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Eco-friendly production of waterborne paint

MUDP report

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Contents

Foreword

This project *Eco-friendly production of waterborne paint* has been approved and funded by the Environmental Technology Development Program (MUDP) under the Ministry of Environment and Food of Denmark in 2017 and was carried out as a collaboration between Flügger A/S and Danish Technological Institute (DTI). The project was headed by Jan Lorenzen, DTI.
This final report covers the work carried out during the period 1 May 2018 – 31 October 2020. The project period was shortened by 6 months compared to the original planning.
A special thanks to the Ministry of Environment and Food of Denmark for funding the project and to everyone involved who contributed to the work and the completion of the project:

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Abbreviations

BIT:	Benzisothiazolinone
BPR:	Biocidal Products Regulation
CFU:	Colony Forming Units
CIT/CMIT:	Chloromethylisothiazolinone
CLP:	Classification, Labelling and Packaging
MIT:	Methylisothiazolinone
PT:	Product type under the Biocidal Products Regulation
PVA:	Polyvinyl Acetate Ethene
ZnPt:	Zinc Pyrithione
NaPt:	Sodium Pyrithione

Sammenfatning og konklusion

Med projektet ønskedes det gennem reduceret forbrug af biocider og ressourcer at opnå en mere bæredygtig produktion af maling. Biociderne anvendes som konserveringsmiddel, der skal forhindre ødelæggende vækst af bakterier i malingsspanden. I løbet af de seneste år har biocidreguleringen imidlertid medført, at der kan anvendes færre biocider til konservering af maling, hvilket kan indebære en forøget risiko for, at malingen kan blive inficeret og ødelagt af bakterier. Med henblik på at minimere risikoen for inficeret maling har projektet dels fokuseret på at finde kilder til kontaminering, dels undersøgt nye løsninger, som kan gøre det muligt at opnå god konservering med mindre biocid.

Indledningsvis koncentrerede projektet sig om de mikroorganismer, der var årsag til inficeret, ødelagt maling. Flügger har implementeret et registreringssystem, der omfatter registrering af forbrugerreklamationer med fuld sporbarhed til produktionsdata og anvendt produktrecept. Registret afslørede, at de fleste reklamationer omhandlede maling, der var ødelagt af bakterier allerede inden anvendelse af selve malingen, hvilket indikerede, at infektionen af malingen hovedsageligt fandt sted under produktionen. Gennem undersøgelse af inficeret maling fandt vi, at maling hovedsageligt blev ødelagt af bakterier af slægten *Pseudomonas*. Denne bakterie blev ikke påvist i råvarer, men derimod som bakteriebelægninger (biofilm) på svært tilgængelige steder og på overflader, der er svære af rengøre i selve produktionssystemet. Nærmere undersøgelser af disse bakterier viste, at der ikke var tale om biocidresistente stammer, og at en kraftig infektion med mange bakterier betød, at der skulle bruges mere biocid for at undertrykke deres vækst. Samlet set pegede de indsamlede data og resultater på, at ødelæggelse af maling ved bakterievækst kunne forklares ved periodevis kontaminering forårsaget af bakterie-hot spots på områder i produktionssystemet, der er svære at rengøre.

Med afsæt i disse erkendelser igangsatte Flügger en omfattende renovering af deres produktionssystem med henblik på at forbedre muligheden for rengøring af fabrikken, bl.a. gennem installation af rensegrise til rengøring af hele anlæggets rørsystem. Ydermere har forbedret overvågning af den mikrobiologiske kvalitet af råvarer, produktionshygiejne og registrering af reklamationer ført til, at kontaminerede partier af råvarer og færdig maling har kunnet identificeres tidligere. Derved kan Flügger hurtigere igangsætte afværgende foranstaltninger, hvilket har resulteret i et faldende antal reklamationer.

Parallelt med den store indsats for forbedring af produktionshygiejnen er der i projektet udviklet en ny mikrobiologisk testmetode, som blev anvendt i forbindelse med udvikling og formulering af maling med mindre biocid til konservering. Den nye testmetode viste en god og forbedret overensstemmelse med både reklamationsstatistik og hygiejnedata fra fabrikken. Det lykkedes at reducere mængden af biocider i forskellige typer maling, men det er ikke muligt med de eksisterende teknologier at undgå biocider til konservering af maling. På nuværende tidspunkt er det udelukkende lykkedes at producere meget mat indendørsmaling (som næsten ikke anvendes i Norden) med et meget stærkt reduceret biocidindhold.

Projektet har gjort det muligt at reducere mængden af biocider i maling ved forbedret hygiejne i Flüggers malingsfabrik. Der er dog stadig behov for en betydelig indsats for yderligere at forbedre produkternes egenskaber og formulering for at minimere risikoen for ødelæggende infektion af malingerne. En yderligere indsats vil desuden have afgørende betydning, specielt set i lyset af de regulative stramninger, der må forventes mht. brug af biocider til konservering af maling.

Summary and conclusion

The aim of this project was to improve the sustainability of paint production through reduced use of biocides and minimisation of resources. The biocides are used as in-can preservatives to avoid spoilage of the paints due to growth of bacteria. But due to recent years' restrictive legislation in the area, the number of biocides has been reduced, and the risk of microbiological spoilage of paint may thus increase. With the purpose of reducing the risk, the project has focused on the causes of spoilage and explored possible solutions to reduce the use of biocides.

Initially, the project was concentrated on microorganisms in relation to incidences of infected, spoiled paint in-can. Implementation of a track and trace system at Flügger giving full traceability on produced paint documented the level of claims regarding failed in-can preservation, i.e. infected paint. The system revealed that most claims were related to infection appearing before use of the paint, indicating microbial contamination of the paint during production. *Pseudomonas* were found to be the predominant genus in connection with infection in-can. While these bacteria were not found in raw materials, they were found in the form of biofilm at difficult-to-access and difficult-to-clean sites in the paint production facility. It was revealed that the bacteria found in infected paint were not biocide resistant, nevertheless, it became evident that the concentration of biocides needed to control the bacteria in the paint increased with the number of bacteria present in the paint. This indicated that failure of in-can preservation of paint was linked to periodically increased microbial contamination, likely related to bacteria hotspots situated at difficult-to-clean points in the factory.

Based on these findings, Flügger initiated reconstruction of their production facility to further improve cleanability of the plant and to implement pigging systems for cleaning of the whole piping system. In addition, improved monitoring and surveillance of the microbial quality of raw materials, plant hygiene and claims registration have resulted in early detection of contaminated batches. In combination, these actions have resulted in a reduction in the registered claims for failed in-can preservation of paint.

In parallel, a microbiological test method was developed and used in connection with the development of new paint formulations containing reduced amounts of biocides for in-can preservation. This new method resulted in markedly improved correspondence between findings of the needed biocide level during laboratory testing, the hygienic situation in the factory, and statistics on customer claims on failed in-can preservation. While it was possible to reduce the concentration of biocides, it seemed not possible to omit the use of in-can preservatives in paint based on the available technologies. Currently, only very mat indoor paints with low market shares in the Nordics have shown potential for a dramatic reduction of the use of biocides.

The project has made it possible to reduce the use of biocides combined with an improved hygienic situation at the Flügger production facility. The process and findings in this project resulted in positive results. But even more effort is needed to further improve the paint quality against risk of infections with the expected legislative changes related to the biocides.

1. Introduction

1.1 Background

Today biocides are used for in-can preservation of water-based paints. But due to environmental and end-user concerns, the number and amounts of biocides used for in-can preservation have been reduced continuously in recent years with further restrictions coming into effect during this project and beyond.

This project aims at identifying means to supplement and improve in-can preservation of waterborne paints accepting the use of reduced amounts of biocides.

At the same time, it is becoming more important to optimise the use of resources in paint production for economic as well as for environmental reasons. Therefore, the project also aims at a sustainable development of production processes, e.g. to minimise waste of resources particularly in water.

1.2 Overview of activities

The project is executed in three linked work packages over a total period of 2½ years.

Work package 1 Track and Trace addresses the need to identify the sources for microbial contamination of paints resulting in rot and to characterise the rot-causing microorganisms with respect to their tolerance to in-can preservatives.

Work package 2 Biocide Concentration vs. Biocide Activity addresses the apparent paradox that biocides in paint exhibit less antimicrobial activity than biocides in e.g. growth media for microorganisms.

Work package 3 Sustainable Product shall result in well-preserved paint while at the same time using the least possible amounts of resources.

2. Track and Trace contamination in process

The production of paint is more or less a process of mixing chemicals running in a complex production system. The production process was investigated thoroughly to locate and identify sources and hotspots of microorganisms linked to in-can spoilage of paint. In addition, isolated microorganisms were characterised for their tolerance towards in-can preservatives.

2.1 Activity 1.1 Identification and traceability of contamination sources

2.1.1 Routine microbiological analysis at Flügger

Flügger has established a procedure for microbiological control of production processes, equipment, raw materials and finished goods at the paint production plant in Kolding, Denmark. The purpose of the weekly routine collection of microbiological samples throughout the production system is to determine the hygienic status of the equipment to allow for initiation of suitable extraordinary cleaning routines when needed.

