practically implemented pharmaceutical wastewater re-use examples on the basis of the experience gained at JSC Grindeks, a medium-sized multiproduct pharmaceutical company.

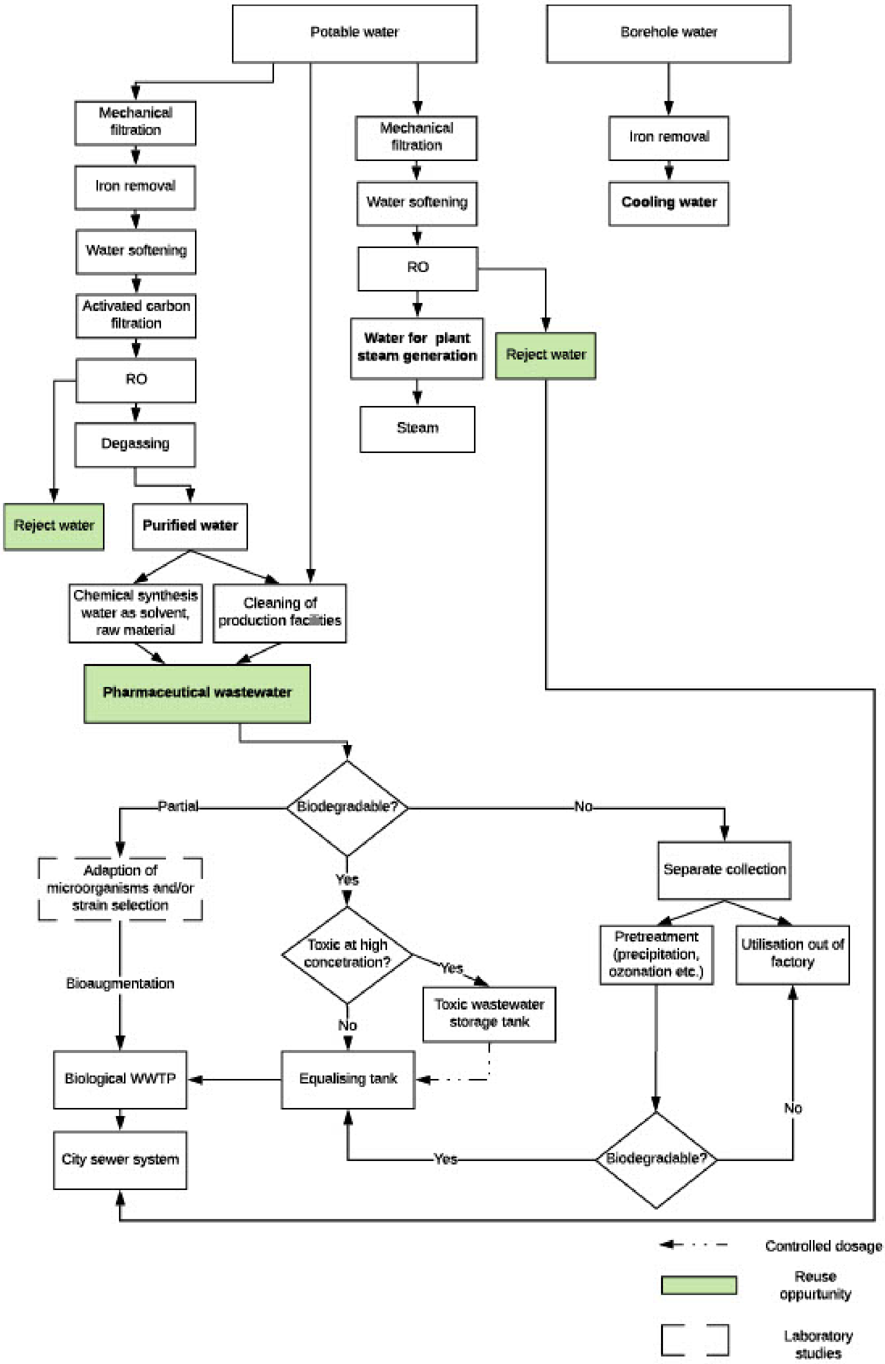

Fig. 1. Flow chart illustrating the water management practices at JSC Grindeks.

2. Materials and methods

2.1. Plant description

The pharmaceutical company JSC Grindeks located in Riga, Latvia was selected for a case study in our article. The multi-product manufacturing facility of JSC Grindeks produces both active pharmaceutical ingredients (APIs) and final dosage forms (FDFs) belonging to the cardiovascular, central nervous system, gastroenterological, and anti-cancer therapeutic groups.

The wastewaters originating from the pharmaceutical manufacturing facilities of JSC Grindeks are polluted with a wide range of difficult-to-degrade and toxic organic compounds, including phenols, while also containing high concentrations of organic nitrogen compounds and inorganic salts in combination with the near absence of phosphorus compounds. The wastewaters are treated in a system of compact moving-bed biofilm reactors (MBBRs). The technological process and main operational parameters of the WWTP are detailed in a previous study by Strade et al. [47]. According to the data from year 2019, the average wastewater flow is 200 m^3/d, while the incoming COD load fluctuates from 0.4 to 2.2 t per day and the total nitrogen load varies from 0.012 to 0.075 t per day. After pre-treatment, the wastewaters are discharged into the municipal sewer system for final treatment.

2.2. Data acquisition

A comprehensive analysis of the incoming and outgoing water flows within the pharmaceutical manufacturing facilities of JSC Grindeks was carried out, including pharmaceutical grade water, cooling water, water for the steam generation, and wastewater. The analysis included plant-specific data about: (i) water supply sources; (ii) quality requirements of water used for industrial purposes; (iii) water preparation (pre-treatment) processes used at the manufacturing facilities; (iv) water consumption; (v) wastewater production: generation, composition, flows and treatments; (vi) water and wastewater reuse practices. The numerical values of water consumption and wastewater reuse were analysed using the monitoring data from year 2019.

The relevant data were obtained from internal technical documentation and standard operating procedures, from the information provided to local regulatory agencies, as well as through interviews with employees. The chemical and microbiological purity standards of the pharmaceutical-grade water and the defined uses during the various manufacturing stages of the APIs and FDFs were summarized and analysed according to Good Manufacturing Practice (GMP) guidelines, Pharmacopoeia monographs, and the guidelines from European Medicines Agency (EMA) and the World Health Organisation (WHO).

2.3. Data evaluation

On the basis of the complete water flow analysis, the overall water flow chart for the pharmaceutical manufacturing facilities of JSC Grindeks was summarized and analysed for the first time. The information obtained was used to identify the bottlenecks in the current water management system and to point out the opportunities to improve water usage efficiency, to implement reuse solutions, and to "close the water loop". Restrictions and limitations related to direct water reuse in the pharmaceutical production were emphasized based on the sector-specific regulatory requirements and product quality and safety risks. The percentage of water savings from water efficiency improvements and reuse actions was estimated using scientific and technical literature sources.

In order to demonstrate the dependence of pharmaceutical industry on water resources, the first part of the article provides an overview of the diverse applications of water in pharmaceutical manufacturing processes. The sector-specific water quality requirements are analysed in this part to underline the difference of pharmaceutical industry from other process industries. Considering that the pharmaceutical sector also is one of the major polluters of water resources, the following section of this article points out the shortcomings of the current legislation regarding the control of environmental emissions from the pharmaceutical industry. Various possibilities for applying the principles of circular economy through the recovery of resources and the reuse of wastewater streams are discussed at the end of this article. The application of the principles of circular economy in the management of chemically polluted wastewater was analysed from three aspects: (i) the reuse of untreated wastewater streams as secondary resources in the biological wastewater treatment processes (ii) the recovery of materials from concentrated wastewater streams; (ii) the possibilities and limitations of wastewater reuse after the application of different treatment strategies.

3. Results and discussion

3.1. Different grades of water in the pharmaceutical industry

According to the World Health Organisation, the most widely used raw material in pharmaceutical manufacturing is water [48]. Consistent and high-quality water supply is required for the production of pharmaceutical products, rinsing and cleaning of equipment, as well as for technical purposes like steam generation and cooling [15]. The various types of water use in pharmaceutical manufacturing requires efficient management of both incoming and outgoing water flows. The water flows at JSC Grindeks are depicted in Fig. 1. This scheme characterises the water sources and the applied water pre-treatment methods in various technological processes, and also outlines the wastewater management practices at the company. A reference to this flow chart is given in the following sections of this article to describe the specific stages of water management at JSC Grindeks.

