

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

19 December 2024 (19.12.2024)



(10) International Publication Number

WO 2024/256747 A1

(51) International Patent Classification:

C12Q 1/689 (2018.01)

(21) International Application Number:

PCT/FI2024/050256

(22) International Filing Date:

23 May 2024 (23.05.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

20235671

15 June 2023 (15.06.2023)

FI

(71) Applicant: KEMIRA OYJ [FI/FI]; Energiakatu 4, 00180 Helsinki (FI).

(72) Inventors: EKMAN, Jaakko; c/o Kemira Oyj, P.O. Box 44, 02271 Espoo (FI). IKÄVALKO, Satu; c/o Kemira Oyj, P.O. Box 44, 02271 Espoo (FI). SIMELL, Jaakko; c/o Kemira Oyj, P.O. Box 44, 02271 Espoo (FI). KANERVA, Tinja; c/o Kemira Oyj, P.O. Box 44, 02271 Espoo (FI). JAAKKOLA, Anu; c/o Kemira Oyj, P.O. Box 44, 02271 Espoo (FI).

(74) Agent: ESPATENT OY; Kaivokatu 10 D, 00100 Helsinki (FI).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

(54) Title: METHOD FOR EVALUATING EFFECT OF MICROBIAL COMMUNITY ON PRODUCTION PROBLEMS OF A PROCESS PRODUCING A CELLULOSE BASED ARTICLE

(57) Abstract: There is provided a method for evaluating effect of microbial community on production problems of a process producing a cellulose based article and/or defects of the produced cellulose based article.

WO 2024/256747 A1

METHOD FOR EVALUATING EFFECT OF MICROBIAL COMMUNITY ON PRODUCTION PROBLEMS OF A PROCESS PRODUCING A CELLULOSE BASED ARTICLE

TECHNICAL FIELD

- 5 The present disclosure generally relates to a method for evaluating effect of microbial community on production problems of a process producing a cellulose based article.

BACKGROUND

- 10 This section illustrates useful background information without admission of any technique described herein representative of the state of the art.

- Cellulose based materials, such as paper, board and molded fiber based article are important packaging materials used in many fields of industry. Said packaging materials can provide physical or chemical protection to the object(s) enclosed. Paper, board and molded fiber based article are usually mainly composed of
15 cellulose fibres, calcium carbonate, starch and hemicellulose. All these are natural components and can serve as growth media for contaminating microorganisms.

- Microbial community, such as bacterial community in paper, board and molded fiber based article manufacturing process can have also a big effect on paper, board and molded fiber based article and their manufacturing process. Different bacterial
20 species can cause for example pH drop, starch and cellulose degradation, poor end product strength, bad smells, runnability problems and end product defects.

- To analyze microbial community from the paper or board manufacturing process, a representative sample must be collected, and it must be transported to laboratory quickly, frozen or preserved with specific preservative chemicals. All these methods
25 are laborious or at least they have some extra cost. Additionally, paper making and board making are continuous processes, the status of which changes all the time, thus, it is important to collect a process sample exactly when a (possibly stochastic) problem occurs.

As the present methods are laborious, time consuming and expensive there is a need for improved methods of determining microbiological status of the process.

SUMMARY

The appended claims define the scope of protection. Any examples and technical descriptions of apparatuses, products and/or methods in the description and/or drawings not covered by the claims are presented not as embodiments of the invention but as background art or examples useful for understanding the invention.

In a first aspect the present invention provides a method for evaluating effect of microbial community on production problems of a process producing a cellulose based article, the method comprising steps of

- (i) providing a sample of an intact cellulose based article;
- (ii) extracting DNA from the sample of the intact cellulose based article; and
- (iii) analyzing microbial community,

wherein analysis results of the intact cellulose based article are compared with historical process data gathered from manufacturing process of the cellulose based article in question during the manufacturing of the cellulose based article in question.