Samples of contact surfaces from equipment are collected with sterile cotton pins and transferred to sterile test tubes. Correspondingly, samples of liquids are collected with sterile pipettes and transferred to sterile test tubes. All samples are diluted with sterilized water to the required dilution. From the final solution, 1 mL is proceeded to petri dishes and the agar (Malt agar / King's agar / Plate Count agar (PCA)) is added. Malt agar is used for detection of fungi, King's agar is particularly good for detection of *Pseudomonas*, while PCA allows growth of most bacteria. The petri dishes are placed upside down at room temperature, except for the PCA plates that are incubated at 37 °C. The petri dishes are incubated for 3 days and checked for microbial growth.

Equipment checked for microbes includes dissolvers, mixing tanks, storage tanks, filters, and filling lines.

Annually, approximately 500 samples are analysed. Compared to 2016, the culture-positive fraction of these samples was reduced by 42% and 85% in 2017 and 2018, respectively.

From each batch of produced paint, a reference sample is collected. The reference sample is taken in connection with the filling process. According to procedure, a small 250 mL plastic jar is filled after 50-100 buckets have been filled in the filling machine. The reference sample is identified by item number and batch number and stored at room temperature. Routinely, these reference samples are not tested for microbiology. Only in situations where there is a claim for rot, the reference sample for the specific batch is checked. Generally, these reference samples do not confirm contamination in a batch.

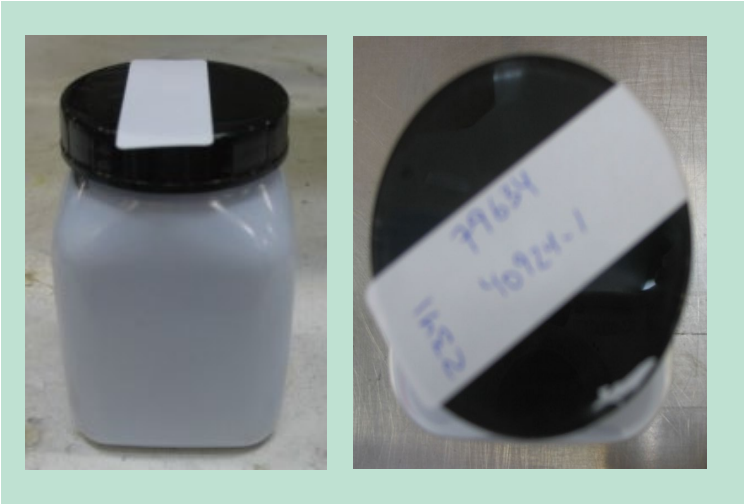

FIGURE 1. Reference samples from each produced batch of paint are collected in 250 mL plastic jars.

Routine microbiological analysis implemented at Flügger in 2018 revealed only limited occurrence of microorganisms, despite an increasing number of claims due to contamination before use were registered (cf. section 2.1.3). Furthermore, even though no formal identification of the few found microorganisms was performed, macroscopic examination of the microbial colonies did not suggest that these microorganisms were the same as typically found in rotten paint, i.e. *Pseudomonas sp.* (cf. section 2.2).

2.1.2 Tracking contamination sources at the Flügger plant, Kolding

As results from routine microbiological monitoring apparently did not correlate with registration of spoiled paint, neither in terms of relative numbers nor in the type of microorganisms found, further investigation of the production plant was initiated. To track the contamination sources in the product system DTI and Flügger conducted a first-round onsite sampling in the Flügger factory, Kolding, in June 2018. This included samples of raw materials and samples collected throughout the production system.

All samples were analysed by culturing to isolate and identify any present microorganisms. Samples were plated on PCA plates for detection of bacteria, while MEA (Malt extract agar) plates were used for isolation of yeasts and molds. The identity of the visible colonies was determined using either 16S rRNA gene-based sequencing methodology or protein-based MALDI-TOF-MS methodology. In general, these methodologies were used for isolation and identification of all the microorganisms in the project.

The microbiological findings related to raw materials are given in TABLE 1. It is clear that one of the analysed raw materials contained culturable bacteria, namely heavy growth of *Staphylococcus* found in a batch of $CaCO_3$.

TABLE 1. Raw materials sampled in June 2018 at the Flügger production plant in Kolding, Denmark and analysed for culturable bacteria.

Sample no.	Sample name	Microbiological finding
1	Clay 1	1 colony/g
2	Diatomite 1	No Growth
3	TiO_2 3	No Growth
4	$CaCO_3$ 2	Heavy growth/ Staphylococcus
5	Cellulose 1	No Growth
6	Cellulose 2	No Growth
7	TiO_2 4	No Growth
8	Thickener 1	No Growth
9	Anti-foam 1	No Growth
10	Dispersion 1	No Growth

In addition, TABLE 2 shows that sampling of sites accessible while production was running at the production plant did not reveal any relevant occurrence of microorganisms in the production system.

TABLE 2. Samples taken throughout the production plant in June 2018 at the Flügger production plant in Kolding, Denmark, and analysed for culturable bacteria.

Sample no.	Sample name	Microbiological finding
11	Process water with chlordioxide	No Growth
12	Filter – Top	1 colony
13	Filter – Inside	No Growth
14	Filter for surface dosing	No Growth
15	Empty bucket 10L	No Growth
16	Mixture used for surface water	No Growth
17	Grease from the surface water	No Growth
18	TM1 Tap head 1	No Growth
19	TM1 Tap head 5	No Growth
20	TM1 Tap head 4	No Growth
21	TM1 Tap head 6	No Growth
22	TM1 Tap head 2	No Growth
23	TM1 Tap head 4	No Growth
24	TM1 Tap head 3	No Growth
25	TM1 surface water	No Growth
28	TM1 Tap head 1 after cleaning	No Growth
29	TM1 Tap head 6 after cleaning	No Growth
30	TM1 Tap head 5 after cleaning	Few colonies/Microbacterium
31	TM1 Tap head 4 after cleaning	No Growth
32	TM1 Tap head 3 after cleaning	No Growth
33	TM1 Tap head 2 after cleaning	1 colony/Staphylococcus

This first extraordinary sampling did not reveal any microbiological hotspots, but instead confirmed the low level of microbial growth found during the routine control. These findings did not

correspond to the registration of claims for paints rotten before use, which increased during summer 2018 compared to the previous year (cf. section 2.1.3). Therefore, another site visit was performed (October 2018) with focus on identification of potential microbiological hotspots. In general, these potential hotspots were not accessible during operation of the paint production plant. The sites included the pressurized air system, an intermediate bulk container (IBC) with cleaning agent used in connection with operation of pigging cleaning of piping, and several filters in connection with mixing tanks. These suspected hotspots were sampled during a planned production stop during the Christmas break 2018-2019.

Due to a handling error, the pressurized air system had been running without dehumidification for a period. This could potentially lead to condensation of water inside the system, allowing growth of microorganisms. In total, seven samples were collected from different sites within the pressurized air system. While four samples were growth negative, two samples revealed presence of *Cupriavidus sp.,* and one sample showed growth of *Paenibacillus sp.* Following these findings, the system was cleaned, and dehumidification was enabled immediately after sampling. Follow-up sampling approximately 1 month later showed no occurrence of bacteria in the pressurized air system.

The IBC was used to allow reuse of cleaning and decontamination solution in connection with the operation of pigging cleaning of the piping. A total of four samples from the IBC and in connection with the cleaning pigs were collected and analysed. Even though the cleaning solution was amended with biocide, both *Comamonas* sp., *Citrobacter* sp., and *Pseudomonas* sp. were found in water samples from the container, while *Pseudomonas* sp. also was found on the pigs themselves. Following these findings, a new steel tank was installed for the cleaning solution. This tank was equipped with automatic valves to allow for daily cleaning of the tank, ensuring fresh cleaning solution at production start every morning. Monitoring of microbial counts showed that it was possible to reuse the biocide-amended pigging water for up to one week. By the end of the project, the said tank has been decommissioned and substituted by a larger pigging station to supply biocide-amended pigging water for cleaning of a substantially larger part of the piping system at the production facility.

The filters in connection with mixing tanks at Flügger's production plant in Kolding are divided into two groups: Group 30xx filters and group 4xxx filters that are installed between the final mixing tanks and the filling lines. These filters are used to ensure product quality in the process plant. They are automatically rinsed and reflushed between each batch of paint. While these filters are also part of the routine microbiological surveillance, extra sampling was performed in connection with manual disassembly and inspection of the filters. Various microorganisms were found in both filter groups, cf. TABLE 3 and TABLE 4 below.

TABLE 3. Microorganisms found during extraordinary sampling of filter group 4xxx, December 2018, Flügger, Kolding.

Sampling site	Microbiological finding
4152	*Citrobacter*, *Pseudomonas*, *Acinetobacter*, *Achromobacter*
4151	*Microbacterium*, *Pseudomonas*, *Stenotrophomonas*
4251	*Pseudomonas*, *Chryseobacterium*, *Stenotrophomonas*
4051	*Pseudomonas*
4351	No growth

TABLE 4. Microorganisms found during extraordinary sampling of filter group 30xx, December 2018, Flügger, Kolding.

Sampling site	Microbiological finding
3082	*Stenotrophomonas*
3081	*Pseudomonas*, *Stenotrophomonas*
3083	*Pseudomonas*, *Stenotrophomonas*, Sphingobacterium, *Microbacterium*
3083	*Acinetobacter*, *Pseudomonas*, *Sphingobacterium*
3082	*Microbacterium*, *Pseudomonas*
3082	*Pseudomonas*
3081	*Pseudomonas*

Following these findings and further sample collection during routine microbiological surveillance exceeding Flügger's internal limits of microbiological load, several remedial actions were initiated. These included disabling of an override option for filter cleaning between paint batches and instructions on weekly manual disassembly and cleaning of the filters.