3.1.1. *Pharmaceutical grade water*

In pharmaceutical manufacturing, different grades of water are used as raw material, ingredient, and solvent in the production, processing, and formulation of active pharmaceutical ingredients (APIs) and final dosage forms (FDFs) for human or veterinary use (Fig. 2). The grades of water used highly depend on the formulation and the route of administration of the pharmaceutical product, and as well as on the stage in the manufacturing process at which water is used [49]. Fig. 2 summarises the minimum acceptable grade of

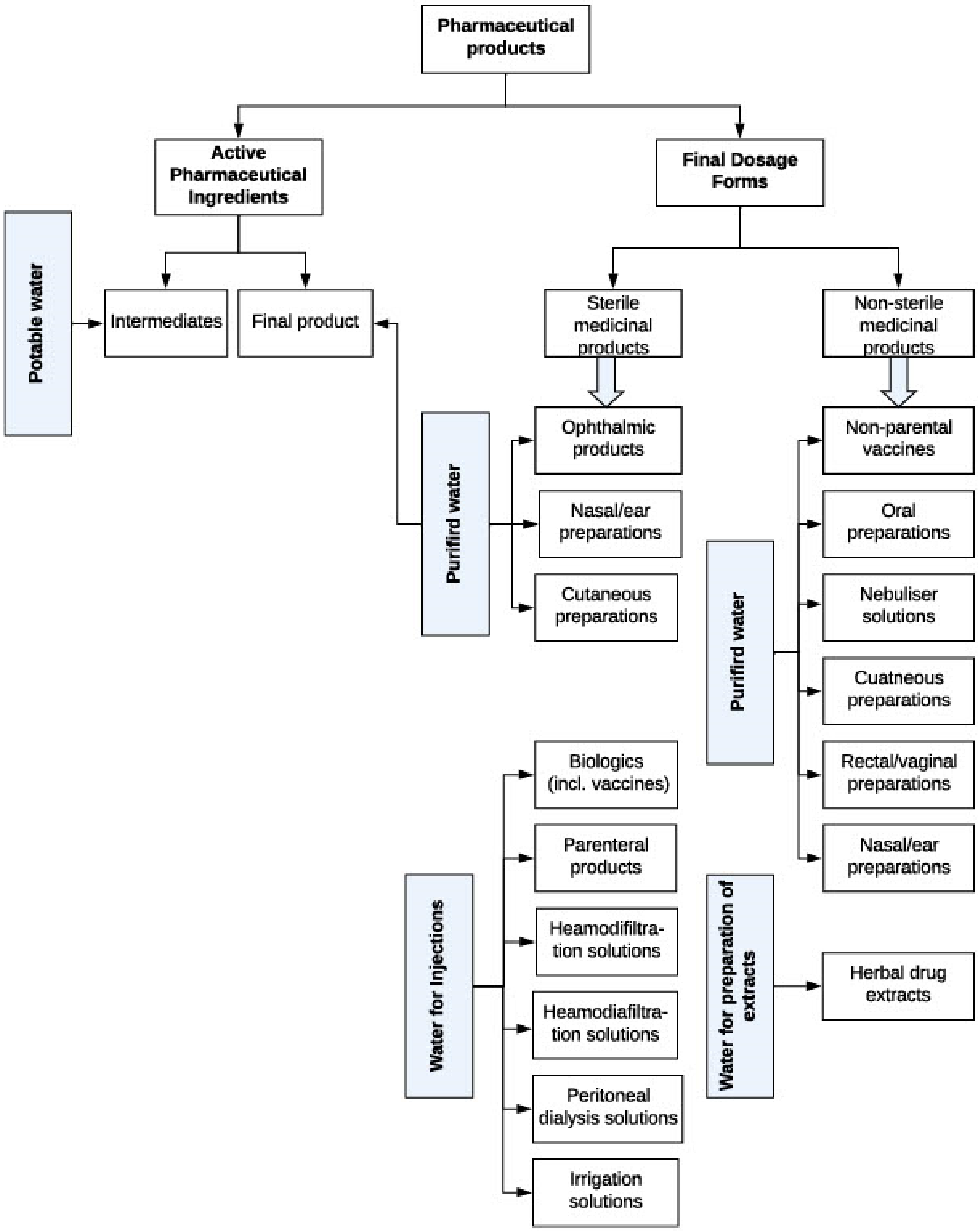

Fig. 2. Different grades of water used in pharmaceutical production.

water used during the manufacture of APIs and FDFs.

Potable water may be used in chemical synthesis of all intermediates of API prior to the final isolation and purification steps. Potable water is also used for the initial rinse of the manufacturing equipment, containers and closures in the manufacturing of APIs and non-sterile FDFs, unless there are specific technical or quality requirements for higher grades of water [49]. Potable water is not covered by a Pharmacopoeia monograph, but should, at a minimum, meet the World Health Organisation (WHO) guidelines for drinking water quality [50]. Potable water is used as feed water for the production of pharmaceutical grade waters: purified water and water for injections.

Purified water (PW). PW is used as an excipient for the final isolation and purification of APIs and for the preparation of nonsterile FDFs. Additionally, PW is used in the granulation and tablet coating processes, preparation of ointments, creams and gels, as well as for the formulation of syrups. The water content in each FDF is specified in the registration dossier. PW is the accepted grade of water for the final rinse of equipment, containers and closures in the manufacture of non-parenteral FDFs. PW is typically prepared by ion exchange, ultrafiltration, and reverse osmosis (RO) or distillation.

JSC Grindeks uses a six-step purification system for obtaining PW for pharmaceutical production (Fig. 1). The purification system provides mechanical filtration, iron removal, softening, filtration through activated carbon, a two-stage RO process, and degassing. The PW storage and distribution system consists of a water storage tank, a circulation pump, a UV irradiation cell and a PW circulation loop pipeline with water outlet valves at the production sites. The average annual PW consumption of JSC Grindeks is about 6500–7000 m^3.

Water for injections (WFI). WFI is the highest quality water used by the pharmaceutical industry. WFI is used in the manufacturing of sterile pharmaceutical products for parenteral administration and other pharmaceutical products where endotoxin content must be controlled. WFI is also used for the final rinsing of primary packaging materials. WFI must be sterile and non-pyrogenic in order to avoid the introduction of contamination and to prevent the growth of microorganisms in pharmaceutical products. Until 2017, the European Union Good Manufacturing Practice (GMP) regulations disallowed the production of WFI by non-distillation methods because of concerns about biofilm formation and endotoxin contamination. After recent changes in the European Pharmacopoeia, it is now also allowed to produce WFI by reverse osmosis coupled to other suitable techniques, such as electro-deionisation, ultrafiltration or nano-filtration. JSC Grindeks uses very small quantities of WFI (<1 m^3/year), which are purchased from external suppliers. This type of water is used in the final production stage for the oxytocin API, due to the tight specification for microbiological purity and the absence of endotoxins.

Water for the preparation of extracts. Water for the preparation of herbal drug extracts must comply with the requirements for PW or water intended for human consumption of a quality equivalent to that defined in Directive 98/83/EC [49].

The chemical and microbiological purity requirements for pharmaceutical grade water are defined in the relevant pharmacopoeias. Table 1 represents the current quality specifications for PW and WFI defined by the European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopoeia (USP). Unlike USP, Ph. Eur. specifies a limit for heavy metals in PW and a limit for nitrate concentration in PW and WFI. Both pharmacopoeias also have different requirements for the electrical conductivity of pharmaceutical grade water. The control of bacterial endotoxins (lipopolysaccharide cell wall components of gram-negative bacteria) in water used in the manufacture of FDFs intended for parenteral administration is critical, as reaction to endotoxins can lead to potentially lethal anaphylactic shock in patients [51].

A less commonly discussed aspect is the continued quality assurance of the various water grades. Pharmaceutical water production, storage, and distribution systems should be designed, installed, commissioned, qualified, and maintained to ensure reliable production of water at the specified quality [48]. Failure to meet water quality requirements poses an unacceptable risk to public health, therefore, continuous quality control of water used in the manufacturing of pharmaceutical products is of vital importance. The frequency of quality control and the water sampling points are usually specified by the water monitoring programs. Failures in the purification process pose critical levels of risk and the lack of proper quality water can result in manufacturing downtime or product recall.

Despite the use of validated purification systems and stringent operational controls, microbial contamination is the most challenging issue in the purification process of pharmaceutical grade water. Recent recall surveys from the USA have found that the presence of water-borne bacteria *Burkholderia cepacia* represent the vast majority of microbiological contamination cases that have triggered Food and Drug Administration (FDA) recalls of non-sterile drug products [52]. Various design features, like continual circulation at a flowrate of 1–3 m/s, heating options and the inclusion of high intensity ultraviolet (UV) light lamps may be incorporated to prevent the growth of biofilms and to control microbial contamination in pharmaceutical grade water systems [51].

Table 1
Quality specifications for different grades of water, as defined by Ph. Eur. and USP.

Parameter	Purified water (PW)		Water for Injection (WFI)	
	Ph. Eur.	USP	Ph. Eur.	USP
TOC (μg/L)	<500	<500	<500	<500
Conductivity (μS/cm)	≤4.3 at 20 °C	≤1.3 at 25 °C	≤1.1 at 20 °C	≤1.3 at 25 °C
Nitrate (mg/L)	≤0.2	NA	≤0.2	NA
Heavy metals (mg/L as Pb)	≤0.1	NA	NA	NA
Aerobic bacteria	≤100 CFU/mL	≤100 CFU/mL	≤10 CFU/100 mL	≤10 CFU/100 mL
Bacterial endotoxins (I.U./mL)	NA	NA	≤0.25	≤0.25

NA – not an applicable requirement.

