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ORIGINAL PAPER

Heterogeneity and spatial distribution of bacterial background contamination in pulp and process water of a paper mill

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Abstract Identifying the source and the distribution of bacterial contaminant communities in water circuits of industrial applications is critical even when the process may not show signs of acute biofouling. The endemic contamination of facilities can cause adverse effects on process runability but may be masked by the observed daily variability. The distribution of background communities of bacterial contaminants may therefore be critical in the development of new site-specific antifouling strategies. In a paper mill as one example for a full-scale production process, bacterial contaminants in process water and pulp suspensions were mapped using molecular fingerprints at representative locations throughout the plant. These ecological data were analyzed in the process–engineering context of pulp and water flow in the facilities. Dispersal limits within the plant environment led to the presence of distinct groups of contaminant communities in the primary units of the plant, despite high flows of water and paper pulp between units. In the paper machine circuit, community profiles were more homogeneous than in the other primary units. The variability between sampled communities in each primary unit was used to identify a possible point source of microbial contamination, in this case a storage silo for reused pulp. Part of the contamination problem in the paper mill is likely related to indirect effects of microbial activity under the local conditions in the silo rather than to the direct presence of accumulated microbial biomass.

Keywords Bacterial community analysis · Contamination · Dispersal · Storage silo · Paper mill

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Introduction

The discharge of used water from modern paper mills has continuously decreased over the last decades because of environmental concerns [1, 3]. In order to maintain the necessary flow rates of process water, water reuse has increased at the cost of spreading microbially contaminated process water throughout the facilities, leading to increased fouling of equipment and the deterioration of end products. Minimizing the contamination of production facilities has therefore been a major objective in papermaking. Also, manufacturers in the food, chemical, or oil industries pay substantial amounts of money for controlling biofouling and microbial contamination. In paper mills, these amounts are justified by achieving more stable operating conditions (e.g., pH and reduction–oxidation potential, dissolved organic carbon concentrations) while minimizing the use of additives (e.g., hydrogen peroxide, caustic soda).

In modern paper mills, the continuous presence of a microbial contamination in the process water directly leads to visible slime formation on air-exposed surfaces receiving aerosols and projections from the paper machine. Less visible but equally severe indirect effects are caused by the degradation of paper additives and the unfavorable alteration of pulp properties [3]. For example, released organic acids from fermentation reactions may dissolve the common additive calcium carbonate and increase the ionic strength in the water phase. Among other problems, this

results in the coagulation of pulp constituents, which in turn causes runability problems in the paper machine [9, 21]. Paper mills with successful antifouling procedures show enhanced deinking of recovered paper and improved runability of the paper machine, ultimately leading to higher product quality and reduced operating costs (e.g., [6, 8]). Even paper mills without explicit symptoms of severe biofouling likely contain an endemic contamination flora whose negative effects are masked by the routinely observed variability in runability. Reducing this endemic source of contamination may thus improve process performance even in production facilities that are not thought of as especially affected by biofouling. For the development of efficient strategies to reduce the endemic contamination of water in production circuits, knowledge of how a contamination is spread in the facilities is required. If a microbial contamination results from a point source, a different treatment strategy is necessary than for diffuse sources. Once a microbial contamination is dispersed, it is beneficial to know in what parts of the plant growth of community members is actually supported and whether biofouling is directly caused by the presence of microbial biomass in the water (e.g., slime formation) or by indirect effects (e.g., spoilage or degradation of additives).

Various contamination scenarios are plausible hypotheses for explaining hidden biofouling in production facilities. A possible point source of a microbial contamination could be brought on site with raw materials, e.g., recovered paper in a paper mill [20] and may consequently be spread in process water and pulp suspensions throughout the plant. A microbial contamination may also be endemic when water is extensively reused in order to minimize the environmental impact of the process. In paper mills, endemic contaminations are common when plants operate with zero or low water discharge. Under these conditions, process water and excess pulp are reused within the system for prolonged periods and continuously maintain a diffuse source of a suspended seed community [4]. Another diffuse source of microbial contamination may be the exposure of process water to biofilms in ducts and pipelines that remain in place even after thorough physical cleaning of the facilities. Bacterial communities in natural environments or industrial facilities, like ultra-clean water supplies of power plants [5], water-desalination plants [10] or in this case a paper mill, can be followed temporally or spatially by the comparison of molecular fingerprints obtained from DNA extracts. With these fingerprints, unit operations or larger parts of the plant can be classified based on similarities of the contaminant community.