2.1.3 Tracking of complaints

To gain in-depth knowledge about complaints related to insufficient in-can preservation of waterborne paints, Flügger has used their in-house complaints registry.

Customer complaints are registered in Flügger´s ERP system. To monitor the claims and identify trends, Flügger has developed a Power Business Intelligence solution that processes the registered complaint data into a visual overview of complained item numbers and problem types.

The most frequent claim type is 'Rotten Paint'. FIGURE 2 shows the relative number of registered claims per month in 2018-2020 (only paint produced in Kolding) with the problem type Rot. From the time a batch of paint is produced and until a claim is received at Flügger, there is a natural delay. In FIGURE 3, the claim type 'Rotten Paint' is depicted as a function of production date showing that batches produced in June 2018 have the highest number of claims.

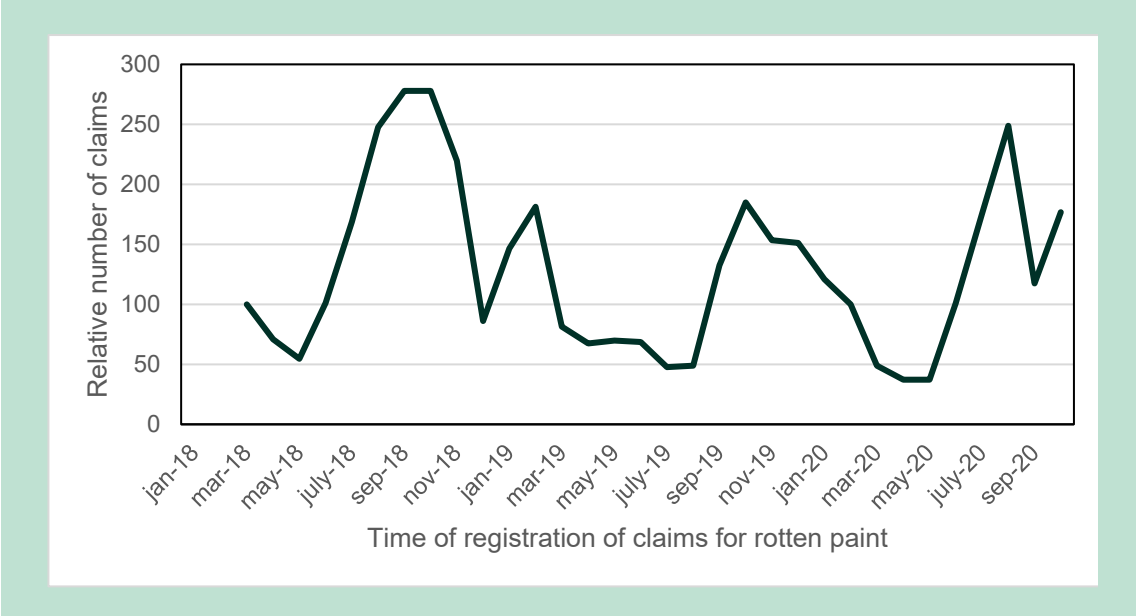

FIGURE 2. Relative occurrence of claim type 'Rotten Paint' recorded when received for items produced at the Flügger paint production plant in Kolding. March 2018 was used as index 100.

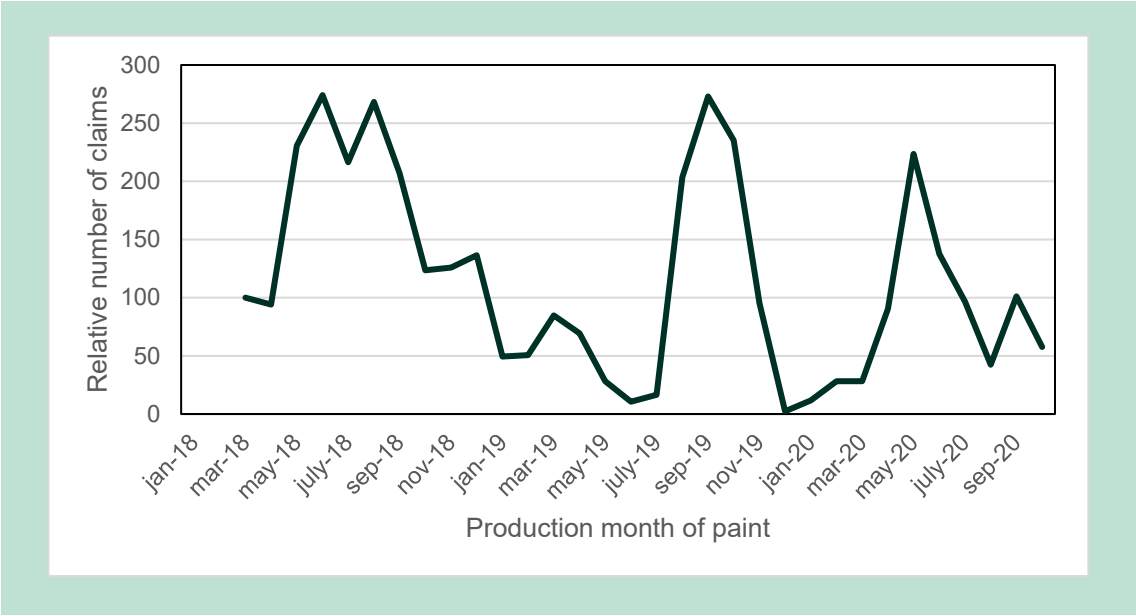

FIGURE 3. Production date for the items with claim type Rotten Paint produced at the paint production plant in Kolding, Denmark. March 2018 was used as index 100.

Interestingly, items produced in June 2018 had the highest incidence of complaints for rotten paint, while at the same time the samples taken for microbiological analysis (2.1.2, TABLE 2) did not reveal significant growth in any of the collected samples.

As mentioned previously, the Power Business Intelligence solution provides a total overview of e.g. claimed items, and when it comes to rot, there is especially one product group in focus (identity is kept as confidential information). This product group differs from other products mainly in its use of different raw materials.

Furthermore, registration shows that spoilage of paint seems to be linked to contamination during production and not by the end-user, as only 22 % of claims are registered as ‘rotten after use’, while 50 % are registered as ‘rotten before use’ FIGURE 4.

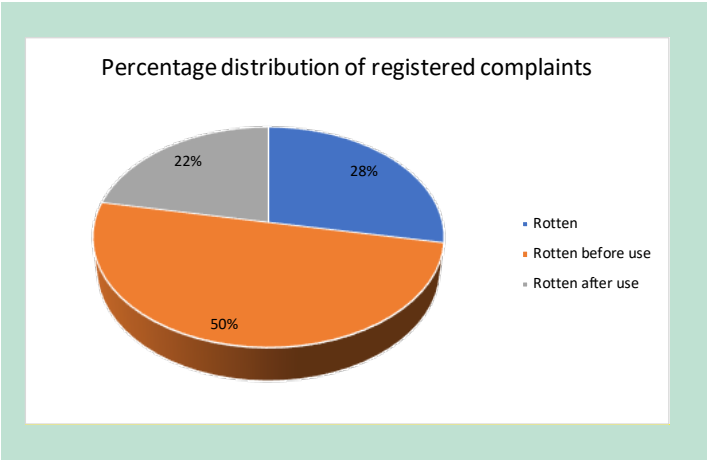

FIGURE 4. Relative number of registered claims for spoiled paint from January 2017 to December 2018.

In addition, some of the returned paints were subjected to microbiological analysis. The microbiological findings are given in FIGURE 5. Clearly, *Pseudomonas* is the one genus identified most often in the analysed spoiled paint.

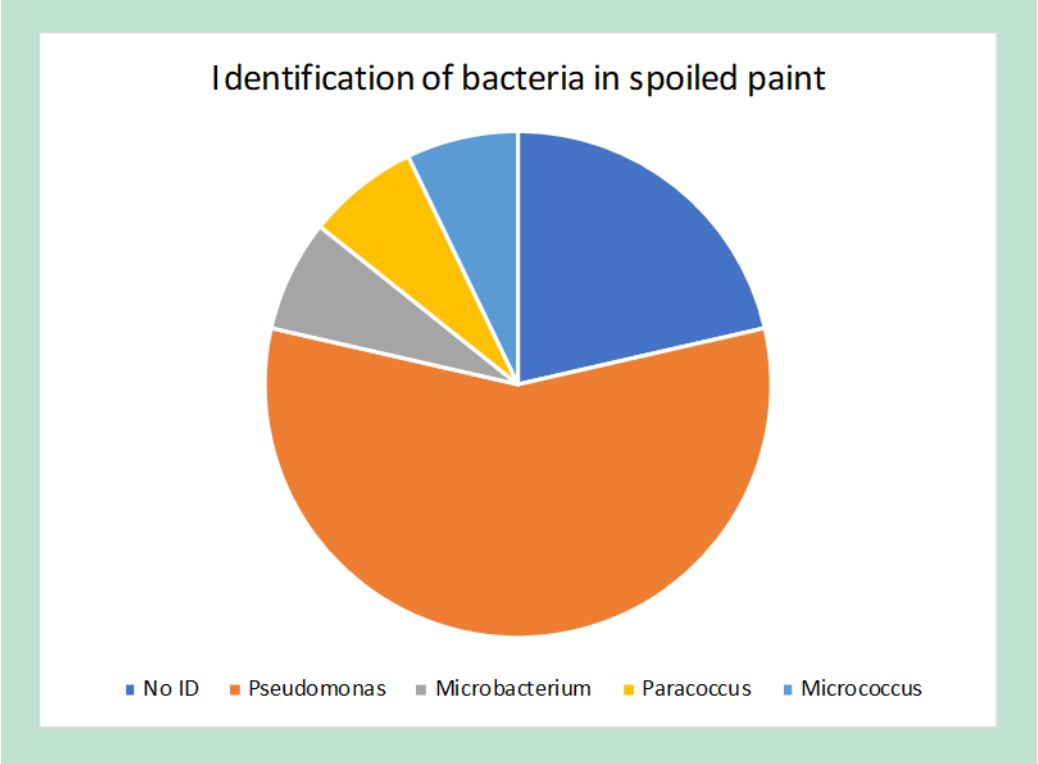

FIGURE 5. Identification of bacteria in spoiled paint performed using either 16S-rRNA sequencing or Maldi-TOF-MS on obtained pure cultures. A total of 14 samples were analysed.