The experience at JSC Grindeks shows that proper sanitation and regular maintenance procedures are essential to preventing microbial proliferation and recontamination of the water. For example, a validated sanitization procedure of the PW distribution and storage system in the API manufacturing facility is performed with 3% or 10% hydrogen peroxide solution: 1) at least once a year; 2) if the PW system has been shut down for more than 14 days; 3) in cases in which the parameters of microbiological contamination do not meet the requirements of the relevant Pharmacopoeia. Thermal sanitization (85 °C/30 min) of the PW system in the FDF manufacturing facility is used for routine operations (twice per year) and if the TOC or total number of aerobic bacteria of the PW circulating in the loop exceed the maximum level or if the loop has been emptied, if reconstruction work in the loop has been performed or the system has been exposed to air. Special attention is paid to the plastic tubing at PW outlet points. To avoid the introduction of microbiological contamination into the PW circulation loop, they are disconnected, rinsed with 70% ethanol, and dried after each use, as well as replaced at least every 6 months.

From a water efficiency standpoint, the preparation of pharmaceutical grade water is associated with relatively high losses of water. The experience of JSC Grindeks confirms that due to the very high purity requirements of PW, up to 50% of the feed water is discharged as waste from the RO modules (Fig. 1). However, this water is still of sufficiently high quality for re-use as an alternative onsite water source in units that do not come into a direct contact with pharmaceutical products, for example, in cooling systems. This inefficiency has been identified as one of the bottlenecks in the water management system of JSC Grindeks where the circular economy approach could be applied to partially close the water loop and to reduce the water consumption by about 7000 m^3 annually (equal to 12% of the total amount of potable water used in production processes at JSC Grindeks).

3.1.2. *Process water*

In addition to direct use in pharmaceutical production where water comes into contact with pharmaceutical substances, it is also used as a key resource for technical purposes, for example, cooling and steam generation.

3.1.2.1. Cooling water. To provide reliable pharmaceutical manufacturing process conditions, the correct process temperature must be guaranteed at all times, which may require a cooling capacity. The main pharmaceutical manufacturing processes that require an effective cooling system are:

- Batch processing in multipurpose reactors (for example, temperature sensitive chemical reactions, crystallization of final products)
- Cooling of creams and ointments before pouring and packaging
- Wet granulation processes for tablet production
- Moulding of gelatine capsules
- Sterilisation of liquid FDFs.

Due to its high heat capacity and low cost, water is the most common coolant. Cooling water should be pre-treated to ensure efficient heat transfer, reduce total energy consumption, and avoid equipment failure. Pre-treatment depends on feed water quality and usually includes iron removal, softening, the addition of corrosion and scale inhibitors, and treatment with biocides to prevent biological fouling in the heat exchange systems [53]. It is also important to control the pH value of cooling water, as acidic environment promotes corrosion, while alkaline pH increases scale formation [54].

The traditionally used cooling towers or once-through (single pass) cooling systems often represent the largest percentage of water consumption in pharmaceutical manufacturing [55], and JSC Grindeks is not an exception. The once-through cooling system used at JSC Grindeks consumes up to 250 000 m^3 of water annually, which exceeds the total amount of potable water used for pharmaceutical manufacturing processes by a factor of about 4.2. After a single pass through heat exchangers and/or condensers, the heated water is discharged into the storm water drainage system. The reduction of high water consumption by once-through cooling systems requires a change towards closed-loop recirculating cooling systems that consume an order of magnitude lower volume of cooling water compared to once-through systems [53]. It has been noted by other authors that the installation of adiabatic cooling systems allows pharmaceutical manufacturers to reduce water consumption by up to 98% compared to the traditionally used open-loop cooling systems [53,56]. Besides, reusing the same clean water into closed circuit also eliminates the costs associated with the treatment and disposal of water, and also eliminates the concerns associated with Legionnaires' disease [56].

JSC Grindeks obtains its cooling water from 2 artesian boreholes located on the territory of the plant. The natural and regulatory limits to water extraction from an artesian aquifer prevent further expansion of production, unless more water-efficient cooling technologies are introduced. Otherwise, the available groundwater resources may be insufficient for the required cooling capacity, and

Table 2
The types of steam used in pharmaceutical industry.

Type of steam	Intended use	Direct contact with pharmaceutical products	Quality requirements of feed water or condensate
Plant steam	Indirect contact process heating	–	Produced from pre-treated potable water
Chemical-free steam	Humidification (HVAC systems)	–	Produced from pre-treated water (without volatile boiler additives)
Pure steam	Sterilisation	+	Condensate meets quality characteristics of WFI

extensive use of this resource will incur regulatory and taxation costs.

3.1.2.2. Water for steam generation. Steam used in pharmaceutical facilities can be separated into three categories: plant steam, chemical-free steam, and pure steam (Table 2).

Depending on the intended use of the steam, there are different requirements of feed water quality and steam generation methods. Feed water of the plant steam boiler must be derived from water that is of drinking quality. It is treated to remove the bulk of dissolved solids, hardness, and silica, which could cause scaling and corrosion of the steam generator and pipelines. The manufacturing facilities of JSC Grindeks consume about 17–18 000 m^3 of potable water per year for plant steam production. Boiler feed water is purified by passing through mechanical filters, cation exchange (softening) resins and an RO system that greatly reduces the content of scale forming salts, improves boiler efficiency and steam purity. Chemical-free steam is typically a separate type of steam generated without chemical boiler additives [57]. It is used for humidity control of processes and critical cleanrooms where pharmaceutical products are directly exposed to the room atmosphere. JSC Grindeks also uses humidification to eliminate the build-up of static electricity in manufacturing environments where flammable gases or substances are being used. Several local steam boilers are installed in the manufacturing facilities for such purposes. Pure steam is the highest quality of steam used in pharmaceutical manufacturing. It is used for the sterilisation of pharmaceutical products, equipment or piping. This type of steam is critical to biopharmaceutical production and for the production of injectable solutions. Unlike plant steam, no chemical additives are permitted in the treatment of water used in pure steam generators. As pure steam comes into direct contact with pharmaceutical products or product contact surfaces, additional treatment of feed water should be performed, to ensure that the condensate of pure steam meets WFI requirements for conductivity, TOC, microbial content, and endotoxins [58]. As JSC Grindeks does not produce sterile pharmaceutical products, pure steam is not used in the manufacturing processes.

The recovery and on-site re-use of steam condensate is one of the key actions that can improve the water efficiency at manufacturing facilities. The re-use of high purity condensate also reduces chemical use, feed-water treatment costs, and saves energy, since after returning to the boiler the hot condensate requires much less energy to reheat for repeated steam production.

Despite the potential water savings, steam condensate is not recovered and reused as boiler feed water at JSC Grindeks. The major obstacle here is that the boiler house is located about 500 m from the manufacturing facilities. The relatively long distance and the initial investment that would be necessary to build a condensate return line have prevented the re-use of plant steam condensate. From a water efficiency point of view, in instances in which the installation costs of condensate recovery equipment are too high to be justified, factories should evaluate alternative options for re-use of condensate. Due to its inherent heat content, condensate can be reused in various heating systems or as a source of clean, hot water for washing purposes.

There are not only financial and technical, but also product quality and safety issues that need to be considered when analysing condensate re-use options in a pharmaceutical plant. As pure steam comes into contact with pharmaceutical products, the condensate must not be directly returned to a pure steam generator because of contamination risk [58]. Potential microbiological contamination of condensate from HVAC systems also is one of the risk factors that could limit condensate re-use. Scientific publications report that HVAC condensate can be contaminated with *Legionella* and other airborne bacteria, therefore, the harvested condensate water must be properly disinfected before re-use as an alternative water resource [59].

Our case study confirms the conclusions of the United Nations [60] that the industry often prioritizes production capacity over water efficiency and conservation. A common reason for this is that investments in efficient cooling processes or water reuse systems may have longer return on investment than short-term improvements in production capacity. Moreover, low water prices in the regions with good freshwater availability also do not encourage investment in water efficiency at the plant level and the potential benefits of water efficiency improvements in industry are often underestimated. In order to properly evaluate and provide justifications for modifications to equipment or operating practices, the new and true value of water should be assessed, accounting not only for the water supply costs, but also for the related treatment, pumping, storage, purification, and disposal costs [6].

3.1.3. Water recovered from drug manufacturing processes

Pharmaceutical products are typically produced in multi-stage batch processes that create highly complex waste streams with variable chemical composition, toxicity, and volume [15]. Such variation in the quality and quantity of wastewater may cause shock and overload to the treatment systems, which leads to malfunctioning or even failure of treatment processes, particularly biological treatment [61].

In order to ensure effective treatment of pharmaceutical effluents, manufacturing plants must separately manage different types of wastewater, distinguish highly toxic and concentrated streams and providing pre-treatment if necessary. Wastewater biodegradability and toxicity as well as the concentration of specific pollutants in the individual batch streams must be taken into account for selecting the most appropriate treatment methods. The evaluation and separation of pharmaceutical batch streams according to the nature of the contamination is a key activity not only for finding the most appropriate treatment solution, but also for assessing the recovery or re-use potential of materials in the effluents.

