INTRODUCTION

Currently, the leading challenge for municipal wastewater treatment plants (WWTPs) staff is to ensure both a high-quality effluent wastewater and reduction of operating costs [Shahzad et al., 2015; Drewnowski et al., 2019; Masłoń et al., 2020]. In particular, large facilities must meet stringent requirements with regard to nitrogen and phosphorus for wastewater discharge to the environment [Mucha and Mikosz, 2021; Jaromin-Gleń et al., 2013; Barbusiński et al., 2020]. Therefore, advanced treatment technologies should be applied [Roots et al., 2020; Shourjeh et al., 2020; Czarnota et al., 2020]. It is noteworthy that WWTPs are modern facilities with high energy consumption, while the treatment process itself is multi-stage and complicated [Zubrowska-Sudol et al., 2018; Kudlek and Dudziak, 2018; Szeląg and Barbusinski 2020], so the additional method of monitoring and control are developed [Guz et al., 2015; Łagóð et al., 2019; Byliński et al., 2019]. WWTP employees have to deal with many operational problems such as variable influent composition, illegal wastewater discharges and frequent breakdowns [Bartkiewicz et al., 2016; Jaromin-Gleń et al., 2020]. Additionally, temporary unstable conditions at each stage of treatment are common [Fernando Morgan-Sagastume and Grant Allen, 2003]. Among them, biological processes are the most sensitive to the occurring changes [Babko et al., 2016]. At many WWTPs, the biological nutrient removal (BNR) is realized through a nitrification-denitrification processes [Regmi et al., 2014; Roots et al., 2019]. In the first step, ammonia is sequentially oxidized to nitrite and then into nitrate. Subsequently in denitrification process, nitrates are reduced to gaseous...
nitrogen. Processes of nitrification and denitrification involve different groups of microorganisms and require different process conditions [Majtacz et al., 2017; Mehrani et al., 2020; Al-Hazmi et al. 2021]. Their efficiency depends on many factors, including wastewater composition, the adopted technology as well as operational conditions [Metcalf and Eddy 2004; Alisawi, 2020; Majtacz et al., 2020].

The temperature is considered as a main parameter that can effect biological treatment [Metcalf and Eddy 2004]. Its fluctuation results from seasonal variations and industrial wastewater discharge. Typically, the temperature difference between inlet and outlet of WWTP is approx. 0.5 and 1°C. Thought, many facilities have to deal with permanent diurnal temperature fluctuations [Mańkinia et al., 2005]. Importantly, this parameter influences the reaction rates of most chemical and biological processes [Alisawi, 2020]. It also affects the fluid viscosity and dissolved oxygen levels as well as the settling properties of biomass. Particularly, the nitrification process is considerably susceptible to its fluctuations. Its optimal level should be varied between 28–32°C [Rodziewicz et al., 2019]. It should be noted that with its decline the nitrification rate significantly decreased [Henze et al., 2008]. Additionally, it is confirmed that at temperatures below 8–10°C an accumulation of nitrates in effluent is observed [Young et al., 2017, Kim et al., 2008]. In contrast, the inhibition of nitrification is found above the temperature of 50°C [Metcalf and Eddy 2004]. Moreover, sudden fluctuations in this parameter are also unfavorable. The WWTP operation at varying temperatures is especially challenging [Alisawi, 2020].

In this context, the problem of reject waters has become a matter of a concern at many WWTPs. This side-stream is generated during the sewage sludge processing. Although its flow is relatively small and approx. 1.5–3%. It can contribute about 30% of the ammonium nitrogen discharged to the main flow [Wett et al., 1998]. Moreover, it is characterized by unfavorable COD:N ratio for conventional nitrification/denitrification and increased temperature [Noutsopoulos et al., 2018, Tae Kim, 2020]. The nitrogen removal from reject water can be realized thought various methods including OLAND (oxygen-limited autotrophic nitrification-denitrification), CANON (Completely Autotrophic Nitrogen Removal Over Nitrite) and ANAMMOX (anaerobic ammonium oxidation) as well as SHARON (Single reactor system for High activity Ammonium Removal Over Nitrite)-ANAMMOX processes [Al-Hazmi et al., 2019; Kim I.T. et al., 2020; Shourjehi et al., 2021]. However, they require the construction of separate devices [Meyer and Wilderer, 2004]. Still, at many WWTPs, such side-streams are recycled to the bioreactors influent without separate treatment, often resulting in its temporary overloading [Podstawczyk et al., 2017]. Besides, it might contribute to increased costs of treatment related with aeration [Kwon et al., 2019; Suschka and Grubel, 2014]. On the other hand, many WWTPs cannot afford to build an additional object for the reject water treatment.