2.1.4 Conclusion for task 1.1

Most claims regarding spoiled paint are registered as 'rotten before use', indicating contamination during production. Identification of bacteria isolated from spoiled paint were dominantly of the genus *Pseudomonas*. Analysis of whole pallets of paint showed sporadic occurrence of spoilage apparently randomly distributed throughout the pallet. Extraordinary collection of microbiological samples at the Flügger production site in Kolding, Denmark, revealed areas with very low levels or even absence of culturable microorganisms (i.e. filling stations, water supply), while other areas were more heavily inhabited by bacteria. Specific actions have been implemented resulting in the successful reduction of the microbial load at these sites. Interestingly, at some of these sites, bacteria of the genus *Pseudomonas* were found attached to surfaces of the production equipment. These findings indicate a direct link between bacterial biofilm in the production system and sporadic occurrence of paint spoiled by the same genus. Steps were taken to avoid the identified bacteria *Pseudomonas* in spoiled paints, including more frequent sampling, improved cleaning processes, and constructional changes at the production plant.

2.2 Activity 1.2 Identification of microorganisms tolerant to biocides

It has been hypothesized that bacteria in spoiled paint might be particularly tolerant or even resistant to the biocides used for preservation of paint. As the track and trace investigations point towards contamination during production, the majority of claims being 'rotten before use', the biocide tolerance of isolates from rotten paint was investigated by determination of minimum inhibitory concentrations (MIC).

The biocides used in the MIC tests are some of the most common biocides and represent a variety of what is used in the industry. The biocides are listed in TABLE 5 along with CAS number and status on the Biocidal Products Regulation (ECHA, 2020) as of December 2020.

TABLE 5. Overview of relevant biocides for in-can preservation of paint. Product Type 6 (PT6) under BPR concerns preservatives for products during storage. * CLP classifications that have consequences for use of the compounds in paint.

Compounds	CAS No.	Biocidal Products Regulation	CLP*
5-Chlor-2-methyl-2H-isothiazol-3-on/2-Methyl-2H-isothiazol-3-on (3:1) (CMIT/MIT (3:1))	55965-84-9	Approved in PT6 and on the art. 95 list	Skin sensitizer > 15 ppm
1,2-Benzisothiazol-3(2H)-on (BIT)	2634-33-5	Under review PT6 and on art. 95	Skin sensitizer > 500 ppm Risk for skin sensitizer > 15 ppm
2-Methyl-2H-isothiazol-3-on (MIT)	2682-20-4	Under review PT6 and on art. 95	Skin sensitizer > 15 ppm
2-Bromo-nitropropan-1,3-diol (Bronopol)	52-51-7	Under review PT6 and on art. 95	
Bis [1-hydroxy-2(1H)-pyridinethionato-O,S](T-4)-zinc (ZnPT)	13463-41-7	Under review. Candidate for substitution	Classified as Repr. 1B
Pyridine-2-thiol 1-oxide, sodium salt (Sodium pyrithione, NaPT)	15922-78-8	Under review	

In the spoiled paints, the bacteria survived addition of high concentrations of biocides and multiplied in numbers, causing e.g. malodour and discolouration of the paints. It is hypothesized that the bacteria are either biocide-tolerant or living in biofilm. To evaluate biocide tolerance of the bacteria isolated from the spoiled paints, minimum inhibitory concentration (MIC) of 12 biocides/biocide combinations (cf. TABLE 5 and further confidential compounds) were determined for three strains according to a standard MIC microbroth dilution method (Wiegand et al. 2008). In brief, the biocide-containing suspension was inoculated with bacterial suspension of 1-5 x 10^5 CFU/mL (CFU: colony forming unit). Following overnight incubation at 30°C, the solution was examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of biocide that prevents growth represents the MIC. The test was done in 96-well microtiter plates allowing multiple biocides in a range of concentrations to be tested in parallel.

The resulting data presented in TABLE 6 showed that all MIC values were below the biocide concentrations typically added to paint, suggesting absence of biocide resistance. However, MIC values are dependent on many factors, such as inoculum size, bacteria species and even bacterial strain and the growth medium. MIC for all biocides increased substantially when the number of bacteria subjected to biocide was increased as shown in TABLE 7. Many of the MIC values exceeded the typically added concentrations of biocides in paint, when higher numbers of bacteria were added in the test.

TABLE 6. MIC values of 12 biocides/biocide combinations in ppm. The bacterial inoculum size for testing was approximately 5 x 10^5 CFU/mL. Typical concentration of biocides in paints by 2018: 100 ppm MIT, 100 ppm BIT, 14.5 ppm CIT/MIT, 100 ppm ZnPT, 100 ppm Bronopol, and 100 ppm amine.

Biocides	Typical concentrations of biocides in paint (ppm)	P. aeruginosa (ATCC 15442) MIC ppm	Pseudomonas isolate 2 MIC ppm	Microbacterium (on site sampling #27) MIC ppm
MIT	100	20	10	80
BIT	100	176	44	11
CIT/MIT	14.5	3.8	7.5	3.8
MIT/BIT		20	10	20
NaPT		240	15	120
Bronopol	100	32	16	32
ZnPT/BIT		20	10	10
ZnPT	100	80	20	10
Amine	100	12.8	3.2	12.8
Amine/ZnPT		6.4/20	3.2/10	6.4/20
Amine/NaPT		6.4/30	3.2/15	6.4/30
Amine/BIT		6.4/10	3.2/5.5	3.2/5.5

TABLE 7. MIC values of 10 biocides/biocide combinations in ppm. The bacterial inoculum size for testing was 10^8 – 10^9 CFU/mL. Typical concentration of biocides in paints by 2018: 100 ppm MIT, 100 ppm BIT, 14.5 ppm CIT/MIT, 100 ppm ZnPT, 100 ppm Bronopol, and 100 ppm amine.

Biocides	Pseudomonas isolate 1 MIC ppm	Pseudomonas isolate 2 MIC ppm	Pseudomonas isolate 3 MIC ppm	Microbacterium (on site sampling #27) MIC ppm
MIT	>80	>80	>80	>80
BIT	350	700	350	88
CIT/MIT	>30	>30	>30	>30
MIT/BIT	>80	80	>80	>80
NaPT	120	120	>480	>480
Bronopol	>64	>64	>64	64
ZnPT/BIT	80	80	80	80
ZnPT	160	>160	>160	-
Amine	50	50	100	50
Amine/ZnPT	25.6/80	>25.6/80	25.6/80	25.6/80

In addition to these findings, literature (e.g. Garrod and Waterford, 1969, Buyck et al. 2012) and the experience of the project team indicate that MIC is also dependent on the growth media used for testing. In the industry, it is accepted knowledge that at least 2-3 times higher concentrations of biocides are needed in paint than in standard growth media to inhibit microbial growth. This general knowledge does indicate that MIC levels as given in TABLE 6 might underestimate the tolerance of bacteria to the investigated biocides in formulated paint.

Furthermore, identification of biofilm hotspots of spoilage bacteria in combination with the registered, sporadic occurrence of in-can spoilage indicates that individual buckets of paint might become contaminated by dislocated biofilm. And bacteria living in biofilm are notoriously known to have markedly elevated tolerance towards biocides.

2.2.1 Conclusion for task 1.2

The MIC tests suggest that the tested bacteria from the spoiled paint were not biocide resistant. Furthermore, MIC values are strongly dependent on the bacterial inoculum size, and on the growth medium. Given the localization of biofilm hotspots of typical spoilage bacteria (*Pseudomonas*), dislocation of biofilm, formed and dominated by *Pseudomonas*, could explain the occurrence of in-can spoilage of individual paint buckets,

3. Biocide concentration vs. biocide activity

Previously, it has been shown that the antimicrobial effect of added biocides in paint is markedly reduced compared to standard test media. Whether this is caused e.g. by negative interaction of biocides with paint components or by increased tolerance of the microorganisms towards biocides, e.g. due to different growth conditions, is not resolved. Here, different approaches are investigated to achieve a better understanding of this phenomenon and to provide novel solutions to ensure optimal activity of biocides in paint.