A cost effective and simple method for toxicity screening of pharmaceutical wastewater has been implemented in laboratory practice at the WWTP of JSC Grindeks, which is based on BOD measurements over a wide range of initial concentrations [47]. By using a simple BOD/COD relationship for different batch loads it is possible to identify the most toxic flows and to determine safe rates of discharge into biological wastewater treatment systems. The extensive initial concentration range used in the experiments, which corresponds to the real amount of wastewater generated per day, while also accounting for the dilution in equalization tank, allows determination of the toxic threshold concentration of the sample. These data are used as a basis for assessing the risks and predicting the process performance in the case of production increases or changes in the presence of specific pollutants in wastewater. Primary attention should be paid to the most toxic compounds that enter the wastewater as synthesis by-products or unreacted raw materials. If the screening indicates that wastewaters are toxic to activated sludge already at low concentration levels, separate collection at the source and further research should be performed. Pre-treatment options are then evaluated or waste disposal by specialised service providers may be required.

3.2. The role of legislation in controlling the emissions of APIs from manufacturing plants

A very important issue at a global level is the role of the pharmaceutical industry in reducing the discharge of pharmaceutical substances into the aquatic environment. Despite the recent concerns regarding the risks to human health and ecosystems posed by pharmaceuticals, current EU legislation does not regulate the emissions of APIs at the manufacturing stage. The Industrial Emissions Directive (2010/75/EU), which also applies to pharmaceutical manufacturing sites, does not include APIs on the list of polluting substances and there are no threshold values established for pharmaceutical discharge. APIs that are already registered with the European Medicines Agency (EMA) as ingredients of medicinal products for human and veterinary use are also exempt from the REACH regulations. GMP guidelines essentially focus on pharmaceutical product quality and safety parameters and do not include any requirements for environmental protection.

There are a number of shortcomings in the existing EMA guidance for environmental risk assessment. First, there are insufficient or no Environmental Risk Assessment (ERA) data available for numerous widely consumed pharmaceutical products that entered the market before the respective guidelines came into force. In the EU, ERA is a mandatory requirement for all new marketing authorization applications for medicinal products intended for human and veterinary use only since 2006 and 2001, respectively [62]. Second, the risks associated with API discharges from manufacturing sites are not included in the ERAs [63]. In contrast to veterinary products, the results of ERA for human medicinal products should not constitute a criterion for the refusal of marketing authorization [62,63].

A recent communication of the European Commission that refers to the European Union Strategic Approach to Pharmaceuticals in the Environment emphasizes that the pharmaceutical industry needs to reduce its environmental impact from the product lifecycle perspective, with the greatest focus on the research and development, as well as the manufacturing stages [64]. The development of pharmaceutical products that degrade more readily to harmless substances in WWTP and the environment and are produced by greener manufacturing methods are identified as the future priorities of the pharmaceutical industry [64].

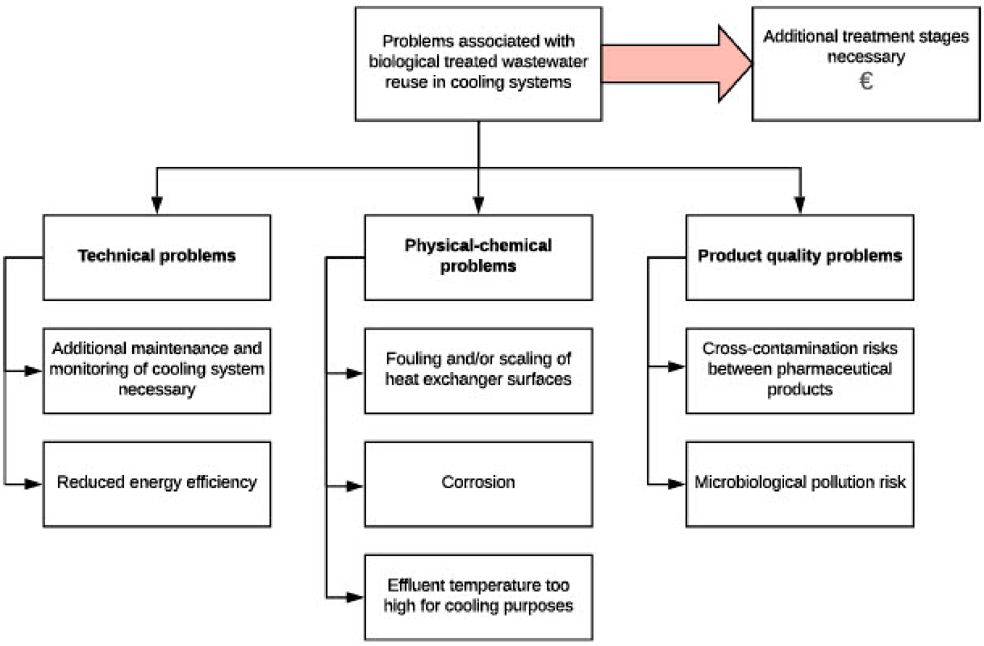

Fig. 3. Problems arising from re-use of biologically treated pharmaceutical wastewaters in cooling systems.

3.3. Opportunities and limitations of treated pharmaceutical wastewater re-use in drug manufacturing processes

From the sustainability point of view, water re-use in manufacturing is essential because it could drastically reduce freshwater requirements and enable the expansion of production capacity without exceeding water discharge limits [15]. The opportunities for reusing treated pharmaceutical wastewaters as a secondary resource in pharmaceutical manufacturing are quite limited due to the high risk of product contamination, which may have serious impact on patient health, and is contrary to the GMP requirements [50]. To avoid cross-contamination between products, re-use of treated wastewater for industrial purposes might be acceptable only after complete removal of API residues during the wastewater treatment process, if the quality of reclaimed water is not inferior to drinking water.

It should be stressed that all changes in pharmaceutical manufacturing, such as alternative sources of starting materials (including water reuse) should be validated and accepted by the relevant regulatory authorities, as well as approved by industrial customers. This is a time-consuming and expensive process that can seriously impact the economic viability of any changes.

Furthermore, investments in wastewater treatment for re-use should be weighed against the cost and local availability of freshwater resources. Among the EU Member states, Latvia ranks 4th in the availability of freshwater resources, with the average of about 18 000 m^3 per inhabitant per year [65]. In contrast to Latvia, the available water resources in Poland, the Czech Republic, Cyprus, and Malta are below 1700 m^3 per inhabitant per year, indicating that those countries may experience shortages of water for industrial uses. Since chemically and energetically intensive wastewater treatment processes are needed to improve the wastewater quality according to the specifications for re-use, in some cases water re-use may cause higher environmental impact than the water use itself [66].

The effluent from the biological WWTP of JSC Grindeks is not suitable for internal re-use purposes. The applied MBBR system ensures pre-treatment in accordance to the local regulations in terms of COD, as well as N and P removal, however, this level of treatment is still insufficient for subsequent re-use of the treated wastewater, for example, in cooling systems. Fig. 3 summarises the main problematic issues that can arise from the re-use of biologically treated pharmaceutical wastewaters in cooling systems.

First, the use of biologically treated pharmaceutical wastewater streams increases the risk of scaling and corrosion due to the high levels of dissolved salts. Second, the re-use of effluents from biological WWTP promotes higher microbiological activity and biofouling due to the remaining dissolved nutrients. Additional fouling problems may be associated with residual suspended solids (activated sludge). Biofilm growth and deposits on heat transfer surfaces tend to cause loss of heat transfer efficiency. Any of these problems or a combination of them can result in costly unscheduled downtime, reduced process capacity and reliability.

Besides that, the chemical compatibility of effluent water with materials used in the cooling systems should be assessed. Direct contact of products with wastewater is not allowed in any situation, therefore, the pipelines should be permanently monitored and maintained to prevent any leakage into the reaction vessels and the associated product contamination risks. Fluctuations of effluent water quality and quantity cause additional challenges and should be taken into consideration. Furthermore, pharmaceutical wastewater after biological treatment is often too warm for cooling purposes. For example, the temperature of effluent water from the biological WWTP of JSC Grindeks varies between 28 and 42 °C, making it unsuitable for cooling purposes. Elevated temperature is one of the stress factors to nitrifying bacteria that may disrupt the nitrification process, therefore, a heat exchanger for wastewater cooling is installed at the WWTP of JSC Grindeks.

3.4. Processing of pharmaceutical waste streams for resource recovery and re-use

For a typical batch chemical process in the pharmaceutical industry, solvents can constitute up to 80–90% of the non-aqueous mass [67]. Due to their high proportion in process mixtures, spent solvents also represent a major part of the waste generated by the pharmaceutical industry. The minimisation of solvent use, substitution of hazardous solvents, as well as solvent recovery and re-use are becoming key priorities for the purposes of greener manufacturing [68–70].

In practice, about 50% of waste solvents are recovered and reused in the batch chemical processes at JSC Grindeks, while the remaining part is either disposed through incineration or biologically degraded as dilute aqueous solutions during wastewater treatment in the post denitrification process (see more details in Section 3.5.4). The main barriers to solvent recovery, especially in the manufacturing of low volume APIs, include the technical difficulty and cost of recovery, purity requirements equal to those for fresh solvents, and the time-consuming analytical and administrative procedures necessary to verify the quality and to approve solvent re-use in the manufacturing. Although many pharmaceutical manufacturers have centralised solvent recovery facilities, the distillation equipment at JSC Grindeks is integrated at separate production sites. Such an approach allows better optimisation of the solvent recovery processes and eliminates the risks of cross-contamination between pharmaceutical products by using shared equipment for solvent recovery.