The goal of this study is to identify the pattern of a bacterial contamination in a paper mill without obvious biofouling problems. Contaminant communities are determined at various points along the flow of process water and pulp suspensions. We demonstrate how distribution of ecological data is put to use in the process–engineering context of plant operation for the identification of the masked contamination pattern, dispersal limits, and possible sources of the contamination. The experimental and analytical approach is applied to a paper mill as a model for a full-scale industrial process. The approach can be applied equally well to other industries, e.g., the oil, chemical, drinking water, or food-production. Our findings may justify the development of new site-specific antifouling strategies in the paper mill.

Materials and methods

Layout of the sampling site

In Fig. 1, a simplified layout is presented of the paper mill Norske Skog Golbey in Golbey, France. The paper mill produces newsprint paper from a mixture of deinked recovered paper and a smaller portion of thermo-mechanical pulp from fresh wood. The plant layout can be roughly divided into three sections: (1) the deinking pulp circuit (DIP), where incoming recovered paper is processed to paper pulp, (2) the thermo-mechanical pulp (TMP) preparation, where fresh wood is processed to TMP pulp, and (3) the paper machine circuit, where pulp is converted to newsprint paper. The DIP is further subdivided into the alkaline and neutral DIP loops. In this manuscript, the two DIP loops and the paper machine circuit are termed the three primary units of the paper machine. The TMP circuit, with the exception of the junction to the paper machine circuit, was disregarded in this work. Pulp storage silos connect DIP and TMP with the paper machine circuit. Pulp suspensions from the DIP and TMP circuits are blended with reused paper pulp from within the paper machine circuit and sent towards the paper machine. Reused pulp is a mixture of excess pulp from the paper machine and paper waste from cutting and trimming the final product. In the language of papermaking, this internally reused pulp is called the “paper machine broke” and is not to be confused with pulp made from recovered paper in the DIP or fresh wood in the TMP. Before blending, the reused pulp is temporarily stored in a 3,000-m^3 silo. Net flows of water and pulp in the paper mill are in opposite directions. The largest loss of water occurs in the DIP circuit with the disposal of deinked sludge from processing the recovered paper. Water in the DIP is constantly replenished from the paper machine circuit while pulp is transported from the DIP towards the paper machine circuit.

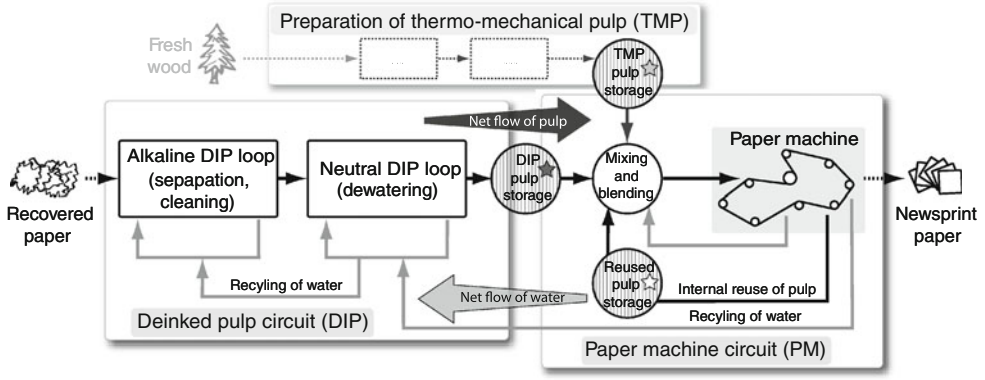

Fig. 1 Simplified flow chart of the paper mill. Paper pulp is prepared from recovered paper in the deinked pulp circuit (DIP) and from wood fiber in the thermo-mechanical pulp circuit (TMP). The two kinds of paper pulp enter the paper machine circuit after temporary storage in the respective silos. From the thermo-mechanical pulp circuit, only samples from the storage silo are further considered. The deinked pulp circuit is subdivided into two functionally distinct primary units: the alkaline loop (pH = 8.3 ± 0.4 standard deviation (SD)) and the neutral loop (pH = 7.5 ± 0.2 SD). Water is recirculation within and between the loops. The paper machine circuit is slightly more acidic (pH = 7.0 ± 0.4 SD) and contains a silo where reused pulp is temporarily stored. The *star symbols* are introduced in the figure to identify samples from these silos in subsequent plots