In a second aspect the present invention provides use of the method according to the present invention for evaluating effect of microbial community on production problems of the process producing the cellulose based article and/or defects of the produced cellulose based article.

It has been now found that microbial community such as bacterial community in a process for producing a cellulose based article, such as a paper or a board manufacturing process can be analyzed easily and less laboriously from an intact cellulose based article, such as intact paper or intact board that was produced on the mill. DNA is well preserved in an intact cellulose based article, such as intact paper or intact board and can be analyzed even after long time periods. A sample of an intact cellulose based article is easy to collect and send in normal mail at ambient temperature for analysis of microbial community in the intact cellulose based article.

As DNA is well preserved in an intact cellulose based article, it is advantageous to collect a sample of an intact cellulose based article, such as intact paper or intact board samples instead of process samples. Additionally, microbial community in a process sample changes after collection of the sample during time, for example
5 bacterial community may change or the proportional quantities of different bacterial species may increase or decrease, thus the sample does not any more represent the situation of the process when the sample was collected.

With the method of the present invention it is possible to find out the microbial community, such as bacterial community in process waters and pulps of the
10 manufacturing process of cellulose based article by analyzing an intact cellulose based article, such as intact paper or intact board produced with paper or board manufacturing process. The analysis results can be compared with process data of the manufacturing process, i.e. the effect of the microbial community can be evaluated on production problems and/or defects of the produced product.

The present invention provides an easier sampling method for DNA analysis from a mill producing cellulose based article, such as DNA analysis from a paper or board mill. By making the analysis from intact cellulose based article, such as intact paper or intact board samples instead of process water or pulp samples several advantages can be obtained. One of the major advantages is that by analyzing a
15 sample of an intact cellulose based article, such as intact paper or intact board samples from archive, microbiological data from the past during problematic situation even several months earlier can be obtained.
20

The present invention has also at least the following advantages:

- Mills producing cellulose based articles, such as paper or board mills usually store
25 samples of their products. In many cases samples are collected from each machine frequently, such as from each machine roll, i.e. even tens of times per day. Thus, it is possible to obtain historical samples, for example during previous 24 months, and select samples from different production situations (efficient production and problematic situation). Therefore, it is possible to analyze if past problems have
30 been caused by microbes.

- Obtaining historical data can speed up the demonstration of the treatment benefits. For example during a trial, such as biocide trial, reference data could be obtained easier, more thoroughly and with fewer mill visits.

- 5 - Collecting a sample of an intact cellulose based article, such as intact paper or intact board samples is easy and mill employees can do it. Thus, they could collect samples for analysis any time, whether or not they have equipment or knowhow for normal microbiological sampling.

The appended claims define the scope of protection.

DETAILED DESCRIPTION

- 10 In a first aspect the present invention provides a method for evaluating effect of microbial community on production problems of a process producing a cellulose based article, the method comprising steps of

- 15 (i) providing a sample of an intact cellulose based article;
 (ii) extracting DNA from the intact cellulose based article; and
 (iii) analyzing microbial community,
 wherein analysis results of the intact cellulose based article are compared with historical process data gathered from manufacturing process of the cellulose based article in question during the manufacturing of the cellulose based article in question.

- 20 In one embodiment the intact cellulose based article is produced at most 24 months before the extraction of the DNA, such as from 1 day to 24 months before the extraction of the DNA, preferably at most 12 months before the extraction of the DNA, such as from 1 week to 12 months before the extraction of the DNA.

- 25 In one embodiment the intact cellulose based article is produced from 1 day to 24 months before the extraction of the DNA, preferably from 1 day to 12 months before the extraction of the DNA.

By term "intact" is meant that the cellulose based article is of normal quality and it meets the visual quality parameters of the cellulose based article in question.

- 30 The cellulose based article can be any suitable cellulose based article known in the art. In one embodiment the cellulose based article comprises paper, board, tissue

paper, task wipes, molded fiber based articles, non-woven material, textile fiber or the like.