Transition metal catalysts play an important role in the pharmaceutical industry and are extensively used for the development of new drug candidates and large-scale synthesis of APIs [71]. Wastewater containing transition metals may not be discharged into biological WWTP, as these metals tend to accumulate in the sludge, causing toxic effects on microorganisms and having an effect on further processing of sludge [72,73]. In accordance with Latvian legislation, elevated content of heavy metals in the sludge is a basic criterion for their classification as hazardous waste [74]. Due to the efforts to prevent heavy metal emissions in the pharmaceutical wastewater stream, the sludge generated at the WWTP of JSC Grindeks typically corresponds to Class 1 or 2 (Class 5 is considered to be hazardous waste), which allows use of the sludge for soil fertilisation and re-cultivation of degraded areas. Spent catalysts and/or contaminated wastewater streams from metal-catalysed chemical synthesis steps could be utilised as a resource for the recovery of toxic heavy metals [75]. Due to the relatively small volumes of heavy metal-containing wastewater and the need for specific processing, JSC Grindeks does not carry out on-site recovery of heavy metals from wastewater streams. Spent Pd and Ni catalysts along with concentrated metal-containing wastewaters from chemical synthesis are collected separately and sent to specialised organisations for processing, while copper ions are precipitated and removed from the wastewater in the form of insoluble hydroxide prior to the discharge into the biological WWTP.

There are several other wastewater streams arising from pharmaceutical manufacturing processes that can be directly reused as resources and secondary raw materials, such as alkaline streams, effluents containing nutrients, such as phosphates, and metals. Practical examples focusing on the re-use of these flows are detailed in Section 3.5. The successful use of such wastes as valuable materials serves as an example of the circular economy approach, providing a motivation for improved waste stream management at the chemical plant level and/or enhanced cooperation both with other companies i.e. to develop industrial symbiosis and with universities.

3.5. Implementing circular economy elements into pharmaceutical wastewater treatment procedures

The practical experience at the WWTP of JSC Grindeks shows that there are several possible ways for reusing contaminated wastewater streams from batch chemical synthesis operations as raw materials in the biological WWTP. To identify and manage the possible ways of wastewater re-use, each batch process should be analysed separately and effective communication between production sites and wastewater treatment plant should be facilitated to share information that is relevant for the WWTP. Obviously, such

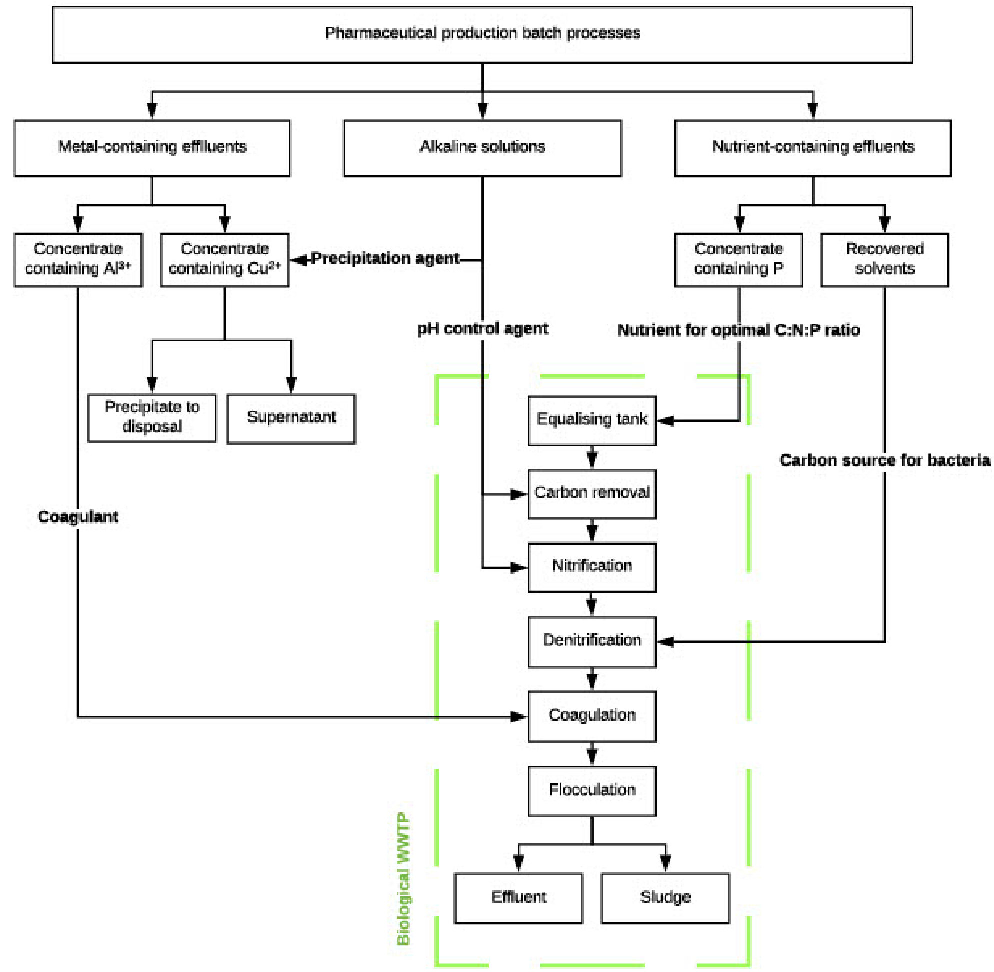

Fig. 4. Scenarios for the use of selected pharmaceutical wastewater batch streams as raw materials in a biological WWTP.

commitment also requires technical possibilities for collecting the individual wastewater streams in separate containers for temporary storage, which might be a limiting factor in some cases.

The examples of reusing untreated pharmaceutical wastewater batch streams as raw materials in the biological WWTP of JSC Grindeks are given in Fig. 4. Practical implementation is demonstrated for metal-containing wastewater streams, alkaline solutions, and nutrient-containing effluents. The given examples indicate that certain pharmaceutical wastewater batch streams should be considered not as a waste but rather as a valuable resource to be handled according to the principles of circular economy. The implementation of circular economy elements through pharmaceutical wastewater re-use allows reduction of the chemical consumption and operational costs of WWTP.

3.5.1. The use of Al^{3+} containing wastewater as coagulant in sludge removal processes

Aluminium chloride is used as a Lewis acid in the synthesis of Milnacipran Hydrochloride API [76]. An acidic aqueous Al^{3+} solution is generated as a synthesis by-product. Aluminium acts as a toxic agent to aquatic organisms, including fish, invertebrates, and plants [77]. To avoid the disruption of the activated sludge process, it is crucial to assess the toxicity risks of such waste streams to biological water treatment plants before large-scale production and the biocenosis from the particular WWTP must be employed in the toxicity tests [78]. In the performed toxicity tests, Al^{3+} containing wastewater showed highly toxic effects on the activated sludge microorganisms at the WWTP of JSC Grindeks [47]. In a separate pilot study, it was found that this type of wastewater is suitable for re-use as a coagulant in the sludge removal process and excess phosphorus precipitation at full-scale WWTP. For the best process performance the optimum pH range and dosage should be adjusted depending on case-specific effluent characteristics. In many situations the pH drop upon the addition of aluminium salts needs to be counteracted by the addition of an alkaline solution. pH control is important also to maintain minimum levels of dissolved residual aluminium in treated wastewater [79].

At the current production capacity, about 15 m^3 of Al^{3+} containing wastewater is generated each year, and 100% of this wastewater is reused in the sludge removal process at the on-site WWTP of JSC Grindeks. The implementation of such circular economy elements as the introduction of Al^{3+} containing wastewater to the sludge removal process allows for replacement of the traditionally used commercial aqueous 40% $FeCl_3$ solution.

3.5.2. The use of inorganic acids or bases for pH control in moving-bed biofilm reactors (MBBRs)

The pH values of pharmaceutical wastewater from different batch processes can vary greatly, ranging from highly acidic to alkaline. If high amounts of acids or bases are discharged into wastewaters, considerable amounts of chemicals are required to return the pH value to a neutral level that is optimal for activated sludge microorganisms, especially if the buffer capacity of wastewater is low.

The pH of the wastewater received at the WWTP of JSC Grindeks is adjusted by adding 50% H_2SO_4 or 30% NaOH solutions according to necessity. In general, the wastewaters of JSC Grindeks are highly alkaline (pH ~ 10 in the equalizing tank). Due to biodegradation processes, the pH value in the first MBBRs drops down to a neutral level, therefore, the addition of H_2SO_4 is usually not necessary. Higher volumes of NaOH solution are consumed for pH control at the nitrification stage.