Bioaugmentation is a strategy that has been widely applied to biological reactors to support or to improve the process [Ji et al., 2020]. Therewith, the selected strains or mixed cultures are added to reactors to improve the catabolism of specific compounds. It is considered a promising technique to overcome many practical difficulties in WWTPs, as well as to enhance the removal efficiency [Herrero and Stuckeya, 2015; Grabas et al., 2016]. Importantly, compared to other techniques, it allows reacting to the changes that appear periodically. Therefore, it has considerable flexibility and is associated with lower investment and operating costs related e.g. with the construction of additional advanced devices at WWTP [Montusiewicz, 2014]. It has been applied to many WWTPs when the existing facilities become insufficient to treat the increased flow or load contained in wastewater [Ma et al., 2009]. It has proven effectiveness in supporting the activated sludge process (mainly nitrification) operated at low temperature [Plaza et al., 2001; Head and Oleszkiewicz, 2004].

Bioaugmentation has also been used to protect the structure and function of the activated sludge microbial community against a various harmful and toxic substances e.g. xenobiotics [Boon et al., 2003] and landfill leachate [Michalska et al., 2020]. Additionally, this technique has been adopted to improve the biodegradation of recalcitrant organic pollutants presented in municipal and industrial wastewater e.g. endocrinedisrupting compounds (EDCs), pharmaceutical [Boonorrh et al., 2018, Nizla et al., 2016], azo dye, [Kv N.S., 2021] pesticides, surfactants, and heavy metals [Almeida et al., 2017; Ji et al., 2019; Nguyen et al., 2019]. However, the utilization of bioaugmentation might be unsuccessful, it can be caused by improper selection
of microorganisms, application of insufficient ac-
climatization period, inadequate inoculum size as well as substrate availability [Herrero and Stuck-
eya, 2015; Lebiocka et al., 2018]. Therefore, there is still a need to conduct research in this area.

In this work the influence of bioaugmentation on the efficiency of reject water and municipal wastewater co-treatment under different temperature conditions was examined. For bioaugmentation, a solution of wild-living bacteria and Ar-
chaea from Yellowstone National Park (Archaea Solutions Inc.) was used.

MATERIALS AND METHODS

Lab-scale Sequencing Batch Reactor (SBR)

Experiment was conducted in two lab-scale SBRs with an active volume of 8 L and a diam-
eter of 0.25 m (Fig. 1). To keep the operating tem-
perature both reactors were placed in water bath. The reactors were equipped with a mechanical agitators, DO and pH probes, an air membrane diffusers, influent and effluent tanks. Moreover, the installation was monitored by control unit.

The SBRs were operated in two 12 hour cycles per day. Each one consisted of the subse-
quent repeated stages: supplying (0.5 h), reaction including mixing (2 h) and aeration (7 h), sedi-
mentation (1.5 h) discharging (0.5 h) as well as operational phase for sampling and additional technical services.

The reactor was inoculated with a mixed li-
quor from WWTP in Lublin. This sample was taken from the activated sludge reactor using a modified Bardenpho method. It was character-
ized by the following parameters: mixed liquor suspended solids (MLSS) 3.21 g/L, mixed liquor volatile suspended solids (MLVSS) 2.45 g/L and sludge retention time (SRT) of 14.9 days.

The sludge volume index (SVI) was 236 mL/g, while the food to microorganism ratio (F/M ratio) – 0.12 gBOD₅/g MLVSS·d [Szaja and Szulżyk-Cieplak, 2020].