Sampling

Seventeen pulp samples and ten water samples were taken from a sequence of sampling points in the paper factory during a measuring campaign on June 16 2009. For all but one pulp sample, approximately 10 g of pulp material was taken directly from the production line through existing sampling ports after wasting a substantial amount of possibly aged pulp in the port. The ports are normally used for quality control of the paper pulp. For the remaining pulp sample, a comparably large sample of 100 ml pulp material was taken to test the heterogeneity of the material. Water samples had a size of at least 10 ml and were taken directly from the water circuit through sampling ports when possible. Some samples were grab samples directly from an open water surface. Samples were immediately transported to the laboratory, aliquoted in portions of 500 mg for pulp samples and 500 µl for liquid samples, and kept at −20 °C in 2-ml screw-cap vials until further processing. The sampling points included all major unit processes of the plant and thus represented the sequence of necessary steps in the functioning of the paper mill. Representing each sampling location in Fig. 1 would require an additional amount of detail that in turn would defeat the purpose of the figure as a schematic overview of the paper mill. At each sampling location for water and pulp, also pH, conductivity, chemical oxygen demand (COD), temperature, and reduction–oxidation (redox) potential among other parameters were measured at the time of sampling. Physico-chemical measurements were taken using a WTW Photolab S12 micro-kit and a portable WTW 340i multiline F/7–2 (WTW, Alès, France). Before and during the sampling campaign, monochloramine was automatically added as several pulse injections per hour at two points in the alkaline loop of the deinked pulp circuit and at one point in the paper machine circuit using the proprietary Bromide-Activated Chloramine procedure (Ashland Hercules Water Technologies, Hercules SA, France).

DNA extraction and PCR and CE-SSCP

DNA was extracted from the aliquoted samples using the procedure by Rochex et al. [18]. The extraction protocol consists of bead-beating and a heat-treatment step and is especially suited for samples containing high levels of impurities. PCR primers specific to the V3 region of the bacterial 16S rDNA molecule were used in a 50-µl PCR reaction with 36.9 µl molecular grade water, 5 µl of 10 × pfu turbo buffer, 4 µl of 2.5 mM dNTP mixture, 1.3 µl of forward primer (426 nM) and reverse primer (412 nM), 0.5 µl of 2.5 U/µl Stratagene PfuTurbo DNA Polymerase (Stratagene, La Jolla, CA, USA). Initial denaturation was done for 2 min at 94 °C; melting for 1 min at 94 °C, annealing at 61 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min after 25 cycles. The (5′–3′) sequences for the forward- and reverse-primers are ACGGTCCAGACTCCTACGGG (*Escherichia coli* position F331) and TTACCGCGGCTGCTGGCAC (*E. coli* position R500), respectively [18]. The bacterial communities of the PCR-amplified DNA samples were analyzed using capillary electrophoresis single-strand conformation polymorphisms (CE-SSCP) under conditions described in [18]. In the CE-SSCP profiles, a relative fluorescence signal greater than zero can be interpreted as the presence of a distinct member of the bacterial community. Conceptually, the interpretation of CE-SSCP

profiles is similar to the interpretation of banding patterns in DGGE gels. For each sample, at least two but up to six independent PCR and SSCP analyses were done, in several cases also with repeated DNA extractions from multiple aliquots. To test the reproducibility of the system, the heterogeneity in pulp samples and a possible sample size-effect, six replicate DNA extractions were done from one relatively large sample of 100 ml of pulp material. The extractions were done on an initial pulp mass of approximately 200, 300, 400, 500, 600, and 700 mg. We did not find significant variability within the replicates, indicating that heterogeneity in the tested range of sample sizes within 100 ml of pulp material was negligible. Reproducibility was considered sufficiently high and heterogeneity low so that it was appropriate to average independent CE-SSCP profiles from one sample to yield one average community fingerprint per sampling location. These averaged profiles were used for the analyses.