To avoid extreme pH fluctuations, a large volume equalizing tank is required. Alternatively, concentrated water solutions of inorganic acids or bases, if available as by-products from the manufacturing processes, can be collected in separate containers and subsequently reused for pH control in the biological treatment reactors. If alkaline aqueous solutions from manufacturing are intended for pH control during the nitrification process, they must not contain biodegradable organic compounds that would directly promote the growth of heterotrophic bacteria. The oxygen affinity and growth rate of nitrifying bacteria is low in comparison to heterotrophic bacteria, therefore, an increase in heterotrophic bacteria may adversely affect the nitrifying bacteria by competing for space, oxygen, and other nutrients, resulting in the inhibition of nitrification [80]. A strongly alkaline NaOH solution (Fig. 4) can also be used as a precipitant to remove copper from wastewaters generated during other batch processes.

3.5.3. The use of phosphorus containing wastewater as a nutrient source for activated sludge microorganisms

A characteristic feature for pharmaceutical wastewater treatment plants is the situation that influent wastewater can be deficient in some nutrients and rich in others. In general, the industrial wastewaters of JSC Grindeks are rich in carbon and nitrogen, while deficient in phosphorus. The median COD and nitrogen concentration of the influent water are 5500 mg/L and 160 mg/L, respectively, yet the median phosphorus concentration in the inlet water is only 3 mg/L. To ensure optimal phosphorus levels at the biological treatment stages at the WWTP of JSC Grindeks, H_3PO_4 is added as a phosphorus source in the first MBBR, and the level of inorganic phosphates is monitored in the first and last MBBRs, because low phosphorus availability strongly affects the biodegradation efficiency and favours the growth of filamentous bacteria [81].

The only production process giving high amounts of phosphates (C = 10 g/L PO_4–P) in the wastewaters of JSC Grindeks is the batch synthesis of Ipidacrine API, which performed in campaigns lasting only several weeks every year. In order to avoid extreme peak loadings and overdose of phosphorus at these times, about 10 m^3 of wastewater from this batch process is collected separately and reused as a phosphorus source during the periods when its concentration is low in the influent wastewater. The substitution of H_3PO_4 with phosphorus-rich wastewater allows reduction of the consumption of phosphorus as a critical nutrient and lowers the operational costs of WWTP, leading to improved plant efficiency.

3.5.4. The use of recovered solvents as easily degradable carbon source for the denitrification processes

Spent solvents that after several recovery cycles are no longer suitable for the manufacturing of pharmaceutical products, represent a large proportion of the liquid waste generated by the pharmaceutical industry (see more details in Section 3.4). In most cases, elevated water content is the reason why spent solvents are no longer suitable for re-use in chemical synthesis. Solvents in general are toxic and inhibit the enzymatic activity of activated sludge microorganisms [82], however, controlled addition of some solvents to wastewater streams may be beneficial for the activated sludge microorganisms.

At the biological WWTP of JSC Grindeks, about 9.5 tons of water-miscible spent solvents per year are utilised as external carbon sources in the post-denitrification process. Due to its relatively low toxicity, highly biodegradable nature, and chemical compatibility with the dosing line materials, ethanol has been selected as the most suitable spent solvent for this purpose. The addition of a carbon source is based on an experimentally determined optimal C:N ratio of 4:1, where C is COD and N refers to both NO_3^--N and NO_2^--N. The spent ethanol is diluted with water to a concentration of about 50% in order to reduce its flammability and to mitigate explosion risks at the WWTP.

Although the current re-use rate of spent solvents in the denitrification process represents only about 3.5% of the total amount of spent solvents generated at JSC Grindeks, there are opportunities to also utilise these solvents at municipal WWTPs. As the denitrification efficiency at municipal WWTPs is often limited by insufficient carbon content in the untreated wastewater [83], spent solvents can be potentially introduced as external carbon sources at municipal WWTPs. Considering that spent solvents are recovered separately from each batch process at JSC Grindeks, it is possible to accurately determine the presence of pharmaceutical residues in such solvents. Additionally, it allows to choose solvents from the production of those APIs that show comparatively better biodegradability at WWTPs and does not represent therapeutic classes that have significant adverse effects on the ecosystem [84]. The implementation of such practices allows reduction of operating costs of the WWTPs, an increase in nitrogen removal efficiency, and general improvement of pharmaceutical waste management.

4. Conclusions

Comprehensive analysis of various water flows and their quality requirements allows identification of opportunities and limitations for water reuse at the manufacturing plant level. Concerns about product contamination, the strict chemical and microbiological purity requirements and current GMP regulations restrict the use of reclaimed water in direct contact with pharmaceutical products. Water steam condensate and reject water re-use from RO systems, and a transition to water-efficient closed-loop cooling systems were recognized as necessary actions to reduce water consumption in a particular pharmaceutical factory. Due to extreme variability of pharmaceutical wastewater in a multiproduct factory, treatability studies and toxicity screening should be conducted for individual batch streams to distinguish highly toxic and concentrated streams and provide pre-treatment if necessary. Our case study highlights a synergy between manufacturing facilities and on-site WWTP that provides a logical path for implementing the elements of circular economy in the pharmaceutical factory. As a result, specific wastewater streams from pharmaceutical batch processes can be reused as sources of nutrients, coagulants or a pH control agent in a biological WWTP, providing both environmental and economic benefits.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] J. Schellekens, L. Heidecke, N. Nguyen, W. Spit, The Economic Value of Water - Water as a Key Resource for Economic Growth in the EU, 2018. https://ec.europa.eu/environment/blue2_study/pdf/BLUE2%20Task%20A2%20Final%20Report_CLEAN.pdf. accessed 5 February 2020.