3.1 Activity 2.1 Evaluation of biocide activity in selected components

To ensure proper preservative effect in paint, it is necessary to choose the optimal in-can biocides. According to the Biocidal Products Regulation (BPR), there are around 50 biocides for in-can preservation (European Chemicals Agency, 2020). Due to reasons such as release of formaldehyde (regulated in ecolabel requirements), environmental demands for problematic substances (e.g. limits on volatile organic compounds, regulated in Directive 2004/42/EC on the limitation of emissions of volatile organic compounds), pH-stability of the biocide, risk of discolouration of paint, activity towards relevant microorganisms, and stability in paint, the shortlist of useful biocides for in-can preservation of paint is not 50 but between 5-10 depending on the formulation. Therefore, it is a matter of utmost importance that the biocides used in paint work optimally with the components in the paint system.

In our previous project *Reducing Biocide Concentrations for preservation of water-based paints* funded by the Danish Environmental Protection Agency (Poulsen et al., 2018), it was discovered that the biocidal efficacy in paint of in-can preservatives was much lower than expected based on standard test methods, i.e. when the same biocides were tested using standard growth media. Initial steps were taken to study the effect of paint components on the antimicrobial effect of biocides to identify components with a particularly negative effect.

3.1.1 Investigating the thermal interactions between paint components and biocides

It was hypothesised that the observed reduced inhibitory effect of biocides in paint systems could be due to molecular interactions of the biocides with paint components. As HPLC analysis of biocides added to paint can recover almost 100% of the added biocide, it is accepted that these biocides have neither been chemically altered in the paint nor are they covalently bound to other paint components. Based on this, it was decided to investigate whether isothermal titration calorimetry (ITC) could be a useful tool to investigate the proposed molecular interactions of biocides with paint components. ITC registers minute changes in temperature of a sample when a component (e.g. a biocide) is added to the sample (e.g. a binder). Such

changes in temperature can potentially be linked to non-covalent interactions such as hydrogen bonds between the sample and the added component. Strong non-covalent interactions of biocides with paint components could result in reduced antimicrobial activity of the biocide.

ITC analyses the thermodynamic interactions in a solution and is used in medical applications to study the binding of small molecules to larger macromolecules (e.g. Velázquez-Campoy et al. 2004). In the present project, it was used to investigate any thermodynamic interactions between selected binders and biocides.

Two binders based on vinyl acetate and styrene acrylate were tested with three different biocides, namely MIT, BIT, and Amine in a 2 x 3 matrix on the ITC. While the binders and biocides were diluted in demineralized water, the ratios between the binder and biocides were kept in a range relevant to paint formulation.

The initial results showed that both binders interacted with BIT and amine at elevated concentrations, but no thermodynamic interaction with MIT was observed. The binder based on styrene acrylate gave exothermal signals, whereas the vinyl acetate binder gave endothermal signals. While data were interesting as such, the observed interactions were at a very low level and could only be observed at very high concentrations of binder and biocide. It was not considered relevant to continue this line of investigation.

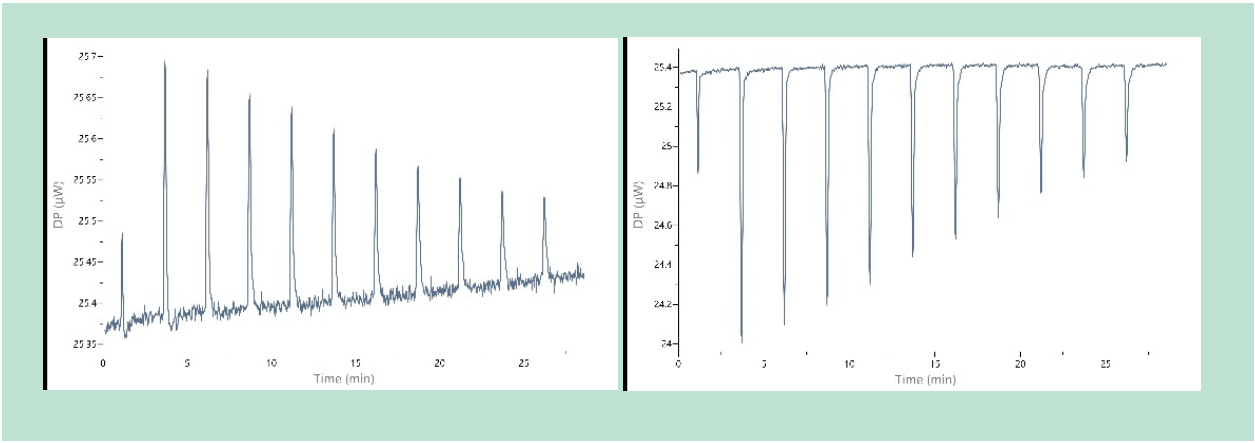

FIGURE 6. ITC results of the thermal reactions between BIT and the binders based on styrene acrylate (left) showing exothermal signals and vinyl acetate (right) showing endothermal signals.

3.1.2 Modified challenge test

In parallel to the investigation into thermodynamic interactions on a molecular level mentioned above, a less technically advanced, but possibly more practical approach to gain new insights on biocide efficacy in paint formulation, was initiated.

Based on the gained knowledge from work package 1, a modified challenge test for paint was developed. In particular, the following lessons learned from WP1 were useful:

- Most cases of spoiled paint were registered as ‘rotten before use’ and are thus associated with contamination during production.
- The biofilm-producing microorganism *Pseudomonas* is associated with most cases of spoiled paint.
- Spoiled paint is mostly stochastically distributed throughout a batch and rarely observed in whole batches.
- MIC concentrations of biocides are strongly dependent on the amounts of bacteria used as inoculum.

- There seems to be no indication that the identified bacteria are resistant to the biocides.

Standard challenge testing prescribes repeated, temporally spaced inoculation of the paint with 10^6 bacteria (ASTM, 2020). Such a test regimen does not represent the observations mentioned above. And as the data collected using the standard test method also did not correlate well with differences between paint types as observed in the registered claims for spoiled paint, a modified challenge test was developed.

This modified challenge test differs mainly in two aspects from the standard test:

1. Paint is only challenged with bacteria once, representing contamination during production.
2. Paint is challenged with different amounts of bacteria, representing the idea of detached biofilm from production to be the main source of contamination: up to eight 10-fold dilutions of an overnight culture of challenge bacteria (typically *Pseudomonas*) are produced and 1 mL of each dilution is added to each 9 mL of paint, typically resulting in a bacterial concentration in the challenged paint ranging from 10^1 to 10^8 CFU per mL paint. Plating and determination of CFU are performed 1 to 7 days after challenge.

TABLE 8. Data from an initial testing of the modified challenge test. Two types of paint were subjected to inoculum of 10-fold dilutions of *Pseudomonas putida* (DSM 1991). Read-out was a semiquantitative assessment of a number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 1 day after challenge		Plating 4 days after challenge		Plating 8 days after challenge 1:10 dilution
		Plate reading 3 days	Plate reading 8 days	Plate reading 4 days	Plate reading 7 days	Plate reading 6 days
Styrene acrylate-based paint	1.36×10^8	0	0	0	0	2
	1.36×10^7	0	0	2	2	2
	1.36×10^6	0	0	0	0	0
	1.36×10^5	0	0	0	0	0
PVA-based paint	1.36×10^8	0	0	2	2	3
	1.36×10^7	0	0	0	1	3
	1.36×10^6	0	0	0	0	3
	1.36×10^5	0	0	0	0	3

TABLE 8 shows data from an initial test of the modified challenge test. In this initial test, two different types of indoor paint with comparable standard biocide packages for in-can preservation were compared. From statistics on claims for spoiled paint (cf. work package 1), it is known that the polyvinyl acetate ethene-based paint is more prone to in-can spoilage than the styrene acrylate-based paint. This finding is reflected in the obtained data from the modified challenge test presented.

Similar differences in in-can preservation between these two types of paints were found when the test was repeated several months later, using a different in-house strain of *Pseudomonas* as challenge microorganism (TABLE 9), *Pseudomonas* isolate #1, isolated by Flügger.

TABLE 9. Comparison of preservation of two types of paint using the modified challenge test. Each of the 8 different 10 mL samples of each paint was subjected to one inoculum of a series of 10-fold dilutions of *Pseudomonas* isolate #1 obtained from rotten PVA-based paint. Read-out was a semiquantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 1 day after challenge		Plating 7 days after challenge	
		Plate reading 3 days	Plate reading 7 days	Plate reading 2 days	Plate reading 7 days
Styrene acrylate-based paint	6.04×10^8	3	3	0	3
	6.04×10^7	2	3	0	3
	6.04×10^6	2	2	0	1
	6.04×10^5	0	2	0	1
	6.04×10^4	1	1	0	1
	6.04×10^3	0	0	0	1
	6.04×10^2	1	1	0	1
	6.04×10^1	1	1	0	1
	0	1	1	3	3
PVA-based paint	6.04×10^8	3	4	3	3
	6.04×10^7	3	4	1	3
	6.04×10^6	3	3	1	2
	6.04×10^5	2	2	1	1
	6.04×10^4	0	2	1	1
	6.04×10^3	2	2	1	1
	6.04×10^2	1	1	0	1
	6.04×10^1	1	1	1	2
	0	1	1	0	1

Based on these data (and on data presented in activity 2.2), the modified challenge test was standardised to include 1) bacterial inoculum from 0 to 10^8 CFU per mL paint, 2) plating 1-2 days after challenge and 6-8 days after challenge, 3) use of 1:10 dilution when plating the challenged paint samples. Furthermore, it was decided to use the modified challenge test to investigate the effect of paint components on biocide activity and to evaluate novel biocide packages for paint.