Analysis of contaminant communities and physico-chemical parameters

All data analyses were done in the software environment R (version 2.10.1, [16]) using functions from the package "vegan" [13], unless otherwise noted. Community data as CE-SSCP profiles were aligned and normalized with the package "StatFingerprints" [11]. Frequently, community fingerprints are dominated by a small number of operational taxonomic units compared to the theoretical number of possible community members that could be resolved by any given method. Shared absence of operational taxonomic units in two community profiles were disregarded in the analysis by using principal coordinate analysis (PCoA) (function cmdscale from package "stats") with a quantitative variant of Jaccard distance matrices (function vegdist) as suggested by Oksanen in [12]. PCoA is a method to present multivariate data in a reduced number of dimensions. In this kind of analysis, the maximum possible amount of variability between the data points is conserved. In the PCoA plot, CE-SSCP profiles from similar communities appear close to each other while the longest distances between points indicate the most distantly related contaminant communities. Scaled physico-chemical data were visualized using principal component analysis (PCA). Principal component analysis is a method to reduce the number of dimensions of a multivariate dataset like environmental data. The R base-package function prcomp was used for PCA. A Euclidean distance matrix was used for calculating multiple pairwise distances between environmental data points. A quantitative variant of Jaccard distances and Euclidean distance matrices for the respective data sets were used for Mantel tests (function mantel) to test the correlation between physico-chemical and community data. Alternatively, ordinations of community and physico-chemical data were compared using Procrustes superimposition tests [15] as implemented in function protest. The qualitative variable "plant layout" with the three primary units as states were fitted using a linear model to the environmental and community data sets with the function envfit. The significance of differences in variability of community fingerprints and environmental data were tested using Student's t test on mean Jaccard distances for communities and Euclidean distances on scaled environmental data.

Results

Occurrence of bacterial contaminants follows plant layout

Bacterial contaminant communities in all samples grouped by the three physically separated primary units in the paper mill. This separation is visualized by the relative positions of bacterial community fingerprints (e.g., CE-SSCP profiles as in Fig. 2a) in the principal coordinate analysis plot in Fig. 2b. The correlation between the qualitative variable "primary unit" and the position of bacterial communities in Fig. 2b underlines this observation ($R^2 = 0.78, p < 0.0001$). Bacterial communities are thus specific to primary units of the paper mill despite high volume flow of pulp and water between the units as indicated in Fig. 1. The data from five CE-SSCP profiles in Fig. 2a were highlighted for illustration purposes in Fig. 2b by the letters A–E. The fingerprint from the paper machine circuit annotated with '1' in Fig. 2b was determined in an unusual environment for the PM, as it is not in direct contact with the paper pulp. It is not surprising that this point showed no similarity with the other PM communities but was more related to one other pulp-free aqueous sample from the alkaline DIP marked as '2'. Samples '1' and '2' were considered outliers because of their unusual nature and were consequently disregarded in the following analyses.

Differences between contaminant communities at the exits of storage silos

Special attention was paid to the bacterial contaminants leaving storage silos (silos highlighted in Fig. 1). Samples from the three main silos (i.e., DIP pulp storage, TMP pulp storage, reused-pulp storage) were emphasized by star symbols in Fig. 2b. Even though DIP and TMP pulp are produced in different manners, the community profiles at the exit of their respective storage silos are comparably closely related to each other but not clearly a part of any of the three primary units. These two silos mark the junction between