[2] Oecd, OECD Environmental Outlook to 2050, 2012. https://read.oecd-ilibrary.org/environment/oecd-environmental-outlook-to-2050_9789264122246-en#page4. accessed 25 January 2020.
[3] United Nations, The United Nations World Water Development Report 2020: Water and Climate Change, 2020. https://unesdoc.unesco.org/ark:/48223/pf0000372985.locale=en. accessed 5 May 2020.
[4] J. Willet, K. Wetser, J. Vreeburg, H.H.M. Rijnaarts, Review of methods to assess sustainability of industrial water use, Water Resour. Ind. 21 (2019) 100110, https://doi.org/10.1016/j.wri.2019.100110.
[5] D.P. Webb, G. Skouteris, S. Rahimifard, In-plant real-time manufacturing water content characterisation, Water Resour, For. Ind. 20 (2018) 37–45, https://doi.org/10.1016/j.wri.2018.08.003.
[6] B.P. Walsh, S.N. Murray, D.T.J. O'Sullivan, The water energy nexus, an ISO50001 water case study and the need for a water value system, Water Resour. Ind. 10 (2015) 15–28, https://doi.org/10.1016/j.wri.2015.02.001.
[7] T. Tong, M. Elimelech, The global rise of zero liquid discharge for wastewater management: drivers, technologies, and future directions, Environ. Sci. Technol. 50 (2016) 6846–6855, https://doi.org/10.1021/acs.est.6b01000.
[8] A.J. Toth, E. Haaz, T. Nagy, A.J. Tarjani, D. Fozer, A. Andre, N. Valentinyi, P. Mizsey, Novel method for the removal of organic halogens from process wastewaters enabling water reuse, Desalin. Water Treat. 130 (2018) 54–62, https://doi.org/10.5004/dwt.2018.22987.
[9] H.I. Abdel-Shafy, M.S.M. Mansour, Treatment of pharmaceutical industrial wastewater via anaerobic/aerobic system for unrestricted reuse, J. Sci. Ind. Res. 76 (2017) 119–127.
[10] L. Ling, Y. Liu, D. Pan, W. Lyu, X. Xu, X. Xiang, M. Lyu, L. Zhu, Catalytic detoxification of pharmaceutical wastewater by Fenton-like reaction with activated alumina supported CoMnAl composite metal oxides catalyst, Chem. Eng. J. 381 (2020) 122607, https://doi.org/10.1016/j.cej.2019.122607.
[11] E. Linclau, J. Ceulemans, K.D. Sitter, P. Cauwenberg, Water and detergent recovery from rinsing water in an industrial environment, Water Resour. Ind. 14 (2016) 3–10, https://doi.org/10.1016/j.wri.2016.03.001.
[12] S. Ayatollahi, D. Kalnina, W. Song, B.A. Cottrell, M. Gonsior, W.J. Cooper, Recent advances in structure and reactivity of dissolved organic matter: radiation chemistry of non-isolated natural organic matter and selected model compounds, Water Sci. Technol. 66 (2012) 1941–1949, https://doi.org/10.2166/wst.2012.404.
[13] S. Ayatollahi, D. Kalnina, W. Song, M. Turks, W.J. Cooper, Radiation chemistry of salicylic and methyl substituted salicylic acids: models for the radiation chemistry of pharmaceutical compounds, Radiat. Phys. Chem. 92 (2013) 93–98, https://doi.org/10.1016/j.radphyschem.2013.06.007.
[14] E. Szabados, A. Jobbágy, A.J. Tóth, P. Mizsey, G. Tardy, C. Pulgarin, S. Giannakis, E. Takács, L. Wojnárovits, M. Makó, Z. Trócsányi, A. Tungler, Complex treatment for the disposal and utilization of process wastewaters of the pharmaceutical industry, period, Polytech. Chem. Eng. 62 (2018) 76–90, https://doi.org/10.3311/PPch.10543.
[15] C. Gadipelly, A. Pérez-González, G.D. Yadav, I. Ortiz, R. Ibáñez, V.K. Rathod, K.V. Marathe, Pharmaceutical industry wastewater: review of the technologies for water treatment and reuse, Ind. Eng. Chem. Res. 53 (2014) 11571–11592, https://doi.org/10.1021/ie501210j.
[16] A.M. Deegan, B. Shaik, K. Nolan, K. Urell, M. Oelgemöller, J. Tobin, A. Morrissey, Treatment options for wastewater effluents from pharmaceutical companies, Int. J. Environ. Sci. Tech. 8 (2011) 649–666, https://doi.org/10.1007/BF03326250.
[17] R. Changotra, H. Rajput, A. Dhir, Treatment of real pharmaceutical wastewater using combined approach of Fenton applications and aerobic biological treatment, J. Photochem. Photobiol., A 376 (2019) 175–184, https://doi.org/10.1016/j.jphotochem.2019.02.029.
[18] D. Kanakaraju, B.D. Glass, M. Oelgemöller, Advanced oxidation process-mediated removal of pharmaceuticals from water: a review, J. Environ. Manag. 219 (2018) 189–207, https://doi.org/10.1016/j.jenvman.2018.04.103.
[19] C. Grandclément, I. Seyssiecq, A. Piram, P. Wong-Wang-Chung, G. Vanot, N. Tiliacos, N. Roche, P. Doumenq, From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review, Water Res. 111 (2017) 297–317, https://doi.org/10.1016/j.watres.2017.01.005.
[20] J. Rivera-Utrilla, M. Sánchez-Polo, M.Á. Ferro-García, G. Prados-Joya, R. Ocampo-Pérez, Pharmaceuticals as emerging contaminants and their removal from water. A review, Chemosphere 93 (2013) 1268–1287, https://doi.org/10.1016/j.chemosphere.2013.07.059.
[21] D.G.J. Larsson, Pollution from drug manufacturing: review and perspectives, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (2014) 20130571, https://doi.org/10.1098/rstb.2013.0571.
[22] M.O. Awaleh, Y.D. Soubaneh, Waste water treatment in chemical industries: the concept and current technologies, Hydrol. Curr. Res. 5 (2014) 164, https://doi.org/10.4172/2157-7587.1000164.
[23] Editorial, Integrated water management in the chemical industry (E4Water), Water Resour, For. Ind. 14 (2016) 1–2, https://doi.org/10.1016/j.wri.2016.05.002.
[24] S.A. Mueller, A. Carlile, B. Bras, T.A. Niemann, S.M. Rokosz, H.L. McKenzie, H.C. Kim, T.J. Wallington, Requirements for water assessment tools: an automotive industry perspective, Water Resour. Ind. 9 (2015) 30–44, https://doi.org/10.1016/j.wri.2014.12.001.
[25] A. Manzardo, J. Ren, A. Piantella, A. Mazzi, A. Fedele, A. Scipioni, Integration of water footprint accounting and costs for optimal chemical pulp supply mix in paper industry, J. Clean. Prod. 72 (2014) 167–173, https://doi.org/10.1016/j.jclepro.2014.03.014.
[26] F.S. Francisco, R.C. Mirre, E.E.S. Calixto, F.L.P. Pessoa, E.M. Queiroz, Management of water consumption in pulp and paper industry – a case study using water sources diagram, Chem. Eng. Trans. 39 (2014) 1255–1260, https://doi.org/10.3303/CET1439210.
[27] V. Colla, I. Matino, T.A. Branca, B. Fornai, L. Romaniello, F. Rosito, Efficient use of water resources in the steel industry, Water 9 (2017) 874, https://doi.org/10.3390/w9110874.
[28] P.W. Gerbens-Leenes, A.Y. Hoekstra, R. Bosman, The blue and grey water footprint of construction materials: steel, cement and glass, Water Resour. Ind. 19 (2018) 1–12, https://doi.org/10.1016/j.wri.2017.11.002.
[29] A.S.M. Raja, A. Arputharaj, S. Saxena, P.G. Patil, Water requirement and sustainability of textile processing industries, in: S.S. Muthu (Ed.), Water in Textiles and Fashion, Consumption, Footprint, and Life Cycle Assessment, Woodhead Publishing, Kidlington, 2019, pp. 155–173, https://doi.org/10.1016/B978-0-08-102633-5.00009-9.
[30] L. Zhou, K. Xu, X. Cheng, Y. Xu, Q. Jia, Study on optimizing production scheduling for water-saving in textile dyeing industry, J. Clean. Prod. 141 (2017) 721–727, https://doi.org/10.1016/j.jclepro.2016.09.047.
[31] K. Valta, T. Kosanovic, D. Malamis, K. Moustakas, M. Loizidou, Overview of water usage and wastewater management in the food and beverage industry, Desalin. Water Treat. 53 (2015) 3335–3347, https://doi.org/10.1080/19443994.2014.934100.
[32] L. Pettigrew, V. Blomenhofer, S. Hubert, F. Groß, A. Delgado, Optimisation of water usage in a brewery clean-in-place system using reference nets, J. Clean. Prod. 87 (2015) 583–593, https://doi.org/10.1016/j.jclepro.2014.10.072.
[33] R.P. Taylor, C.L.W. Jones, M. Laing, J. Dames, The potential use of treated brewery effluent as a water and nutrient source in irrigated crop production, Water Resour. Ind. 19 (2018) 47–60, https://doi.org/10.1016/j.wri.2018.02.001.
[34] O. Frédéric, P. Yves, Pharmaceuticals in hospital wastewater: their ecotoxicity and contribution to the environmental hazard of the effluent, Chemosphere 115 (2014) 31–39, https://doi.org/10.1016/j.chemosphere.2014.01.016.
[35] T. Asfaw, Review on hospital wastewater as a source of emerging drug resistance pathogens, J. Res. Environ. Sci. Toxicol. 7 (2018) 47–52.
[36] B.I. Escher, R. Baumgartner, M. Koller, K. Treyer, J. Lienert, C.S. McArdell, Environmental toxicology and risk assessment of pharmaceuticals from hospital wastewater, Water Res. 45 (2011) 75–92, https://doi.org/10.1016/j.watres.2010.08.019.
[37] G.K. Paulus, L.M. Hornstra, N. Alygizakis, J. Slobodnik, N. Thomaidis, G. Medema, The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes, Int. J. Hyg Environ. Health 222 (2019) 635–644, https://doi.org/10.1016/j.ijheh.2019.01.004.
[38] F. Kaczala, S.E. Blum, The occurrence of veterinary pharmaceuticals in the environment: a review, Curr. Anal. Chem. 12 (2016) 169–182, https://doi.org/10.10217/2F1573411012666151009193108.
[39] V. Lazarova, T. Asano, A. Bahri, J. Anderson, Milestones in Water Reuse. The Best Success Stories, IWA Publishing, London, 2013.