Substrate characteristic

The wastewater used as influent for SBRs was taken from the effluent of primary sedimentation tank. In turn, the reject water used in the experiment was originated from dewatering belt press. At Lublin WWTP, the sewage sludge were treated involving the following devices: gravity-
and mechanical thickeners, anaerobic digesters (mesophilic conditions), belt presses and a ther-
mal drying unit.

The samples of wastewater and reject water were collected twice a week. Then these were immediately delivered to the laboratory, where they were kept at 4°C in a refrigerator. Before feeding the reactors, the samples were adjusted to reach the determined temperature; then, they were mixed in the assumed proportions by a low-
speed agitator. The characteristic of both samples is presented in Table 1.

![Figure 1. The laboratory SBRs utilized in the present study](image-url)
For bioaugmentation, the wild-living bacteria and Archaea from Yellowstone National Park, USA were applied (Archea Solutions Inc.). To SBRs it was added as a solution made of a solid substrate. Its production was realized in a specially constructed preparation unit operating in a continuous mode. The procedure of the mixture preparation was presented in the study performed by Lebiocka et al., 2018. Its composition was as follows: COD – 22.0 ± 1.0 mg/L, TSS – 6.0 ± 1.0 mg/L, TN – 75 ± 1.0 mg/L, N-NH₄ – 0.4 ± 0.02 mg/L, TP – 0.17 ± 0.03 mg/L, pH 7.16 [Szaja et al., 2018].

Experimental set-up

The start-up period for biomass adaptation lasted 2 weeks. After this time, the SBR A was supplied by the bioaugmentation product in amount of 0.25 L. The second reactor – SBR B was the control one. In this case, the bioaugmentation product was replaced by an analogous amount of distilled water to keep constant retention time. The acclimatization of biomass to the bioaugmentation product was achieved after 3 weeks. During this period, a constant temperature of 20±0.5°C was maintained in both SBRs. Subsequently, both reactors were fed with a mixture of wastewater and 13% v/v reject water (Table 2).

During the experiment, 5 phases with different temperature range were distinguished, each one lasted 14 d. The consecutive temperatures were investigated 20, 25, 30, 25 and 20°C. In this work, the effect of both the increase and decrease of this parameter was examined.

Analytical methods

The composition of wastewater and reject water was determined after their delivery to the laboratory. While the characteristic of seed sludge and bioaugmentation product was analyzed once. The following parameters were controlled: the total chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen (NH₄⁺–N), nitrate nitrogen (NO₃⁻–N) and nitrite nitrogen (NO₂⁻–N). These were made using Hach Lange UV–VIS DR 5000 (standard test cuvettes). The pH and DO values were monitored by a HQ 40D Hach-Lange multimeter (Hach, Loveland, CO, USA). Moreover, turbidity, TSS (total suspended solid) and VSS (volatile suspended solid) were monitored. Total and volatile suspended solids were measured on the basis of the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The average values are presented, while the differences were assumed to be statistically significant at p < 0.05.

RESULTS AND DISCUSSION

The composition of reject water mainly depends on the stage of sludge treatment. However, the type of applied devices and the adopted process conditions are also significant. The highest concentrations of ammonium nitrogen, total phosphorus and nitrogen as well as alkalinity are found in the samples originated after anaerobic digestion process. These wastewaters are characterized by higher pH level and increased temperature. It is noteworthy that its flow is lower than these generated in sludge thickening processes. The reject water from primary and waste thickening units contains a significant share of non-biodegradable organic matter fraction and increased solids content [van Loosdrecht and Salen, 2006; Noutsopoulos et al., 2018; Mucha and Mikosz, 2021]. As it was mentioned above, the reject water that supplied the SBR was collected from dewatering belt press (Table 1). As compared to the results presented in different studies, it indicates an increased content of NH₄⁺–N, while COD, TP as well as TSS were reduced [Noutsopoulos et al., 2018; Mucha and Mikosz, 2021].
The observed differences might have resulted from variable sewage sludge composition and adopted process conditions (Table 1). Moreover, the reject water share in the influent was considerable and it reached 13% v/v. Therefore, its contribution to SBR without pre-treatment might cause a process instability and deterioration of treatment efficiency [Kim et al., 2020].