In one embodiment majority of the cellulose based article is cellulose, preferably at least 0.1 wt. % is cellulose, such as 0.1-100 wt.% is cellulose, preferably 2-100 wt.% is cellulose, more preferable 40-100 %, even more preferably 80-100%.

In one embodiment the sample of the intact cellulose based article is as dry as dried in manufacturing process of the intact cellulose based article. In one embodiment the intact cellulose based article is air dry, i.e. the intact cellulose based article has moisture content equal to air humidity.

10 In one embodiment a suspension is produced from the sample of the intact cellulose based article before extracting DNA from the sample of the intact cellulose based article.

The suspension can be produced in any suitable method known in the art. An example of such method is in which cellulose based article, such as paper or board is pulpered to suspension. In another method cellulose based article is cut into small pieces or torn to small pieces followed by mixing, preferably mixing vigorously, the small pieces in a liquid medium, preferably aqueous liquid medium.

In one embodiment the suspension is 0.5%-15% suspension, preferably 1%-15% suspension, more preferably 3%-13% suspension, even more preferably 3%-10% suspension, such as 6% suspension.

Preferably the suspension is aqueous suspension.

The method according to the present invention may be more sensitive and give more reliable results when a suspension of the sample of the intact cellulose based article is formed prior the extraction. On the other hand, when the intact cellulose based article is dry the method according to the present invention is a bit faster than the suspension based extraction to do in laboratory.

In one embodiment the microbial community comprises bacterial community, fungal community, archaeal community, viral community or a mixture thereof, preferably the microbial community is bacterial community.

In one embodiment the method comprises at least partial sequencing the extracted DNA after the extraction of the DNA.

In one embodiment the method comprises sequencing the extracted DNA after the extraction of the DNA.

- 5 In one embodiment the method comprises at least partial sequencing of the 16S rRNA gene of the extracted DNA after the extraction of the DNA.

In one embodiment the method comprises qPCR of the extracted DNA after the extraction of the DNA.

- 10 In one embodiment the method comprises qPCR, preferably specific qPCR for one or more microbes associated with cellulose based article production, such as microbes related to paper or board production runnability problems such as fermentation or biofouling.

- 15 In one embodiment the extraction comprises chemical cell lysis, mechanical cell lysis, thermal cell lysis, cell lysis by sonication, column-based purification, magnetic purification, precipitation-based purification or any combination thereof.

In one embodiment microbial taxa of the microbial community are determined in step (iii).

- 20 In one embodiment proportion, i.e. percentage of at least one microbial taxon and/or quantity of at least one microbial taxon of the microbial community is measured in step (iii).

In one embodiment, the microbial taxon is bacterial taxon and can be any suitable bacterial taxon.

- 25 In one embodiment the taxon comprises bacterial phyla Actinobacteriota, Bacteroidota, Chloroflexi, Cyanobacteria, Deinococcota, Desulfobacterota, Firmicutes, Myxococcota, Proteobacteria or a mixture thereof.

In one embodiment the bacterial taxon comprises Lacibacter, Cloacibacterium, Meiothermus, Anoxybacillus, Porphyrobacter, Comamonadaceae, Schlegelella, Tepidimonas, Hydrogenophilus, Pseudomonadaceae, Pseudomonas, Pseudoxanthomonas, Xanthomonadaceae, Chitinophagaceae, Weeksellaceae,

Deinococcus, Rhizobiaceae, Sphingomonadaceae, Bordetella, Burkholderia-Caballeronia-Paraburkholderia, Burkholderiaceae, Cupriavidus, Ralstonia, Methyloversatilis, Acinetobacter, Thermomonas, Bifidobacteriaceae, Bifidobacterium, Ethanoligenens, Microvirga, Pleomorphomonas, Rhizobiales, 5 Rhodobacteraceae, Sphingomonas, Rhodocyclaceae, Acidibacter, Vulcaniibacterium, Corynebacterium, Frankia, Micromonospora, Streptomycetaceae, Flectobacillus, Sphingobacterium, Roseiflexaceae, Bacillales, Lactobacillales, Paenibacillus, Thermoanaerobacterium, Pajaroellobacter, Sandaracinus, Azospirillum, Asticcacaulis, Bosea, Methylobacterium- 10 Methylo rubrum, Sphingobium, Rivicola, Vogesella, Paucibacter, Duganella, Niveibacterium, Zoogloea, Enterobacteriaceae or a mixture thereof.