3.1.3 Investigation of the influence of paint components on biocide activity using the modified challenge test

In our previous environmental project (Poulsen et al. 2018), it was hypothesised that the difference in binder between styrene acrylate and PVA-based paints might cause the observed difference in efficacy of the in-can preservation of similar biocide packages. As we could not conclusively link data obtained with ITC to differences in biocide efficacy (cf. section 3.1.1), it was decided to investigate whether such difference could be replicated using the modified challenge test.

A PVA binder on its own and all the other components of the PVA paint together were tested with the modified challenge test together with water as a control and compared to full PVA paint and styrene acrylate-based paint, presented in TABLE 9. While it is evident that the styrene acrylate-based paint is better protected against the used challenge microorganism than

the PVA-based paint, TABLE 10 indicates that the binder itself does not reduce the biocide efficacy to the same extent as the other paint components combined.

TABLE 10. Modified challenge test of the components used in PVA paint and water to compare with the full PVA paint formulation and the styrene acrylate-based paint presented in TABLE 9.

Product	CFU/mL sample	1. plating after 1 day		2. plating after 7days	
		plate reading 3 days	plate reading 7 days	plate reading 2 days	plate reading 7 days
PVA binder + full preservation	6.04×10^8	2	3	4	4
	6.04×10^7	2	3	0	0
	6.04×10^6	2	2	0	0
	6.04×10^5	2	2	0	0
	6.04×10^4	0	1	0	0
	6.04×10^3	0	0	0	0
	6.04×10^2	0	0	0	0
	6.04×10^1	0	0	0	0
	0	1	1	0	0
Other raw material + full preservation	6.04×10^8	4	4	3	4
	6.04×10^7	3	3	3	4
	6.04×10^6	2	3	1	3
	6.04×10^5	2	2	1	1
	6.04×10^4	1	1	1	1
	6.04×10^3	1	1	1	1
	6.04×10^2	1	1	1	1
	6.04×10^1	2	2	1	1
	0	1	1	1	1
Water + full preservation	6.04×10^8	0	0	0	0
	6.04×10^7	0	0	0	0
	6.04×10^6	0	0	0	0
	6.04×10^5	0	0	0	0
	6.04×10^4	0	0	0	1
	6.04×10^3	0	0	0	1
	6.04×10^2	0	0	0	0
	6.04×10^1	0	1	0	0
	0	1	1	0	0

From these results it is evident that change of the used binder but also mixture of additives will influence the ability of biocides to preserve the final paint. Binders will have different pH and added substances for reactions that will influence the stability of the used biocides but could also influence the biocide activity. To obtain full understanding of the influence on raw materials on preservation, detailed investigation needs to be performed, where single components are exchanged in formulations with similar biocide content. This may also include addition of different biocides and investigation of the oil-water distribution of the available biocides. However, these suggested investigations were not conducted in this project.

3.1.4 Testing with co-formulants

Certain raw materials used frequently in the paint industry with the purpose of obtaining specific properties in the paint itself may also support the activity of biocides, but they do not possess any biocidal activity themselves.

Two co-formulants were tested at two concentrations each in a standard preserved paint using the modified challenge test with just four bacteria concentrations (TABLE 11).

TABLE 11. Investigation of the addition of co-formulants on the in-can preservation of styrene acrylate-based paint protected with standard biocide package. Each of the 5 different 10 mL samples of each paint was subjected to one inoculum of a series of hundred-fold dilutions of *Pseudomonas* isolate #1 obtained from rotten PVA-based paint. Read-out was a semiquantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 2 day after challenge		Plating 7 days after challenge	
		Plate reading 4 days	Plate reading 7 days	Plate reading 2 days	Plate reading 7 days
Standard	9.00×10^9	4	4	4	4
	9.00×10^7	4	4	4	4
	9.00×10^5	3	4	4	4
	9.00×10^3	2	3	3	4
	0	0	0	0	0
0.075% Amine 1	9.00×10^9	1	1	4	4
	9.00×10^7	0	0	0	0
	9.00×10^5	1	1	0	0
	9.00×10^3	1	1	0	0
	0	0	0	0	0
0.025% Amine 1	9.00×10^9	4	4	4	4
	9.00×10^7	2	3	4	4
	9.00×10^5	1	1	2	2
	9.00×10^3	0	0	0	0
	0	0	0	0	0
0.075% Amine 2	9.00×10^9	4	4	4	4
	9.00×10^7	1	1	0	0
	9.00×10^5	0	0	0	0
	9.00×10^3	0	0	0	0
	0	0	0	0	0
0.025% Amine 2	9.00×10^9	4	4	4	4
	9.00×10^7	4	4	4	4
	9.00×10^5	2	3	4	4
	9.00×10^3	1	1	3	3
	0	0	0	0	0

All but one (the low concentration of Amine 2) of the formulations showed to have reduced growth of the challenge bacterium and a good preservation effect. This indicates that the

amines may influence the activity of the biocides. It should be noted that the amines themselves have no biocidal effect. It was also established that these recipes would have a low MAL-code and therefore could be approved environmentally. Efforts to include promising co-formulants in paint are continued beyond the scope of the present project.

3.1.5 Conclusion

The developed modified challenge test has provided useful information on the in-can preservation of paint as experience from production and claims registration could be replicated by laboratory testing.

It was demonstrated that certain raw materials otherwise used in the paint industry with no biocidal effect themselves could influence the activity of biocides used. This supports that further investigations and optimization of paint formulation may improve the efficacy of biocides in paints.

3.2 Activity 2.2 Incorporation and development of recipe with improved biocide activity

While the project has not succeeded in an identification of the root cause of poor and good in-can preservation of different paint recipes with comparable biocide packages, an urgent need to improve in-can preservation for certain paint recipes pushed the project team to test substitution of MIT with zinc pyrithione (ZnPt) in PVA-based paint using the modified challenge test. It might be worth mentioning that this decision was taking prior to the reclassification of ZnPt (cf. TABLE 5). The compounds is now classified as Reproductive 1B and aquatic acute 1 with a M-factor 1000, and as a consequence ZnPT will fall under the exclusion criteria within BPR and cannot be used in ecolabelled paints.

3.2.1 Novel biocide packages

As the in-can preservation of polyvinyl acetate-based (PVA) paint was not satisfactory (cf. section 3.1.2 and confidential data from Flügger's claims registry), the modified challenge test was used to test novel biocide packages to identify candidates for implementation in production.

Zinc pyrithione

From production, microbiological and regulatory perspectives it seemed obvious at the time (late 2018) on one hand to minimise the use of MIT and on the other hand to introduce zinc pyrithione in the biocide package for the PVA-based paint. Data from a modified challenge test comparing the original biocide package with the novel package where MIT was substituted by zinc pyrithione are given in TABLE 12. It is evident from TABLE 12 that the new biocide package performs better in the modified challenge test. The standard biocide package could only withstand an inoculum of 25 CFU per mL paint, whereas the new biocide package could withstand 2.5 million CFU per mL paint.

TABLE 12. Two different biocide packages were compared for their preservation of PVA-based paint upon challenge with 10-fold dilutions of *Pseudomonas* isolate #1 obtained from rotten PVA-based paint ranging from 10^8 to zero CFU per mL paint. Read-out was a semi-quantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 1 day after challenge		Plating 8 days after challenge	
		Plate reading 3 days	Plate reading 7 days	Plate reading 3 days	Plate reading 7 days
PVA-based paint Standard biocide package	2.50×10^8	4	4	4	4
	2.50×10^7	4	4	4	4
	2.50×10^6	3	3	4	4
	2.50×10^5	3	3	3	3
	2.50×10^4	2	2	3	3
	2.50×10^3	1	1	2	2
	2.50×10^2	0	0	2	2
	2.50×10^1	0	0	0	0
	0	0	1	0	0
PVA-based paint New biocide package with zinc pyrithione	2.50×10^8	1	1	3	3
	2.50×10^7	1	1	3	3
	2.50×10^6	0	0	1	1
	2.50×10^5	0	0	0	0
	2.50×10^4	0	0	0	0
	2.50×10^3	0	0	1	1
	2.50×10^2	0	0	0	1
	2.50×10^1	0	0	0	1
	0	1	1	0	0

Based on these data and further technical tests, Flügger decided to change in-can preservation of a number of PVA-based indoor wall paints to contain zinc pyrithione instead of MIT in Q1 2019. Collected data on the number of registered claims for rotten paint indicate that this novel biocide package actually performs better on the market (FIGURE 7). Overall, the frequency of claims was lower in 2019 than in 2018. The Flügger track and trace system allowed the identification of the exact product types and production batches related to the sudden increase in claim frequency from September to October 2019. Through cross-referencing of product recipes, a microbiologically contaminated batch of a paint ingredient was identified allowing the removal of the root cause of this incidence.