Figures 2 and 3 present the average composition of effluents from the laboratory reactors.

In the first phase of experiment, the temperature of 20°C was maintained. Therein, the average concentration of NO$_3^-$– N in the effluent of bioaugmented reactor was higher as compared to control one (Fig. 2a). However, the observed differences were not statistically significant. In SBR A, it reached 18.04±1.99 mg/L, while in SBR B it was 16.06±0.59 mg/L. Additionally, in this case greater variations in obtained results were observed in the bioaugmented reactor.

A different tendency was found with regard to NO$_2^-$– N and NH$_4^+$-N (Fig. 2b, c). Importantly, significantly lower concentrations were observed in the bioaugmented reactor. For ammonia nitrogen the average value was 2.2±0.68 mg/L in SBR A, while in control (SBR B) it was 5.46±0.87 mg/L. With regard to NO$_2^-$– N, the average concentrations were 0.29±0.04 and 0.37±0.04 mg/L.

Figure 2. The average concentrations of a) nitrate nitrogen b) nitrite nitrogen c) ammonia nitrogen in the effluent of bioaugmented reactor (SBR A) and non-bioaugmented reactor (SBR B) (average data and standard deviations are presented)
in bioaugmented and non-bioaugmented SBR, respectively.

However, major removal efficiencies of \( \text{NH}_4^+ - \text{N} \) were achieved in both SBRs. In SBR A it was 96%, while in SBR B it was accounted of 86%. It should be noticed that in both reactors, at the beginning of the experiment, significant daily fluctuations in the \( \text{NH}_4^+ - \text{N} \) contents were found. However, in the case of bioaugmented SBR, this observation was noticed after 6th day of operation. It might be related with a change of the wastewater composition that supplied reactors that was
enriched with 13% v/v of reject water. In the start-up period, the SBRs were only fed by the wastewa-
ter taken from the effluent of primary sedimentation tank. The microorganisms needed an additional time for accli-
mization to enhanced NH\textsubscript{4}\textsuperscript{+}– N content discharged to influent. Importantly, in the bioaugmented SBR, the growth of this parameter was observed with a delay. This fact might indicate a protective effect of microorganisms from the Archaea domain on the activated sludge process. It has been confirmed that Archaea play a critical role in nitrification [You et al., 2009]. They showed a considerable resistance under extreme environmental conditions e.g. low/high temperature and low oxygen level [Yin et al., 2018]. They occurred in various environments such as deep ocean, thermal springs, marine and fresh waters, soils and wastewater treatment systems [Liu et al., 2017]. The adaptation ability of Archaea to temperature changes is related with the special structure of glycerol ether in the cell membrane [Yin et al., 2018]. At the existing WWTPs, this effect might allow the operator to quickly counteract or limit the negative effects e.g. to supply of highly concentrated wastewater or illegal discharges.

As is shown in figure 3a, at 20°C the COD concentrations in bioaugmented and non-bioaug-
mented reactor were comparable, in both SBRs the average value was approx. 32 mg/L.

Additionally, in the bioaugmented reactor the lower values of TSS and turbidity were found; however, the observed differences were no of statistical significance (Fig. 3 c, d). The average TSS content reached 4.5 and 5.36 mg/L in bio-
augmented and non-bioaugmented SBR, respectively. In turn, the averages values of turbidity were 3.3±0.85 and 4.32±0.87NTU in SBR A and SBR B, respectively. A significantly increased pH 8.06±0.01 was noticed in SBR A. In turn, in non-bioaugmented reactor it was pH 8.02±0.01 (Fig. 3 b).

