BIOCIDES IN PAPERMAKING CHEMISTRY

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List of Abbreviations

BNPD	2,2-Dibromo-3-nitrilopropionamide
BCDMH	1-Bromo-3-chloro-5,5-dimethylhydantoin
BT	Benzothiazole
BHAP	2-Bromo-4-hydroxyacetophenone
CMIT	5-Chloro-2-methyl-4-isothiazolin-3-one
CFU	Colony forming unit
CRT	Cathode-ray tube
DBNPA	2,2-Dibromo-2-cyanoacetamide
DBNPA	2,2-Dibromo-3-nitrido-propanamide
DDH	1,3-Dichloro-5,5-dimethylhydantoin
GC	Gas chromatography
HOBT	2-Hydroxybenzothiazole
HPLC	High-performance liquid chromatography
MIT	2-Methyl-4-isothiazolin-3-one
MIC	Microbialy influenced corrosion
MBT	Methylene bisthiocynate
MITC	Methyl isothiocyanate
MTBT	2-(Methylthio)benzothiazole
MAE	Microwave assisted extraction
2-MBT	2-Mercaptobenzothiazole
OD	Optical density
OIT	2-n-Octyl-4-isothiazolin-3-one
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SPE	Solid phase extraction
SEM	Scanning electron microscopy
TCMTB	2-(Thiocyanatomethylthio)benzothiazole
THPS	Tetrakis(hydroxymethyl)phosphoniumsulfate
THPO	Tris hydroxymethylphosphine oxide

1 Introduction

Paper consists of a web of pulp fibres derived from wood or other plants from which lignin and other non-cellulose components are separated by cooking in a congruent way with chemicals at a high temperature. In the final stages of papermaking an aqueous slurry of fibre components and additives is deposited on a wire screen and water is removed by gravity, pressing, suction and evaporation /1/. Growth of micro organisms in the above paper manufacturing processes can cause major technical, economic and hygienic problems mainly due to slime formation /2/. The chemical-physical conditions and the compositions of microorganisms may vary widely in one process and between processes. Resistant strains can evolve following repeated treatment by certain biocides. Biocides are added to the wet end process to prevent slime formation. Introduction of neutral or alkaline sizing instead of acidic papermaking, the closed water circuits and the increasing proportions of recycled paper have required changes in biocide types in order to control different microbial populations. Biocides are also used in the pulp and paper industry for the protection of processing materials. The main biocides used in this application fall into the preservative range and can be different from others employed in paper processing.

In the 1920s and 1930s the most common compound used in the paper machine circulations for bacterial control was chlorine, with an addition of copper sulfate to control fungal growth dripped into the white water chest /3/. In the 1950s to 1960s organic mercury compounds like phenylmercuric acetate and ethylmercury salts were introduced to kill microbes in the deposits /3,4/, However, due to their severe environmental impact, these compounds were prohibited or abandoned by the industry /5,6/. They were often used in combination with chlorophenates /3/. Several new organosulfur compounds were also introduced in the 1960s to be used in combination with the chlorophenates. Already in the 1960s, chlorine dioxide was described as a new treatment of machine circuits, with the comment that it cannot be used for more than two weeks due to its bad fungicidal effect /3/. The quaternary ammonium compounds and other surface active so called germicides, at that time, were also introduced and found to keep the machine surfaces cleaner than other substances /3/. Organic tin compounds like distannoxane, bis (tri-n-butyltin) oxide in combination with quaternary ammonium compounds were also used although they caused problems by being

extremely toxic for fish and other aquatic life. Chlorophenols have been banned due to their high fish toxicity /6/.

Annually, over 200 million euros are spent on slime prevention by the paper industry in Europe /7/. Biocide dosing is the most common way to combat microbes. Biocides either stop their activity and multiplication (biostatic) or they permanently destroy the microbe cell (biocidal) /8/. A typical biocide programme is composed of individual treatments of the different parts of the system, such as incoming raw water, additive storages, paper machine water circuits and the broke system. The type of biocide to be selected depends on the point of application, the target and chemical compatibility to the system.

From this discussion it is clear that decent study about the identification of microorganisms and their treatment with decent biocides at every point is of great interest both technically and economically for the paper industry. This is further highlighted in detail in the next topics.