During the course of this project, ZnPt has been reclassified as Repr 2 (European Agency for Safety and Health at Work, 2020) within CLP. This may have an influence on future evaluation and approval of ZnPt within BPR. It is noted as being candidate for substitution due to the reclassification. Therefore, ZnPt should only be seen as an intermediate solution for in-can preservation.

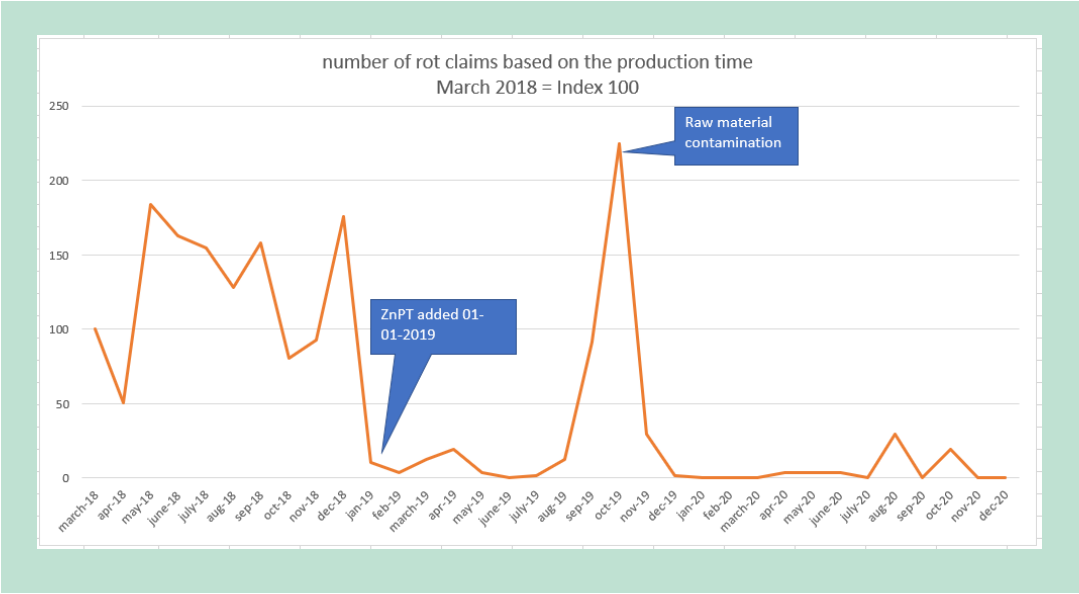

FIGURE 7. Relative number of monthly claims for 'Rotten paint' registered according to date of production for a PVA-based paint produced at the Flügger production plant in Kolding, Denmark. Monthly claims collected March 2018 served as index 100. Introduction of a new biocide package substituting MIT with ZnPt in January 2019 is indicated.

MIT

As MIT is classified as a skin sensitizer at concentrations >15 ppm (European Agency for Safety and Health at Work, 2020), there is a regulatory push to minimise the use of MIT for in-can preservation. Therefore, the modified challenge test was also used to evaluate the effect of removing MIT (100 ppm) from the existing biocide package for styrene acrylate-based paint and substituting the compound with BIT (255 ppm) (TABLE 13). While the original formulation performed very well, the substitution of MIT with BIT made the paint more prone to in-can growth of bacteria.

As MIT, due to the CLP classification as skin sensitizer (>15 ppm), *de facto* is not used for in-can preservation of paint anymore, these findings put further emphasis on the need for minimisation of microbial product contamination during production; further work on the improvement of the biocide package for in-can preservation of styrene acrylate-based paint is being performed beyond the present project.

TABLE 13. Two different biocide packages were compared for their preservation of styrene acrylate-based paint upon challenge with 10-fold dilutions of *Pseudomonas* isolate #1 from PVA-based paint ranging from 10^8 to zero CFU per mL paint: The standard preservation including 100 ppm MIT and a novel recipe where MIT is substituted by 255 ppm BIT. Read-out was a semiquantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 2 days after challenge		Plating 4 days after challenge	
		Plate reading 4 days	Plate reading 7 days	Plate reading 4 days	Plate reading 7 days
Styrene-Acrylate New preservation variant 255 ppm BIT	1 x 10^8	4	4	4	4
	1 x 10^7	4	4	4	4
	1 x 10^6	0	0	0	0
	1 x 10^5	0	0	0	0
	1 x 10^4	1	1	4	4
	1 x 10^3	0	0	0	0
	1 x 10^2	0	0	1	1
	1 x 10^1	0	0	1	1
	0	0	0	0	1
Styrene-Acrylate Standard preservation 100 ppm MIT	1 x 10^8	0	0	0	0
	1 x 10^7	0	0	0	0
	1 x 10^6	0	0	0	0
	1 x 10^5	0	0	0	0
	1 x 10^4	0	0	0	0
	1 x 10^3	0	0	0	0
	1 x 10^2	0	0	0	0
	1 x 10^1	0	0	0	0
	0	0	0	0	0

3.2.2 Paint with increased pH

On various markets new paints have been launched as biocide-free and typically with a much higher pH than for common paints. The high pH is suggested to result in a biostatic effect. The technologies used result in constraints related to gloss as only very low-gloss paints for walls (interior and exterior) are achievable.

In this project, various options to formulate paints with different gloss levels at a high and a medium/high pH level were investigated. In TABLE 14, four different paints formulated at around pH 11.5 were microbially challenged, all of them showing optimal biostatic effect. Over a 6-week storage period, pH was stable (TABLE 15) in these formulations, suggesting that the biostatic effect was maintained.

TABLE 14. Four high-pH paint product prototypes (pH 11.30-11.73) without biocides were tested by challenging with 10-fold dilutions of *Pseudomonas* isolate #1 from PVA-based paint ranging from 10^8 to zero CFU per mL paint. Read-out was a semiquantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product prototype	Inoculum (CFU/mL paint)	Plating 2 days after challenge		Plating 7 days after challenge	
		Plate reading 3 days	Plate reading 7 days	Plate reading 2 days	Plate reading 7 days
A pH 11.50	7.3×10^8	0	1		
	7.3×10^6	0			
	7.3×10^4	0			
	7.3×10^2	0			1
	0	1	1		
B pH 11.30	7.3×10^8	0			
	7.3×10^6	0			
	7.3×10^4	3	3		
	7.3×10^2	2	2		
	0	0			
C pH 11.73	7.3×10^8	0			
	7.3×10^6	0			
	7.3×10^4	0			
	7.3×10^2	0			
	0	0	1		
D pH 11.70	7.3×10^8	0			
	7.3×10^6	0			
	7.3×10^4	0			
	7.3×10^2	0			
	0	0			

TABLE 15. pH measured in high-pH paint product prototypes over time.

Product prototype	Day 0	Day 22	Day 44
A	11.50	11.52	11.30
B	11.30	11.50	11.35
C	11.73	11.54	11.38
D	11.70	11.69	11.53

Only very low-gloss paints may be formulated with the technologies used at high pH as also common on various markets. To achieve a higher gloss required for the majority of paints for interior walls on the markets particular in the Nordics, other technologies were screened with the aim to achieve improved paint characteristics at a lower pH level around pH 10.5. TABLE 16 shows marked differences in the in-can preservation characteristics of three different formulations based on styrene acrylic binders without addition of silicate. Final results and conclusions were not achieved within this project period, but further work is required to investigate if the new technologies will result in sufficient biostatic effects without the use of biocides.

TABLE 16. Three pH paint product prototypes with elevated pH (pH 10.56-10.66) without biocides were tested by challenging with 100-fold dilutions of *Pseudomonas* isolate #1 from PVA-based paint ranging from 10^8 to zero CFU per mL paint. Read-out was a semiquantitative assessment of number of CFUs: 0: no colonies, 1: few (1-10) colonies, 2: 10-100 colonies, 3: many (>100) colonies, 4: overgrown with colonies, too many to count.

Product type	Inoculum (CFU/mL paint)	Plating 4 days after challenge		Plating 7 days after challenge	
		Plate reading 3 days	Plate reading 7 days	Plate reading 2 days	Plate reading 7 days
1 pH 10.66	1.6×10^8	0	0	0	0
	1.6×10^6	0	0	0	1
	1.6×10^4	0	0	0	0
	1.6×10^2	0	0	0	0
	0	0	0	0	0
2 pH 10.62	1.6×10^8	0	0	0	0
	1.6×10^6	0	0	0	0
	1.6×10^4	0	0	0	0
	1.6×10^2	0	0	0	0
	0	0	0	0	0
3 pH 10.56	1.6×10^8	3	3	4	4
	1.6×10^6	3	3	4	4
	1.6×10^4	3	3	4	4
	1.6×10^2	3	3	4	4
	0	3	3	4	4

4. WP3 Sustainable product

Aiming at minimal use of resources in relation to production of water-borne paint, the options for reuse of water and non-biocide-based methods to prevent microbial growth were investigated.

4.1 Activity 3.1 Water treatment

The main liquid components in paint are water and binders. As most binders can support microbial growth, they are supplied to the production plant containing biocides to ascertain their preservation before addition to the paint production process. In general, routine microbiological control and project samples show no or very low levels of microorganisms in binders.