In the following phase, the temperature was increased to a level of 25°C. This change resulted in a reduction of NH\textsubscript{4}\textsuperscript{+}–N concentration in the effluent for both SBRs as compared to the previous stage. However, as previously, in bioaugmented SBR A, statistically lower results were obtained. The average concentrations were 0.25±0.06 and 1.43±0.25 mg/L in bioaugmented reactor and control, respectively. As before, notable removal efficiencies were achieved, in SBR A it was 98%, while in SBR B – 95%. In this stage, after 14 d of operation, –a stabilized concentrations in the effluent of both SBRs were obtained. In the case of NO\textsubscript{3}– N comparable results were found in both SBRs. The average content was 0.27±0.04 and 0.32±0.02 mg/L in SBR A and control SBR, respectively. Regarding NO\textsubscript{3}– N, significantly increased concentrations were found in the non-bioaugmented reactor. Therein, this parameter was established on the level of 23.4±
2.36 mg/L. While, in the bioaugmented SBR A it was 17.0±1.43 mg/L.

It is noteworthy that the average concentration of COD was significantly lower in SBA A, it reached 22.8 ±1.4 mg/L. In turn, in SBR B it was 25.9±1.26 mg/L. However, only up to 6 d more favorable results were obtained in the bioaug-
mented reactor. Compared to the previous phase, lower pH values were obtained for SBR A and SBR B. For both, similar results were obtained, amounting to pH 7.9.

The highest daily variability was shown in the case of TSS. Moreover, the observed differences between reactors were not statistically significant. The average values were 5.29±1.75 and 6.14±2.3 mg/L in SBR A and SBR B, respectively. Regarding turbidity, in bioaugmented reactor, a significantly lower value of 2.61±0.59 NTU was found. In the non-bioaugmented one, it was 3.71±0.4 NTU.

In the 3rd stage, the temperature was increased by further 5°C. At 30°C, greater concentrations of NO\textsubscript{3}–N, NH\textsubscript{4}\textsuperscript{+}–N and COD were observed than in the previous phase. Despite this fact, it should be noticed that the effluent concentrations in the bioaugmented reactor were lower than in the non-bioaugmented one. This might also indicate a beneficial effect of Archaea on the reactor performance under increased temperature.

The average values of NH\textsubscript{4}+–N were 0.73±0.15 and 1.5±1.0 mg/L in SBR A and control SBR, re-
spectively. It is noteworthy that a notable removal efficiency compared to previous phases was still achieved. In SBR A it was 96%, while in SBR B – 89%.

Regarding COD, comparable results were achieved in the bioaugmented and non-bioaug-
mented reactors. In both SBRs, it was approx. 29 mg/L. However, increased concentrations were obtained in comparison to the previous phase. A similar tendency was found for NO\textsubscript{3}– N. In this case, in both SBRs the average concentration was approx. 14 mg/L. However, the obtained results were lower than in the previous stages.
Importantly, the rise of temperature to 30°C caused agrowth in the NO$_2^-$ – N content. In this phase, the highest values were achieved throughout the experiment. The obtained results were 0.54±0.1 and 0.7±0.09 mg/L. During this phase, the pH and TSS values varied significantly. Nevertheless, considering the mean values, comparable results were obtained in both SBRs. It was pH 7.94±0.07 and pH 8.0±0.08 in the bioaugmented and non-bioaugmented reactor, respectively. In turn, TSS was 5.1±0.95 and 6.33±1.85 mg/L in the bioaugmented SBR and control SBR, respectively. Regarding turbidity, similar results in both SBRs were obtained. This parameter reached approx. 2.5 NTU.

In the following phase, the temperature was reduced to 25°C. In this stage, the NO$_2^-$ –N concentrations returned to the level noted for 20 and 25°C. The similar average value of 0.25 mg/L was found in both SBRs. Regarding the NH$_4^+$ – N content, comparable results to the previous phase were found in the bioaugmented reactor with the average concentration of 0.55±0.06 mg/L. However, in the case of the non-bioaugmented one, statistically higher values were observed. In SBR B, the change in temperature contributed to an increase in this parameter as compared to the previous stage. The average concentration was 1.54±0.03 mg/L. In relation to temperature of 30°C, a growth in NO$_2^-$ – N content was noted, greater for the non-bioaugmented SBR. The average values were 16.48±0.53 and 20.85±1.59 mg/L in bioaugmented SBR and control one, respectively.