1.1 Types of micro organisms occuring in papermaking process

The types of microbial species encountered in the papermaking process may be diverse with aerobic and anaerobic bacteria, fungi and yeast appearing throughout the process /9/. In particular, bacterial species, such as *pseudomonas*, are found in the water source. These species utilize the food sources available from the additives to produce extra cellular polysaccharides known as slimes. The types of microorganisms usually encountered in the papermaking process are described in Table 1. The main microorganisms in the overall paper industry are described in Table 2.

Table 1. **Types of micro organisms encountered in the papermaking process /9/**

Type	**Microorganism**	**pH**
Aerobic bacteria	*Acromonas*	3.5 - 9.5
	Escherichia coli	3.5 - 9.5
	Pseudomonas	3.5 - 9.5
	Flavobacter	3.5 - 9.5
Anaerobic bacteria	*Sulfate reducers*	3.5 – 10.0
Fungi	*Aspergillus*	2.0 – 7.0
	Cladosporiun	2.0 – 7.0
	Penicillium	2.0 – 7.0
	Trichoderma	2.0 – 7.0
Others	Yeast/Protozoa/Nematodes	2.0 – 7.0

Table 2. Main microorganisms occurring in the paper industry /10/

1. Aerobic bacteria

Spore forming

Bacillus subtilis, B. cereus, B. megaterium, B. mycoides

Non- sporulating

Achromobacter, Acinetobacter, Aeromonas, Beggiatoa, Citrobacter,Corynebacterium, Enterobacter, Escherichia, Flavobacterium,Gallionella, Klebsiella,Lepthotrix, Micrococcus, Pseudomonas,Sphaerotilus,Staphylococcus, Thiobacillus

2. Anaerobic bacteria

Spore forming

Clostridium

Non-sporulating

Desulfovibrio, Actinomycetes

3. Fungi

Moulds

Alternaria, Aspergillus, Fusarium, Penicillium, Phialophora, Phycomyces,Trichoderma

Yeast

Candida, Geotrichum, Monilia, Rhodotorula, Saccharomyces, Torula

4. Algea

Blue-green

Asterionella, Navicula, Oscillatoria

Green

Chlorococcus

1.2 Runnability and corrosion due to microbes

Unless microbial growth is controlled, e.g. with biocides, these tiny organisms can bring the large, modern, computer-controlled, hi-tech paper machines, up to 9 meters wide and 200 m long, to a standstill /11/. Bacterial metabolism in biofilms may release aggressive metabolites such as organic (acetic, succinic, isobutyric, etc.) or inorganic acids (sulphuric). The patchy nature of biofilms may generate regions with different oxygen content on the underlying steel surface. Both of these properties may promote corrosion /12/. This type of corrosion is called microbial influenced corrosion (MIC). It is not a "new" mechanism of corrosion, but merely indicates that bacterial activities have created conditions for "normal" corrosion to proceed. Some of the microbe slimes that form on papermaking machines are described in Fig. 1 /12/.