Water to be added to the paint, on the other hand, is delivered by the municipality in a microbiological quality according to the rules for drinking water. In Denmark, no antimicrobial strategies such as UV treatment or chlorination are used to achieve drinking water quality. While drinking water quality is good for consumption, the inherent low bacterial load in drinking water might be a source of microbial contamination in paint production. Bacteria such as for instance *Pseudomonas* species might enter the production system in low numbers through the drinking water supply. Proliferation of bacteria in Danish drinking water is limited due to the oligotrophic nature of the water and due to low temperatures of the groundwater-based drinking water supply. When entering a paint production system, the same few bacteria encounter a different environment with higher temperatures and more nutrients which could lead to e.g. biofilm formation in the production system.

To secure sufficient water supply during production of paint, water is often stored in large tanks, as direct supply from the municipal pipes will not supply sufficient quantities during a production. The microbiological quality of these tanks should be carefully monitored to maintain the low number of contaminants.

To minimise the microbiological load into the production system and into the paint itself, Flügger has evaluated different options for decontamination/disinfection of incoming drinking water to be used in paint production. Based on processing, environmental, and economic considerations, Flügger decided to install a continuous chlorination unit on the buffer tank at the drinking water inlet into the production facility in Kolding.

Routine microbiological surveillance and project samples show that this treatment is effective in producing production water of very high microbiological quality with a bacterial load below the detection limit of 0.1 CFU per mL, cf. section 2.1.2 (TABLE 2).

The project ambition to reduce the amount of water used for cleaning and flushing the production system was counterbalanced by a need for improved cleaning to reduce microbial contamination of the produced paint. This was clearly emphasised by the detection of *Pseudomonas* in connection with the reuse of cleaning water in the pigging system investigated in December 2018 (cf. section 2.1.2). As a result of the need for intensified cleaning of the production system to ensure product integrity, it was not possible to reduce the amount of water for cleaning and system flushing during the course of the project (TABLE 17).

TABLE 17. Generation of wastewater in relation to production of paint at the Flügger production plant in Kolding, Denmark.

Year	Production volume (m^3)	L waste water/L product
2017	21,509	0.380
2018	21,543	0.402
2019	23,374	0.375

In order to obtain further reductions of water transferred to wastewater treatment, various initiatives have been initiated. During 2019, the first phase of introducing pipes including a pigging system was implemented. This system is able to reduce the build-up of biofilm dramatically and a reduced amount of water is required for cleaning. Furthermore, a new filter station was installed to improve the ability to clean these difficult-to-clean parts of the production process. The second phase of the novel pigging system will be more extensive and is expected to be operational in the beginning of 2021.

Both pigging systems and pigging stations are new and will include installation of new pipes to make flushing and cleaning with the pigs possible. The system will allow full cleaning of pipes from the beginning of the production all the way to the pipes in the filling process. During autumn 2020, a first investigation was initiated with the purpose of further reducing generation of wastewater by recirculating cleaning water back into the production process. There will be special focus on the biological challenges during the process. The conclusions of the study were not available by the end of this project.

4.2 Activity 3.2 Product treatment

Besides the main components water and binder, paint contains further components delivered either in a solution or as dry raw materials. Investigations performed in the previous MUDP project (Poulsen et al. 2018) and continuous routine microbiological analyses performed by Flügger on these components in general reveal no high bacterial loads. Also, it is of interest to note that the microbiological species most often associated with spoilage of paint (especially *Pseudomonas*) are only rarely found in these raw materials. In general, no direct link between the microbiological quality of raw materials and in-can spoilage has been identified neither in the present nor in the former project (besides the specific incidence illustrated in FIGURE 7 where microbiologically contaminated raw material was delivered to the production facility). Therefore, it is at present not considered relevant to disinfect/treat these materials further to minimise their microbiological load. However, careful monitoring might be necessary to ascertain the microbiological quality of raw materials to reduce the risk of contamination further, in particular in relation to future paint products with even lower biocide concentrations.

The reported findings of work package 1 led to the conclusion that biofilm formation inside the production plant had profound impact on the occurrence of failed in-can preservation detected as claims on rotten paint before use by customers. Furthermore, it was evident that standard cleaning routines and standard plant hygiene surveillance could not always ascertain hygienic production of paint.

Therefore, a task force was established to translate project findings and learnings to technical solutions that could be implemented at full scale on factory level.

This included:

- Simplification of the piping system and plant layout under consideration of hygienic design principles.
- Design of novel automated pigging and cleaning stations based on the intermittent, experimental station investigated in WP1, allowing Flügger to use cleaning pigs in the whole piping system.
- Optimisation of cleaning procedures.
- New biological test method for quality control of selected raw materials.
- Further intensification of biological control of production equipment.

FIGURE 8. Details of renovated factory with filter station, station with pipes assigned for filling station and pipes prepared for optimal cleaning.

As mentioned earlier in this report, the production plant in Kolding is currently undergoing comprehensive rebuilding and redesign with the aim of minimising the risk of introducing spoilage bacteria into paint during production.

The effect of these interventions combined with changes in product formulation and the use of different biocide combinations were followed by daily updated registration of product claims for rotten paint by customers and stores.

As indicated in FIGURE 9, the focus on new cleaning procedures starting 2018/19 did have a positive effect in 2019 on the reduction of claims due to contamination. The introduction of the new pigging system in part of the production and the new filter station by the end of 2019 added to the positive development. An unforeseen contamination in a delivered raw material had a negative effect on claims in 2019, but a further focus on deliveries of materials and hygiene resulted in expected very low incidences of contaminated paints.

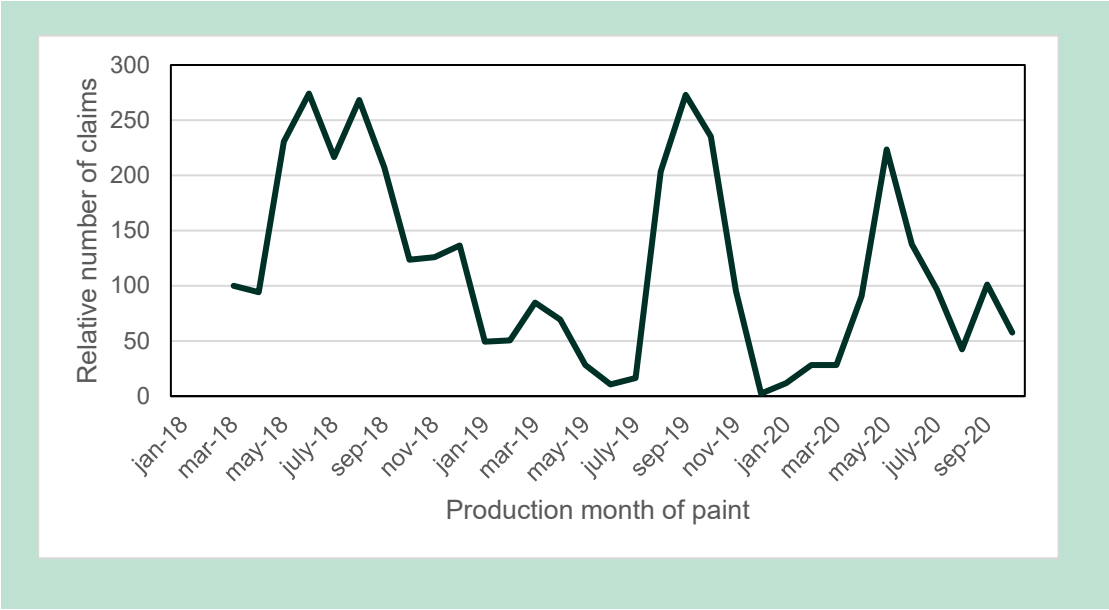

FIGURE 9. Production time for the items with claim type Rotten Paint produced at the paint production plant in Kolding, Denmark. March 2018 was used as index 100. The same data are presented as FIGURE 3.

With the background of the improved plant hygiene, a program was initiated to further reduce the amounts of biocides used. In TABLE 18, the amount of isothiazolinones and amine used in a paint is given. This example indicates that a reduction of biocides used in certain paints is possible but only when the introduced cleaning procedures are followed, equipment for production is installed giving optimal options for cleaning, and the biological quality of essential raw materials including water is in focus.

TABLE 18. Relative amounts of in-can preservatives used in a typical paint over the course of the last five years. Biocide content in 2016 was used as index 100.

Year	Isothiazolinones	Amine	Total
2016	100	100	100
2017	No data	No data	No data
2018	91	100	94
2019	71	100	78
2020	65	100	79

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Eco-friendly production of waterborne paint

The project revealed that contamination of paint was not due to bacteria being resistant to biocides but that biofilm residing at hard-to-clean sites inside the production system was the main reason leading to failed in-can preservation. This finding led to modernization of the production facility, increased focus on cleaning procedures, including deployment of cleaning pigs and improved monitoring of the microbial status of raw materials and the production system. Together these initiatives resulted in minimized contamination of produced paint and a reduction in the number of claims regarding 'rotten paint'. In parallel to improved hygiene, the project group worked to improve the antimicrobial efficacy of biocides for in-can preservation. In this context a new method for fast and reliable testing of the resilience of preserved paint against microbial growth was developed. This method was extensively used to aid the development of improved paint formulations with respect to avoid microbial deterioration. The new paint formulations included implementation of new biocide-packages for more traditional paints with lower biocide concentrations Furthermore new developed paint using medium to high pH as antimicrobial hurdle gave promising results. While marked improvements have been achieved during the project, for the near future it is obvious that in-can preservation of paints remains necessary. However, the tools for preservation may become even more environmentally and societally sustainable.

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