Considering COD, a reduction of this parameter was found in comparison to the prior temperature conditions. In SBR A and B, comparable average concentrations were achieved. In the bioaugmented reactor, this parameter was at the level of 25.9±1.92 mg/L, while in the non-bioaugmented one it was 26.9±0.53 mg/L. Similarly, there were no significant differences in pH between reactors. In both SBRs, it was approx. pH 7.95. An analogous tendency was observed with regard to turbidity. Therein, the average values were 1.62±0.42 and 1.75±0.35 mg/L in SBR A and SBR B, respectively. Moreover, at 25°C lower values were found, as compared to 30°C. In turn, average TSS reached a level of 4.0±0.18 and 4.3±0.97 mg/L in SBR A and SBR B, respectively. However, in the case of non-bioaugmented reactor, a greater daily variation of results was observed.

In the last phase, the temperature returned to the initial conditions. As compared to the previous stage, the NH$_4^+$ – N content has not changed significantly, similar values were observed. In SBR A, it was 0.44±0.03, while in SBR B – 1.41±0.03 mg/L. A similar tendency was found also for COD. Therein, the average concentrations were 24.9±1.43 and 26.9±1.67 mg/L in the bioaugmented and control SBR, respectively. Regarding NO$_2^-$ – N, the lowest concentrations in the experiment were observed. The mean value of 0.09 mg/L in both SBRs was obtained. In the case of NO$_3^-$ – N the increased concentrations in comparison to the previous phases were achieved. In both SBRs, the average content of this parameter was approx. 23 mg/L. Additionally, at temperature of 20°C the pH did not differ in both reactors, with the average value of pH 7.9. Turbidity in SBR A was increased, as compared to SBR B. However, the observed differences were of no statistical significance. This parameter reached a level of 2.5±0.69 and 2.0±0.24 NTU in the bioaugmented and control SBR B, respectively.

The greatest fluctuations in this phase were noticed for TSS. The average concentration of 2.95±0.62 and 4.86±0.93 was found in SBR A and SBR B, respectively.

The obtained results indicated that both reactors showed significant removal efficiencies of all analyzed parameters, especially at a temperature of 20 and 25°C. It is noteworthy that more favorable results were found in the bioaugmented system. This might be related with an increased oxygen utilization rate, as well as its beneficial effect on microbial metabolism in activated sludge system [You et al., 2009; Gray et al., 2002; Lens at al., 1995].

Compared to other studies in the present research, significant removal efficiencies were achieved. However, in many of them reject water constituted the main substrate. In the study conducted by Kim et al. (2020), NH$_4^+$ – N was reduced to 59%, while the highest concentration of 877.3 mg/L was found in the reactor influent as compared to the presented results. In fluidized-bed reactor inoculated with Anammox sludge, the ammonium removal efficiency reached a significant value of 82% [Strous et al., 1997]. Favorable results are also obtained with the use of adsorption/ion exchange processes involving zeolites [Sperczyńska, 2016]. Therein, this parameter varied between 80–90%.
However, the implementation of bioaugmentation with Archaea to an existing SBR might constitute a cost effective solution for reject water co-treatment. Compared to the methods described above, it does not require the construction of additional objects. Moreover, this strategy might support the SBR performance under the influence of various negative factors such as temperature fluctuation.

CONCLUSIONS

In the bioaugmented reactor (SBR A) lower concentrations of NH$_4^+$ – N, TSS, NO$_3^-$–N in effluent were found, in relation to the non-bioaugmented – SBR B. Importantly, in the case of the NH$_4^+$ – N content, the observed differences were statistically significant. In turn, the concentrations of COD and NO$_3^-$ – N as well as turbidity and pH reached comparable levels. The greatest variations in the obtained results were found in the case of TSS in both SBRs. However, also in this case the lowest results were found in the bioaugmented reactor. The exception was the last phase with temperature of 20°C. Importantly, in both SBRs, the process was carried out in a stable way. The obtained results indicate the possibility of using this strategy in the reactors exposed to unfavorable conditions, e.g. variable temperature.

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