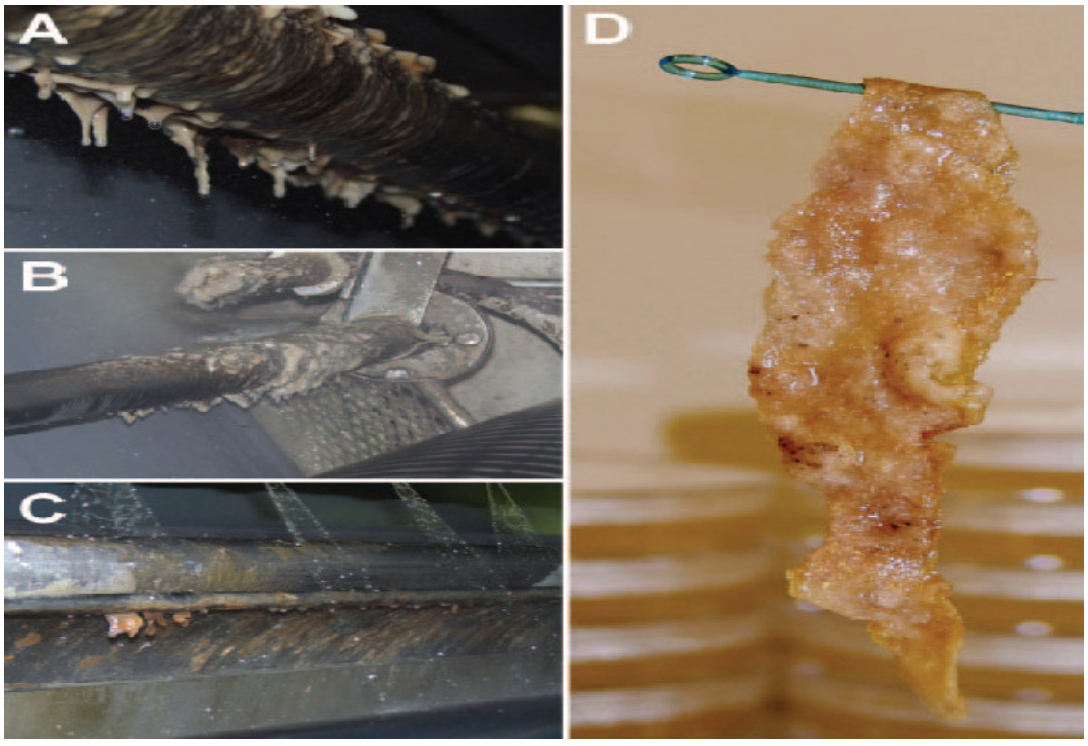


Figure 1. Photographs of biofilms (microbe slimes) that have grown on surfaces in the splash areas of the wet-end part of a paper machine (A-C). Areas that are constantly wet due to mist (B) or water sprays (C) are prone to be biofouled. Microbes can form coherent sheets of slime (D) that are likely to cause serious process disturbances if they detach from the machine surfaces /12/.

1.3 Classification of biocides

Classifying industrial biocides based on their chemical structures is a difficult task /13/. Some articles classify the biocides on group basis and include a miscellaneous group whose member do not fit in any major class. Sometimes biocides are also grouped on the basis of their mode of action. This can be organized based on the target region of the microorganism affected by biocide action. Related to biocides some terms and categories such as membrane active biocide and permeabilizers, cellwall inhibitors, cytotoxic agents (affecting targets in cytoplasm and interfering with metabolism and total cell function), and genotoxic agents (affecting DNA biosynthesis and reacting with DNA) have also been occasionally used.

For industrial use biocides are generally divided into two groups, Oxidizing biocides and non-oxidizing biocides /13/. Oxidizing biocides are relatively fast-killing and the use of oxidizing biocides has continued to increase due to its lower cost than non-oxidizing biocides. In some respects, non-oxidizing biocides are more effective and convenient than oxidizing biocides; therefore both biocides are used together in many conditions such as cooling water systems.

1.4 Types of biocides used in the papermaking process

The types of biocides being used in the papermaking process is still heavily regulated /2/. Slimicides, as they are commonly known, are used to reduce the buildup of slime deposits within the water phase of the process. Many changes have occurred over the last 20 years within the European papermaking industry. Most systems have now changed from acidic processing to alkaline one and the majority has closed up their processing, i.e. the water is now recycled within the plant and not discharged. Hence microbial contamination has shifted in favor of bacterial strains (due to alkaline conditions) and also increased due to the continuous re-introduction of these species and food sources back into the process. Good plant hygiene procedures and proper use of biocides is critical to maintain effective production and low microbiological population to prevent paper failure. The types of biocides commonly used in the papermaking process are described in Table 3.

Table 3. Most common types of biocides being used in the paper making process /2/

Biocides Used
Halogenated hydantoin
2-Bromo-2-nitropropane-1,3-diol
5-Chloro-2-methyl-4-isothiazolin-3-one
2,2- Dibromo-2-cyanoacetamide (DBNPA)
n-Octyl-isothiazolin-3-one
Methylene bisthiocyanate (MBT)
Quaternary ammonium compounds
Tetrakis(hydroxymethyl)phosphonium sulfate (THPS)
Glutaraldehyde

The types of biocides used in the coating and additives for the paper making process differ from the rest of the pulp and paper industry. These require longer lasting, sometimes up to one year's protection in storage tanks, and hence fall into the category of preservatives. The types of preservatives that are commonly encountered in the paper industry are described in Table 4 below.