The analysis results are compared to process data of the process that produced the intact cellulose based article, i.e. process data gathered from the process that produced the cellulose based article, for example the number of interruptions in the 15 process, pH and/or conductivity in the process, strength of cellulose based article, smell issues, defects, such as holes and spots in the cellulose based article. That is, the effect of the microbial community on production problems and/or defects of the produced product can be evaluated.

In one embodiment the analysis results are compared to one or more of the number 20 of interruptions in the process, pH and/or conductivity in the process, strength of cellulose based article, smell issues, and defects, such as holes and spots in the cellulose based article.

In one embodiment the analysis results are compared to process data of the process that produced the intact cellulose based article, i.e. process data gathered from the 25 process that produced the cellulose based article during a period of good or premium quality production.

In one embodiment, the proportion and/or the quantity of at least one microbial taxon of a microbial community of at least two samples of intact cellulose based articles produced at different times are gathered, and is compared to the historical process 30 data. Samples can be selected based on production data so that they are produced during different process conditions.

In one embodiment analysis results of the intact cellulose based article is compared with historical process data at the time when the cellulose based article in question was manufactured.

5 With the term “historical process data” is meant process data gathered during the manufacturing of the cellulose based article in question, i.e. the historical process data is as old as the intact cellulose based article in question is, i.e. the historical process data is from the process that produced the cellulose based article in question.

10 In one embodiment the historical process data comprises the number of interruptions in the process, pH and/or conductivity in the process, strength of cellulose based article, smell issues, defects, such as holes and spots in the cellulose based article, oxidation-reduction potential, consumption and/or quality of different production chemicals (e.g. starch, biocides, dry and/or wet strength chemicals, retention aids, enzymes and/or antiscaling agents), cellulose based raw
15 materials that were used, waste water treatment plant performance, consumption of drying energy, fresh water consumption, process temperature, hygienic quality of the cellulose based article or any combination of these.

Mills, such as paper or board mills using recycled fiber have generally higher bacterial counts in the processes than mills not using recycled fiber. Problems
20 caused by planktic bacteria, e.g. pH drop, smells or strength issues, are often severe on those machines. By term planktic bacteria is meant free swimming bacteria in process water. They are present also in the head box and they can be identified from the paper or board with the method according to the present invention

In recycled containerboard strength is a key parameter and with the method
25 according to the present invention it is possible to study the role of microbes in the strength-related problems in the past. Also, smells may be caused by bacteria, and with the method according to the present invention their role can be studied. The same applies to any problem at cellulose based article, such as paper or board.

In one embodiment the intact cellulose based article comprises recycled fiber,
30 preferably comprises substantially recycled fiber, more preferably the intact cellulose based article is manufactured from recycled fiber.

In one embodiment the recycled fiber originates from old corrugated containers, office waste paper, mixed office waste paper, sorted office waste paper, recycled pre-consumer fiber material, recycled post-consumer fiber material, recycled non-woven material, recycled textile fiber or the like or a mixture thereof.

- 5 In a second aspect the present invention provides use of the method according to the present invention for evaluating effect of microbial community on production problems of the process producing the cellulose based article and/or defects of the produced cellulose based article.

EXAMPLES

- 10 The examples presented herein are according to the present invention. Tables 1, 2, 3 and 4 show results of bacterial community analysis by the method according to the present invention.