[40] M. Yaqub, W. Lee, Zero-liquid discharge (ZLD) technology for resource recovery from wastewater: a review, Sci. Total Environ. 681 (2019) 551–563, https://doi.org/10.1016/j.scitotenv.2019.05.062.
[41] A.K. Rathoure, Zero liquid discharge treatment systems: prerequisite to industries, MOJ Eco, Environ. Sci. 5 (2020) 1–10.
[42] S. Gangavarapu, U.M.R. Ganguru, S. Kulkarni, N.M. Brahmandam, Studies on zero liquid discharge (ZLD) plant in API manufacturing unit, Int. J. Innov. Res. Eng. Manag. 2 (2015) 33–36.
[43] G.V. Reddy, T.R. Kiran, M.V. Reddy, Innovative zero liquid discharge based effluent treatment system for API industry cluster in India, Int. J. Waste Resour. 6 (2016) 70.
[44] M. Andrews, P. Berardo, D. Foster, The sustainable industrial water cycle - a review of the economics and approach, Water Supply 11 (2011) 67–77, https://doi.org/10.2166/ws.2011.010.
[45] O. Miarov, A. Tal, D. Avisar, A critical evaluation of comparative regulatory strategies for monitoring pharmaceuticals in recycled wastewater, J. Environ. Manag. 254 (2020) 109794, https://doi.org/10.1016/j.jenvman.2019.109794.
[46] I. Reinholds, O. Muter, I. Pugajeva, J. Rusko, I. Perkons, V. Bartkevics, Determination of pharmaceutical residues and assessment of their removal efficiency at the Daugavgriva municipal wastewater treatment plant in Riga, Latvia, Water, Sci. Technol. 75 (2017) 387–396, https://doi.org/10.2166/wst.2016.528.
[47] E. Strade, D. Kalnina, Cost effective method for toxicity screening of pharmaceutical wastewater containing inorganic salts and harmful organic compounds, Environ. Clim. Technol. 23 (2019) 52–63, https://doi.org/10.2478/rtuect-2019-0004.
[48] World Health Organization, WHO Good Manufacturing Practices: Water for Pharmaceutical Use, 2011. https://www.who.int/medicines/areas/quality_safety/quality_assurance/GMPWatePharmaceuticalUseTRS970Annex2.pdf?ua=1. accessed 25 January 2020.
[49] European Medicines Agency, Guideline on the Quality of Water for Pharmaceutical Use. Draft, 2018. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-water-pharmaceutical-use_en.pdf. accessed 20 January 2020.
[50] European Commission, The Rules Governing Medicinal Products in the European Union vol. 4, 2014. Good Manufacturing Practice. Medicinal Products for Human and Veterinary Use. Part II: Basic Requirements for Active Substances used as Starting Materials, https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2014-08_gmp_part1.pdf. accessed 5 January 2020.
[51] T. Sandle, Pharmaceutical Microbiology. Essentials for Quality Assurance and Quality Control, Woodhead Publishing, Cambridge, 2016.
[52] L. Jimenez, Microbial diversity in pharmaceutical product recalls and environments, PDA J. Pharm. Sci. Technol. 61 (2007) 383–399.
[53] European Commission, Integrated Pollution Prevention and Control (IPPC). Reference Document on the Application of Best Available Techniques to Industrial Cooling Systems, 2001. https://eippcb.jrc.ec.europa.eu/sites/default/files/2019-11/cvs_bref_1201.pdf. accessed 2 February 2020.
[54] A. Bhatia, Cooling Water Problems and Solutions: Quick Book, CreateSpace Independent Publishing Platform, South Carolina, 2015.
[55] J.G. Cleary, Water conservation and reuse case study in pharmaceutical industry, Proc. Water Environ. Fed. 13 (2006) 111–125.
[56] A. Fosco, Process Cooling as Part of a Sustainable Strategy, 2015, in: https://www.pharmamanufacturing.com/articles/2015/process-cooling-as-part-of-a-sustainable-strategy/. accessed 22 April 2020.
[57] J. Agalloco, F.J. Carleton, Validation of Pharmaceutical Processes, third ed., Informa Healthcare USA, Inc., New York, 2008.
[58] T. Latham, Clean Steam in the Pharmaceutical Industry, 2004. https://pdhonline.com/courses/k109/k109content.pdf. accessed 5 February 2020.
[59] D. Glawe, San Antonio Condensate Collection and Use Manual for Commercial Buildings. San Antonio: San Antonio Water System, 2013. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1022.9110&rep=rep1&type=pdf. accessed 5 December 2019.
[60] United Nations, The United Nations World Water Development Report 2015: Water for a Sustainable World, 2015. https://unesdoc.unesco.org/ark:/48223/pf0000231823. accessed 5 November 2019.
[61] L.K. Wang, Y.T. Hung, H.H. Lo, C. Yapijakis, Handbook of Industrial and Hazardous Wastes Treatment, second ed., Marcel Dekker, Inc., New York, 2004.
[62] A. Küster, N. Adler, Pharmaceuticals in the environment: scientific evidence of risks and its regulation, Philos. Trans. R. Soc. Lond. B Biol. Sci. 369 (2014) 20130587, https://doi.org/10.1098/rstb.2013.0587.
[63] M. Ågerstrand, C. Berg, B. Björlenius, M. Breitholtz, B. Brunström, J. Fick, L. Gunnarsson, D.G.J. Larsson, J.P. Sumpter, M. Tysklind, C. Rudén, Improving environmental risk assessment of human pharmaceuticals, Environ. Sci. Technol. 49 (2015) 5336–5345, https://doi.org/10.1021/acs.est.5b00302.
[64] European Commission, Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee, 2019. European Union Strategic Approach to Pharmaceuticals in the Environment, http://ec.europa.eu/environment/water/water-dangersub/pdf/strategic_approach_pharmaceuticals_env.PDF. accessed 5 January 2020.
[65] Eurostat, Freshwater Resources Per Inhabitant — Long-Term Annual Average, 2017. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Freshwater_resources_per_inhabitant_%E2%80%94_long-term_annual_average_(1_000_m%C2%B3_per_inhabitant).png&oldid=349063. accessed 25 January 2020.
[66] C. Baresel, L. Dalgren, M. Almemark, A. Lazic, Environmental performance of wastewater reuse systems: impact of system boundaries and external conditions, Water Sci. Technol. 73 (2016) 1387–1394, https://doi.org/10.2166/wst.2015.624.
[67] D.J.C. Constable, C. Jimenez-Gonzalez, R.K. Henderson, Perspective on solvent use in the pharmaceutical industry, org. Process Res. Dev. 22 (2007) 133–137, https://doi.org/10.1021/op060170h.
[68] T. Welton, Solvents and sustainable chemistry, Proc. R. Soc. A Math. Phys. Eng. Sci. 471 (2015) 20150502, https://doi.org/10.1098/rspa.2015.0502.
[69] F.P. Byrne, S. Jin, G. Paggiola, T.H.M. Petchey, J.H. Clark, T.J. Farmer, A.J. Hunt, C.R. McElroy, J. Sherwood, Tools and techniques for solvent selection: green solvent selection guides, Sustain. Chem. Process. 4 (2016) 7, https://doi.org/10.1186/s40508-016-0051-z.
[70] M.J. Raymond, C.S. Slater, M.J. Savelski, LCA approach to the analysis of solvent waste issues in the pharmaceutical industry, Green Chem. 12 (2010) 1826–1834, https://doi.org/10.1039/C003666H.
[71] M.L. Crawley, B.M. Trost, Applications of Transition Metal Catalysis in Drug Discovery and Development: an Industrial Perspective, John Wiley & Sons, Inc., Hoboken, 2012.
[72] M. Saidi, Experimental studies on effect of heavy metals presence in industrial wastewater on biological treatment, Int. J. Environ. Sci. 1 (2010) 666–676.
[73] J. Gawdzik, B. Gawdzik, Mobility of heavy metals in municipal sewage sludge from different throughput sewage treatment plants, Pol. J. Environ. Stud. 21 (2012) 1603–1611.
[74] Cabinet Regulation No 362, Republic of Latvia, Regulations Regarding Utilisation, Monitoring and Control of Sewage Sludge and the Compost Thereof, 2006. https://likumi.lv/ta/id/134653-noteikumi-par-notekudenu-dunu-un-to-komposta-izmantosanu-monitoringu-un-kontroli. accessed 22 April 2020.
[75] University of Alicante, Technology offer: Recovering/removing of heavy metals from waste water by electrochemical technology. https://stt.ua.es/es/empresa/documentos/ot-0737-recuperacion-eliminacion-metales-pesados-eng.pdf accessed 5 November 2019.
[76] N.R. Desireddy, A. Glory, K.R. Bhimireddy, Y. Kurra, R. Reddy, An efficient synthesis of milnacipran Hydrochloride via reductive amination of aldehyde, J. Chem. (2017) 5385843, https://doi.org/10.1155/2017/5385843, 2017.
[77] R.W. Gensemer, R.C. Playle, The bioavailability and toxicity of aluminum in aquatic environments, Crit. Rev. Environ. Sci. Technol. 29 (1999) 315–450, https://doi.org/10.1080/10643389991259245.
[78] B. Cebere, E. Faltina, N. Zelcans, D. Kalnina, Toxicity tests for ensuring successful industrial wastewater treatment plant operation, Environ. Clim. Technol. 3 (2009) 41–47.
[79] O.P. Sahu, P.K. Chaudhari, Review on chemical treatment of industrial waste, water, J. Appl. Sci. Environ. Manag. 17 (2013) 241–257.
[80] Y.M. Kim, H. Park, K.H. Cho, J.M. Park, Long term assessment of factors affecting nitrifying bacteria communities and N-removal in a full-scale biological process treating high strength hazardous wastewater, Bioresour. Technol. 134 (2013) 180–189, https://doi.org/10.1016/j.biortech.2013.02.036.
[81] H. Kroiss, H. Rechberger, L. Egle, Phosphorus in water quality and waste management, in: S. Kumar (Ed.), Integrated Waste Management – Volume II, InTech, Rijeka, 2011, pp. 181–214.

[82] Z. Zhao, S. Cheng, Z. Qin, K. Ma, X. Jin, S. Liang, Evaluation of microtoxicity and biodegradability of residual organic solvents in pharmaceutical wastewater by combined prediction-test system, Desalin. Water Treat. 57 (2016) 28187–28194, https://doi.org/10.1080/19443994.2016.1180264.
[83] G.M. Tardy, V. Bakos, A. Jobbágy, Conditions and technologies of biological wastewater treatment in Hungary, Water Sci. Technol. 65 (2012) 1676–1683, https://doi.org/10.2166/wst.2012.062.
[84] H.I. Abdel-Shafy, M.S. Mohamed-Mansour, Issue of pharmaceutical compounds in water and wastewater: sources, impact and elimination, Egypt, J. Chem. 56 (2013) 449–471.

6. pielikums
Appendix 6

Promocijas darbā pētīto farmācijas NAI raksturojums (pieejams pēc pieprasījuma).
A: latviski, B: angliski.
Characterization of pharmaceutical WWTP studied in the thesis (available under request).
A: latvian, B: english.

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