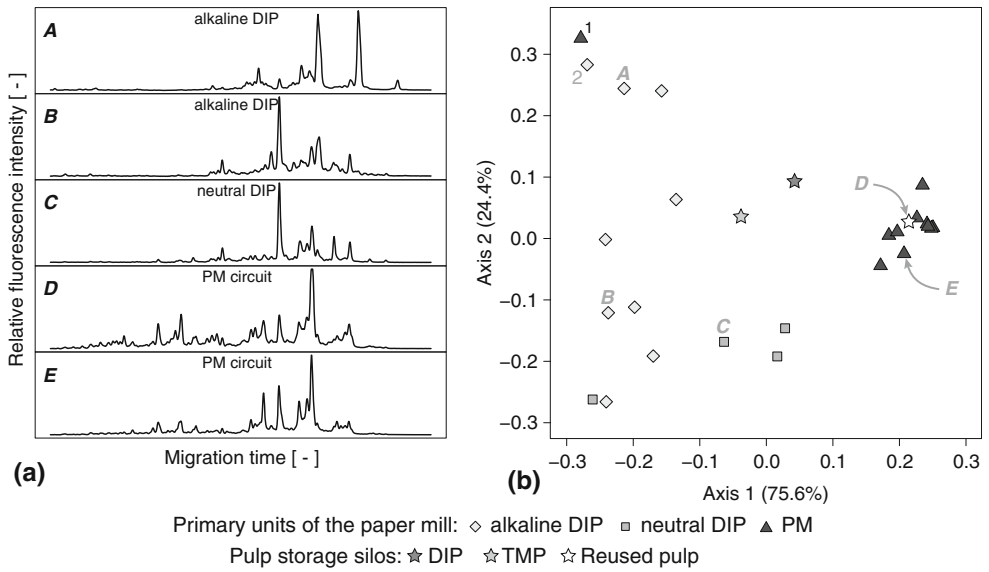

Fig. 2 **a** Five exemplary bacterial community fingerprints determined in pulp and water samples from the three primary units of the plant (alkaline and neutral DIP, paper machine circuit). **b** Community fingerprints represented in a two-dimensional PCoA plot. The difference between community fingerprints is proportional to the distance between points in the plot. *Gray levels* and symbol types were used to differentiate the locations of the samples. Samples from the three storage silos were emphasized with the same *star symbols* as in Fig. 1. *Letters* refer to the profiles in panel (**a**). The samples annotated with *1* and *2* were removed from subsequent analyses (see text)

primary units (pulp preparation and the paper machine circuit). In contrast, the content of the storage silo for reused pulp was from a community perspective not different from the contaminants dominating the paper machine circuit. While DIP and TMP silos bridge units and only freshly prepared pulp is pumped once through, the reused-pulp silo is physically an integral part of the paper machine circuit, continuously receiving already-reused pulp.

Physico-chemical environment correlates with plant layout

In the analysis of the physico-chemical data associated with each sample, a similar grouping of samples was obtained as for the contaminant communities. The various physico-chemical parameters were measured in situ at all sampling locations or in the lab from grab samples. We compared the multidimensional data set of the environmental data in a similar manner as the community fingerprints but here using principal component analysis (PCA). As in PCoA for the bacterial community analysis, also in PCA plots the distance between points indicates the similarity of measured values per sampling location. In Fig. 3, we presented the first two principal components of the environmental data, expressing roughly 83 % of their variability. The third principal component contributes more than 13 % of the variability but does not largely influence the separation of points by primary unit (data not presented).

As expected from the rationale of paper production, the highest chemical oxygen demand (COD), conductivities,

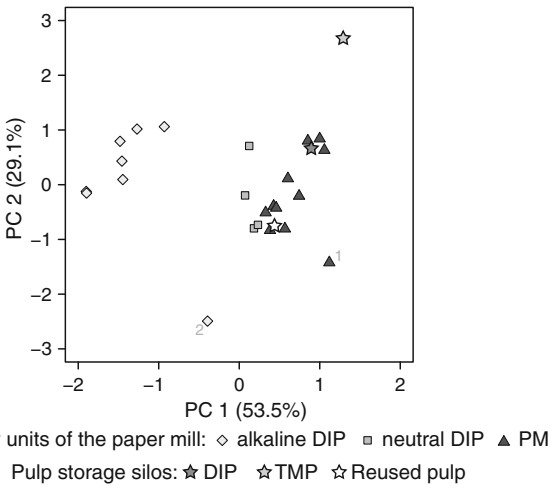

Fig. 3 First two principal components of the analysis of environmental variables pH, conductivity, chemical oxygen demand (COD), temperature, and reduction–oxidation potential at all sampling locations for water and paper pulp. *Gray levels* and symbol types were used to differentiate samples from the three primary units of the plant (alkaline and neutral DIP, PM) and the storage silos for DIP, TMP, and reused pulp