Table 1: Bacterial communities were analyzed by extracting DNA and sequencing 16S gene (partially) from two intact paper samples (intact paper 1 and intact paper 15 2) and one clear filtrate sample from same paper machine. The intact paper 1 was produced one month earlier than the intact paper 2, and the clear filtrate sample was collected between the manufacturing of the papers 1 and 2. Both DNA extractions from the papers were made after one month from production of the intact paper in question. Values in the table are percentages of total bacteria. Bacterial 20 taxa with >1% at least in one sample are shown. It can be seen that the same main bacteria were found from the papers and also from the clear filtrate. There were some differences, which may be due to time differences in sampling or because clear filtrate sample without fibers was analyzed, and not head box sample containing fibers. It can be seen from two paper samples that the bacterial 25 community did not change significantly in this machine in one month.

Table 1.

	Intact paper 1	Intact paper 2	Clear filtrate
Lacibacter	2.4	1.2	9.6
Cloacibacterium	2.1	1.1	3.2

Meiothermus	3.9	1.0	9.7
Anoxybacillus	0.0	1.8	0.0
Porphyrobacter	2.2	1.2	0.5
Comamonadaceae_unclassified	5.0	4.4	2.5
Schlegelella	10.7	8.4	7.5
Tepidimonas	61.1	53.9	51.8
Hydrogenophilus	0.0	1.9	0.3
Pseudomonadaceae_unclassified	6.4	13.4	0.0
Pseudomonas	0.9	1.3	0.0
Pseudoxanthomonas	0.0	4.3	14.1
Xanthomonadaceae_unclassified	1.0	0.2	0.1

Table 2: Bacterial community analysis from intact tissue papers from three different mills. DNA was extracted after ten days from production of the Soft roll sample (Mill 1). The tissue wipe (Mill 2) was a commercial product bought to the laboratory and its' manufacturing date was not known. DNA was extracted after fourteen days from production of the Towel sheet sample (Mill 3). The table shows percentages of total bacteria. Bacterial taxa >1% at least in one sample are shown. It can be seen that the bacterial communities are clearly different. Mill 2 producing tissue wipes had clearly more potential acid producing bacteria that can live in anaerobic conditions (e.g. *Cloacibacterium*, *Weeksellaceae*). On the other hand, paper from Mill 1 contained more biofilm-forming bacteria and for example a well-known primary biofilm former *Deinococcus* was present in significant share. Towel sheet of mill 3 contained significant amount of *Cloacibacterium*, showing that also there is potential for fermentative growth. Based on the results it seems that the Mills 2 and 3 have higher risk for problems in the storage towers of pulp and process water (e.g. pH drop, starch degradation), whereas Mill 1 has more risk for biofouling in the short loop of the paper machine.

Table 2.

Bacterial taxon	Soft-Roll sample, Mill 1	Tissue wipes, Mill 2	Towel sheet, Mill 3
Pseudonocardiaceae_unclassified	1.3	0.0	0.0
Cloacibacterium	0.6	9.9	16.0
Weeksellaceae_unclassified	0.0	3.5	0.0
Deinococcus	9.5	0.0	0.6
Rhizobiaceae_unclassified	0.3	1.0	2.6
Sphingomonadaceae_unclassified	3.3	1.6	8.4
Bordetella	2.0	0.0	0.0
Burkholderia-Caballeronia-Paraburkholderia	1.4	0.2	0.1
Burkholderiaceae_unclassified	25.2	0.2	0.1
Cupriavidus	14.7	0.1	0.2
Ralstonia	4.5	0.0	0.0
Vogesella	0.0	0.0	1.2
Comamonadaceae_unclassified	1.7	5.2	29.7
Schlegelella	2.9	14.0	0.3
Tepidimonas	20.2	36.0	2.4
Methyloversatilis	0.7	1.4	0.4
Niveibacterium	0.0	0.0	1.3
Rhodocyclaceae_unclassified	0.0	0.2	3.1
Zoogloea	0.0	0.0	10.0
Acinetobacter	0.0	7.0	9.8
Pseudomonas	1.2	0.7	0.4
Pseudoxanthomonas	0.6	10.6	0.6
Thermomonas	1.3	0.0	0.0
Xanthomonadaceae_unclassified	4.4	0.3	0.0
Total bacteria, cells/ml	1.2E+06	2.2E+06	2.7E+06

Table 3: Bacterial community from Mill 2 (see table 2) analyzed with two different methods. Tissue wipes were a commercial product bought to the laboratory and its' manufacturing date is not known. Values in the table are percentages from the total bacteria. Bacterial taxa >0.3% at least in one sample are shown. In method 1 intact paper was first pulpered to 6% suspension, and then 0.5 ml of this suspension was taken into the DNA extraction. In method 2 about 50 mg intact tissue paper was directly taken for the DNA extraction. There were more bacterial species present in the analysis with the Method 1 and thus DNA extraction using Method 1 is more efficient and it can analyze more sensitively also gram-positive bacteria that have thicker cell walls and thus their DNA is more difficult to extract. Such bacteria are

for example *Bifidobacteriaceae*, *Bifidobacterium* and *Ethanoligenens*, which were not observed using method 2. Thus Method 1 gives more comprehensive results.

Table 3.

	Tissue wipes, Mill 2, method 1	Tissue wipes, Mill 2, method 2
Bifidobacteriaceae_unclassified	0.6	0
Bifidobacterium	0.3	0
Chitinophagaceae_unclassified	0.6	0.6
Cloacibacterium	9.9	10.7
Weeksellaceae_unclassified	3.5	3.4
Ethanoligenens	0.5	0
Microvirga	0.5	0.6
Pleomorphomonas	0.5	0.4
Rhizobiaceae_unclassified	1	1.1
Rhizobiales_unclassified	0.1	0.3
Rhodobacteraceae_unclassified	0.3	0
Sphingomonadaceae_unclassified	1.6	0.7
Sphingomonas	0.3	0.1
Comamonadaceae_unclassified	5.2	4.1
Schlegelella	14	12.8
Tepidimonas	36	34.8
Methyloversatilis	1.4	1.7
Rhodocyclaceae_unclassified	0.2	0.3
Acidibacter	0.3	0
Acinetobacter	7	7.8
Pseudomonas	0.7	0.3
Pseudoxanthomonas	10.6	17.4
Vulcaniibacterium	0.7	0.6
Xanthomonadaceae_unclassified	0.3	0.2

- 5 **Table 4:** Bacterial community in two samples from Mill 3. DNA was extracted two weeks after production of the tissue with no odor and over one month after the production of the tissue with odor. Values in the table are percentages of total bacteria. Bacterial taxa >0.5% in at least one sample are shown. The lowest line shows total number of bacteria (DNA-based). Bacterial community is clearly
- 10 different in samples with observed unpleasant odor than in flawless sample without odor. Specifically, Actinobacteriota known to produce odorous geosmin and 2-methylisoborneol (*Micromonospora* and unclassified Streptomycetaceae) were

detected in the paper with odor. The same bacteria were detected in some wetlaps used as raw material, allowing to pinpoint the tissue odor origin to wetlap.

Table 4.