and pH values were measured in the alkaline part of the DIP. These three variables dropped towards the neutral part of the DIP and separate the points in x-direction in Fig. 3. The lowest pH and COD values were found in the paper machine circuit. Temperature and redox have the strongest influence on the separation in *y*-direction. The correlation between the qualitative variable "primary units" (alkaline DIP, neutral DIP, and paper machine circuit) and the first

two principal components of the environmental data is highly significant ($p < 0.001$) but with $R^2 = 0.58$, weaker than for the contaminant data. The data indicate that spatially separated environments exist in the paper mill. Of course, these findings did not come as a surprise as different environmental conditions are the prerequisite for the various steps in papermaking. However, it must be emphasized that these differential environmental conditions were maintained despite high flow rates of water and pulp, within and between primary units (up to 90 m^3 min^{-1} in parts of the plant).

Contaminant communities and physico-chemical data are correlated

The physico-chemical environment may be able to sufficiently explain the distribution of bacterial communities along the production process. Therefore, the correlation between the occurrence of bacterial communities and the environmental data was explicitly tested using permutation-based versions of Mantel and Procrustes superimposition tests. Both testing procedures returned highly significant correlations ($p = 0.001$) between the environmental and community data with $R^2 = 0.58$ and $R^2 = 0.52$, respectively. The relatively strong correlations according to the two tests emphasized once more the link between the environmental data and the bacterial communities within each primary unit and at the same time stressed the disconnection between the primary units. At the same time, a high portion of the distribution of the bacterial contaminant communities cannot be explained by the measured environmental data alone.

Higher variability in contaminant communities than in physico-chemical data

The unexplained difference between environment and bacterial communities was likely caused by the different degrees of heterogeneity of the bacterial communities in the primary units compared to the environmental conditions in the units. Communities in the paper machine circuit were the most homogeneous. Their variability was significantly lower than in the other two primary units (Tables 1, 2). The variability of communities in the alkaline DIP was highest. In contrast, the variability of physico-chemical data could not be differentiated between primary units: p values much larger than 0.05 for the environmental data indicate that the seemingly different mean distances in Table 1 were likely caused by chance and that the measured mean distances cannot be differentiated from each other (Table 2). While environmental conditions are equally variable throughout the paper mill, the variability of bacterial communities differs between the primary units.

Table 1 Variability of community fingerprints and environmental data in each primary unit of the paper mill

Circuit	Community fingerprints		Environmental data	
	Mean ± SD	*n*	Mean ± SD	*n*
Alkaline DIP	0.50 ± 0.11	21	1.83 ± 0.70	21
Neutral DIP	0.39 ± 0.10	6	1.38 ± 0.77	6
Paper machine circuit (PM)	0.27 ± 0.09	55	1.54 ± 0.71	55

Variability was expressed as mean pairwise distances between all samples taken within each primary unit of the paper mill. Data in the table were used for significance tests in Table 2

Table 2 p values indicating the significance of calculated differences in mean variability of community fingerprints (*italics, lower triangle*) and environmental data (*upright, upper triangle*) between the three primary units of the paper mill (Table 1)

	Alkaline DIP	Neutral DIP	Paper machine circuit
Alkaline DIP		0.24	0.12
Neutral DIP	*<0.04**		0.64
Paper machine circuit	*<0.001**	*<0.03**	

p values for comparing between community fingerprints and environmental data sets primary units

* p values for significant comparisons at the level of 0.05

Discussion

During our sampling campaign, the paper mill Norske Skog Golbey did not show indications of severe or unexpected biofouling and was operated under normal conditions. Selecting a sampling site without obvious biofouling problems may appear counterintuitive. Despite the absence of acute and severe biofouling, an industrial operation of this size is continuously facing a background of microbial contamination. This contamination, even though it may not be classified as a severe case of biofouling, may nevertheless influence process runability or the quality of end products. It was our objective to identify the distribution of this background contamination in the paper mill and possibly suggest steps to reduce the effects of inevitably present bacterial communities. A community ecological approach using bacterial community fingerprinting was favored for the identification of distribution patterns. The phylogenetic or taxonomic identification of individual populations may be an interesting approach in a more detailed study.