Bacterial genus (%)	Tissue with no odor, Mill 3	Tissue with odor, Mill 3
Bifidobacterium	0.1	0.7
Corynebacterium	0.2	0.9
Frankia	0.0	2.8
Micromonospora	0.0	10.4
Streptomycetaceae_unclassified	0.0	0.9
Flectobacillus	0.7	0.0
Cloacibacterium	13.9	4.5
Sphingobacterium	0.1	1.6
Roseiflexaceae	1.1	0.0
Deinococcus	1.1	1.0
Bacillales_unclassified	0.5	1.9
Lactobacillales_unclassified	1.8	7.1
Paenibacillus	0.1	2.4
Thermoanaerobacterium	0.0	0.5
Pajaroellobacter	0.0	5.2
Sandaracinus	0.0	0.8
Azospirillum	1.1	0.1
Asticcacaulis	0.2	1.1
Bosea	0.2	0.6
Methylobacterium-Methylorubrum	0.2	0.7
Pleomorphomonas	1.4	0.0
Rhizobiaceae_unclassified	2.5	0.6
Sphingobium	0.4	1.1
Sphingomonadaceae_unclassified	8.1	4.2
Sphingomonas	0.6	0.3
Rivicola	1.2	0.6
Vogesella	0.9	0.5
Comamonadaceae_unclassified	29.8	7.1
Paucibacter	0.5	0.1
Tepidimonas	4.3	10.7
Duganella	0.6	0.0
Methyloversatilis	0.7	0.1
Niveibacterium	1.4	0.0
Rhodocyclaceae_unclassified	3.1	0.4
Zoogloea	6.2	0.5
Enterobacteriaceae_unclassified	0.0	0.0
Acinetobacter	7.1	19.0
Pseudomonas	0.2	1.1

Pseudoxanthomonas	0.7	3.3
Total bacteria, cells/ml	8.3E+06	5.7E+06

Various embodiments have been presented. It should be appreciated that in this document, words comprise, include, and contain are each used as open-ended expressions with no intended exclusivity.

- 5 The foregoing description has provided by way of non-limiting examples of particular implementations and embodiments a full and informative description of the best mode presently contemplated by the inventors for carrying out the invention. It is however clear to a person skilled in the art that the invention is not restricted to details of the embodiments presented in the foregoing, but that it can be
- 10 implemented in other embodiments using equivalent means or in different combinations of embodiments without deviating from the characteristics of the invention.

- Furthermore, some of the features of the afore-disclosed example embodiments may be used to advantage without the corresponding use of other features. As such,
- 15 the foregoing description shall be considered as merely illustrative of the principles of the present invention, and not in limitation thereof. Hence, the scope of the invention is only restricted by the appended patent claims.

CLAIMS

1. A method for evaluating the effect of microbial community on production problems of a process producing a cellulose based article, the method comprising steps of
 - 5 (i) providing a sample of an intact cellulose based article;
 - (ii) extracting DNA from the sample of the intact cellulose based article; and
 - (iii) analyzing microbial community,wherein analysis results of the intact cellulose based article are compared with historical process data gathered from manufacturing process of the cellulose
10 based article in question during the manufacturing of the cellulose based article in question.
2. The method of claim 1, wherein the intact cellulose based article is produced at most 24 months before the extraction of the DNA, preferably from 1 day to 24 months before the extraction of the DNA, more preferably from 1 week to 12 months
15 before the extraction of the DNA.
3. The method according to any one of claims 1 or 2, wherein a suspension is produced from the sample of the intact cellulose based article before extracting DNA from the sample of the intact cellulose based article.
4. The method according to any one of claims 1-3, wherein the microbial
20 community comprises bacterial community, fungal community, archaeal community, viral community or a mixture thereof, preferably the microbial community is bacterial community.
5. The method according to any one of claims 1-4, wherein the method comprises at least partial sequencing the extracted DNA after the extraction of the DNA.
- 25 6. The method according to any one of claims 1-5, wherein the method comprises at least partial sequencing of 16S rRNA gene of the extracted DNA after the extraction of the DNA.
7. The method according to any one of claims 1-5, wherein the method comprises qPCR, preferably specific qPCR for one or more microbes associated with cellulose

based article production, such as microbes related to paper or board production
runnability problems such as fermentation or biofouling.

8. The method according to any one of claims 1-7, wherein the DNA extraction
comprises chemical cell lysis, mechanical cell lysis, thermal cell lysis, cell lysis by
5 sonication, column-based purification, magnetic purification, precipitation-based
purification or any combination thereof.

9. The method according to any one of claims 1-8, wherein the analysis results
are compared to one or more of the number of interruptions in the process, pH
and/or conductivity in the process, strength of cellulose based article, smell issues,
10 and defects, such as holes and spots in the cellulose based article.

10. The method according to any one of claims 1-9, wherein proportion and/or
quantity of at least one microbial taxon of the microbial community is measured in
step (iii).

11. The method according to any one of claims 1-10, wherein the proportion and/or
15 the quantity of at least one microbial taxon of a microbial community of at least two
samples of intact cellulose based articles produced at different times are gathered,
and is compared to the historical process data.

12. The method according to any one of claims 1-11, wherein the cellulose based
article comprises recycled fiber, preferably comprises substantially recycled fiber,
20 more preferably the intact cellulose based article is manufactured from recycled
fiber.

13. The method according to claim 12, wherein the recycled fiber originates from
old corrugated containers, office waste paper, mixed office waste paper, sorted
office waste paper, recycled pre-consumer fiber material, recycled post-consumer
25 fiber material, recycled non-woven material, recycled textile fiber or the like or a
mixture thereof.

14. Use of the method according to any one of claims 1-13 for evaluating effect of
microbial community on production problems of the process producing the cellulose
based article and/or defects of the produced cellulose based article.

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2024/050256

A. CLASSIFICATION OF SUBJECT MATTER INV. C12Q1/689 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C12Q		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.-L. SUIHKO ET AL: "Description of heterotrophic bacteria occurring in paper mills and paper products", JOURNAL OF APPLIED MICROBIOLOGY, vol. 97, no. 6, 1 December 2004 (2004-12-01), pages 1228-1235, XP055474505, GB ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2004.02416.x the whole document abstract; p. 1229-1230, Table 1 -----	1 - 14
X	JP 2006 217869 A (KURITA WATER IND LTD) 24 August 2006 (2006-08-24) the whole document para. 1-3, 9, 10, 16, 25 ----- - / - -	1 - 14
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 30 July 2024		Date of mailing of the international search report 12/08/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Sauer, Tincuta

INTERNATIONAL SEARCH REPORT

International application No

PCT/FI2024/050256

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP H07 274999 A (SOMAR CORP) 24 October 1995 (1995-10-24) the whole document para. 1, 5-7, 14, 25, 31; claims -----	1 - 14
X	US 2004/132095 A1 (IIZUMI TARO [JP] ET AL) 8 July 2004 (2004-07-08) the whole document para. 1, 17-20, 26-27, 47, 266 -----	1 - 14
Y	WO 2021/250317 A1 (KEMIRA OYJ [FI]) 16 December 2021 (2021-12-16) the whole document p. 1, 6 -----	1 - 14
Y	ULF GRANHALL ET AL: "Bacterial community diversity in paper mills processing recycled paper", JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 37, no. 10, 11 June 2010 (2010-06-11) , pages 1061-1069, XP019809582, ISSN: 1476-5535 the whole document abstract, p. 1063, 1057 -----	1 - 14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/FI2024/050256

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2006217869 A	24-08-2006	JP 4788150 B2	05-10-2011
		JP 2006217869 A	24-08-2006
JP H07274999 A	24-10-1995	JP 3788999 B2	21-06-2006
		JP H07274999 A	24-10-1995
US 2004132095 A1	08-07-2004	BR 0206375 A	13-07-2004
		CN 1496224 A	12-05-2004
		EP 1350431 A1	08-10-2003
		US 2004132095 A1	08-07-2004
		US 2007117140 A1	24-05-2007
		US 2010227332 A1	09-09-2010
		WO 02054865 A1	18-07-2002
WO 2021250317 A1	16-12-2021	AU 2021289073 A1	02-02-2023
		BR 112022023743 A2	20-12-2022
		CA 3180278 A1	16-12-2021
		CL 2022003460 A1	04-08-2023
		CN 115885051 A	31-03-2023
		EP 4162084 A1	12-04-2023
		KR 20230020435 A	10-02-2023
		WO 2021250317 A1	16-12-2021