Homogenized versus variable contaminant communities in primary units

Considering the large volume flows of microbially contaminated water from the paper machine circuit towards the

pulp preparation and the counterflow of pulp material as indicated in Fig. 1, a homogenization of bacterial contaminant communities in pulp and water samples throughout the production facility could be predicted. The data that is presented in Fig. 2 did not support the hypothesis of a homogenized contamination. Instead, the emerging contamination pattern followed the physical layout of the plant: bacterial contaminations were specific to each primary unit of the paper mill. A more differentiated picture evolved when scrutinizing each primary unit independently. Largely homogenized contaminant communities could be observed within the paper machine circuit while a larger variability of contaminant communities was found in the other two primary units (Tables 1, 2). During the passage of the alkaline DIP, it appeared that bacterial communities were able to differentiate or maintain an initial difference while in the paper machine circuit homogenization prevailed. Also, the physico-chemical properties at each sampling site reflected the division of the paper mill into its three primary units. The correlation of site-specific contaminant communities with the physico-chemistry of the sample may imply that the environmental conditions in each primary unit shape the microbial contamination. If this hypothesis was fully supported by the data, we would expect that the variability of the contaminant communities was reflected in the heterogeneity of the physico-chemical environment. In contrast to the community data, the degree of variability of the physico-chemical parameters in all primary units could not be differentiated from each other.

Heterogeneity of pulp samples

The variability that we saw in the alkaline DIP may be directly related to the heterogeneity of the incoming recovered paper. One alternative explanation for the observed difference in community variability may be the higher pulp content in the alkaline DIP compared to the paper machine circuit. In the alkaline DIP, a local contaminant community may be protected in pockets of concentrated pulp because it is partially disconnected from the bulk environment (e.g., oxygen diffusion) and hydraulic retention. This protection is conceptually similar to flocs or granules in wastewater treatment. Bacterial communities in these protected environments may develop more independently of each other. We tested the variability of microbial communities within 100 ml of pulp material but did not find significant variability between CE-SSCP profiles from six independent DNA extractions. This indicated that if variability was introduced by a heterogeneous environment, the scale of environmental heterogeneity was larger than 100 ml and consequently remained undetected in the replicates of the sampling campaign. In the paper machine circuit where the pulp concentration is lower, less-protected communities are more directly influenced by short hydraulic retention, resulting in frequent and rapid change of environmental conditions (e.g., oxidation-redox potential and temperature) and possibly a homogenization of the communities in different samples throughout the circuit.

Implication of storage silos in biofouling

In storage silos, bacterial contaminant communities may experience more favorable growth conditions than in the high-flow environments of the paper machine circuit. Storage tanks and silos have been implicated in biofouling for example in subterranean storage facilities for crude oil in the petrochemical industry [23] and tanks for refined fuels [2, 19]. Similarly, storage of cereals [14] or food production byproducts [17] have been identified as causes of microbial contamination. The reasons for the frequent contamination of storage tanks are long retention times of the stored materials and the lack of proper hygiene because of inaccessibility and sheer size. Often it is unavoidable that even after attempted drainage and cleaning, a contamination seed remains in the silos, e.g., a contaminated water layer in hydrocarbon tanks [19] or as a surface-attached biofilm community facilitating rapid contamination of fresh product. Three storage silos are depicted in Fig. 1. The DIP and the TMP silos feed freshly prepared paper pulp into the paper machine circuit but do not directly receive material from the paper machine circuit. In contrast, the storage silo for reused pulp is an integral part of the paper machine circuit and is constantly loaded with already contaminated pulp material. The three silos have average pulp retention times on the order of 10–18 h.

Adapted community in reused-pulp silo experiences growth advantage

By comparing the entry and exit of the DIP silo, a drastic change in microbial community over the passage of the silo was observed. Given the long retention time in the silo under comparably stable environmental conditions, this observation was likely related to growth of a new contaminant community and decay of members of the preceding community. The signal of the contaminants leaving the DIP and the TMP silo was immediately lost in the paper machine circuit where another community was established. Granhall et al. [7] report similar findings where pulp preparation and paper machine communities differed at otherwise small within-mill variability.

Out of the three storage silos communities, we were only able to find traces of the contamination leaving the reused pulp silo in the paper machine circuit. It is reasonable to assume that the passage through the reused-pulp silo changes as drastically a foreign bacterial community as

observed in the DIP silo. With this in mind, the resemblance of exit samples from the silo to all other fingerprints from the paper machine circuit (whether upstream or downstream from the silo) was striking. These observations may suggest that the entering community is not foreign to the environmental conditions in the silo. The observed contamination in this unit may therefore be the site-specific contamination of the reused storage silo that was rapidly dispersed throughout the paper machine circuit and overrides any other possible contaminant community. Compared to the once-through silos for DIP and TMP pulp, the reused-pulp silo is continuously loaded with a well-adapted contaminant community that makes use of its growth advantage during its next passage through the silo.

The reused-pulp silo as side-stream incubator for a dispersed community

The storage silo as side-stream incubator may thus act as a point source of a microbial contamination. Because of strong dispersal, the observed contamination of the paper machine circuit may appear as the result of diffuse sources. The use of silos for temporary storage of reused pulp is a typical unit operation in paper mills and other industries. It may be the "common source of contamination" where "similar environmental conditions [across different paper mills]" prevail that Tiirola et al. [22] suggest in their comparison of several paper mills. If growth was indeed restricted to the environmental conditions in the storage silo, it is coherent that the community was not maintained in the alkaline and neutral DIP after being carried over from the paper machine circuit with the reused process water.

Deterioration of pulp ingredients and consequent changes of pulp properties may be a severe side-effect from growth and metabolic activity of a contaminant community under storage conditions. Growth of contaminant communities in storage silos should be limited, for example by directly dosing biocides into the silo rather than to the open process water circuit, as is currently practiced. However, given long enough retention in the silo, the development of a contaminant community in any kind of storage tank is only a matter of time. An alternative with low environmental impact could be tight control and minimization of the retention time and thus reduced bacterial growth and metabolic potential in the silo by means of fill level or flow rates. As a consequence, adverse effects on pulp properties are reduced and plant runability is improved. The practical implementation may be challenging as this approach requires novel control strategies and the reduction of safety margins.

Results from an unpublished independent statistical analysis purely based on overall plant performance and runability parallel our finding and confirm the role of fill level (and thus retention time) of the storage silo as one of the aspects to play a role with respect to plant runability (Armand Klem, Norske Skog Golbey, pers. comm.). Plant runability may therefore be directly related to growth and metabolic activity of a microbial contaminant community with a certain function (e.g., acidification, sulfate reduction) but not necessarily to the unavoidable presence of bacterial biomass in general. In a follow-up study, the changing properties of reused pulp during the passage of the silo will be closely examined to verify this contamination model.

Conclusions

Bacterial contaminant communities follow the general physical layout of the paper mill: all primary units of the plant (alkaline and neutral deinking pulp preparation and the paper machine circuit) harbored unique collections of microbial communities, despite high volume flows of reused water and pulp that link the units. The variability of contaminant communities was used as a measure for homogenization. Contaminant communities in the alkaline deinking pulp preparation loop showed significantly more variability than in the paper machine circuit. In contrast to the variable contaminant communities in the pulp preparation, the similar contaminant communities at each sampling location in the paper machine circuit point towards strong dispersal and homogenization of the bacterial contaminants. The remarkable similarity of the contaminant community at the exit of a storage silo to all other samples in the circuit strongly suggests a prominent role of this silo as a point source of the contamination of the facility. With an identified point source of a microbial contamination, the development of more targeted localized treatment strategies may be justified. This strategy may include disinfecting the storage silo or minimizing bacterial growth by reduced retention times.

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