Table 4. Preservatives found in the coatings/additives application /3/

Preservatives Used
Thione
Glutaradehyde
1,2-Benzo-isothiazolin-3-one
Bronopol
1,2-Dibromo-2,4-dicyanobutane
4,5-Chloro-2-methy-4-isothiazolin-3-one

1.5 Function mechanisms of biocides

Biocide mechanisms of action can be divided into four broad categories (Fig. 2). The oxidants include rapid speed of kill agents such as chlorine and peroxides that work directly via radical-mediated reactions to oxidize organic material /14-17/. The electrophilic agents include inorganic ions such as silver, copper and mercury, and

organic biocides such as formaldehyde and isothiazolones. These biocides react covalently with cellular nucleophiles to inactivate enzymes /18,19/ and there is evidence that they initiate the formation of intracellular free radicals which contribute to their lethal action /20,21/. Cationic membrane active biocides such as chlorhexidine and quaternary ammonium compounds /22,23/, and alcohols such as phenoxyethanol /24/ destabilize membranes leading to rapid cell lysis. Weak acids, such as sorbic and benzoic acids, interfere with the ability of the cell membrane to maintain a proper pH balance. This results in the accumulation of anions and cations inside the cell. The inhibition of cell growth by preservatives has been proposed to be the result of different actions including disruption of membrane, inhibition of metabolic reactions, stress on intracellular pH and the accumulation of toxic anions /25/. Pyrithione is also classified as a weak protonophore /26/. The general mechanism of biocide action over various microbes is described in Fig. 3 /27/.

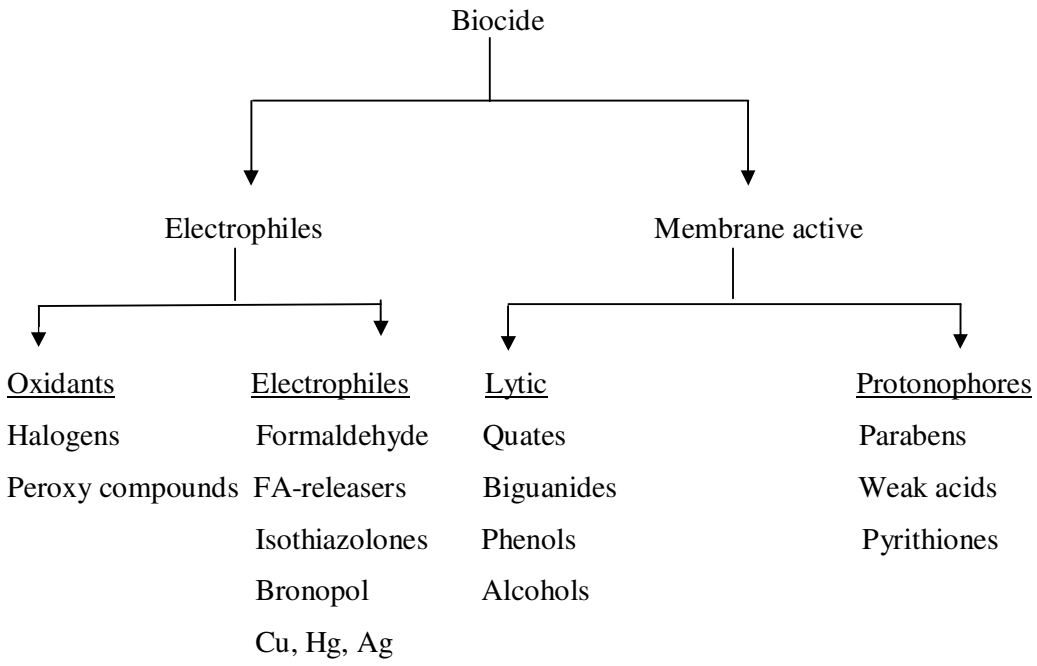


Figure 2. Mechanism of action of biocides /16/.

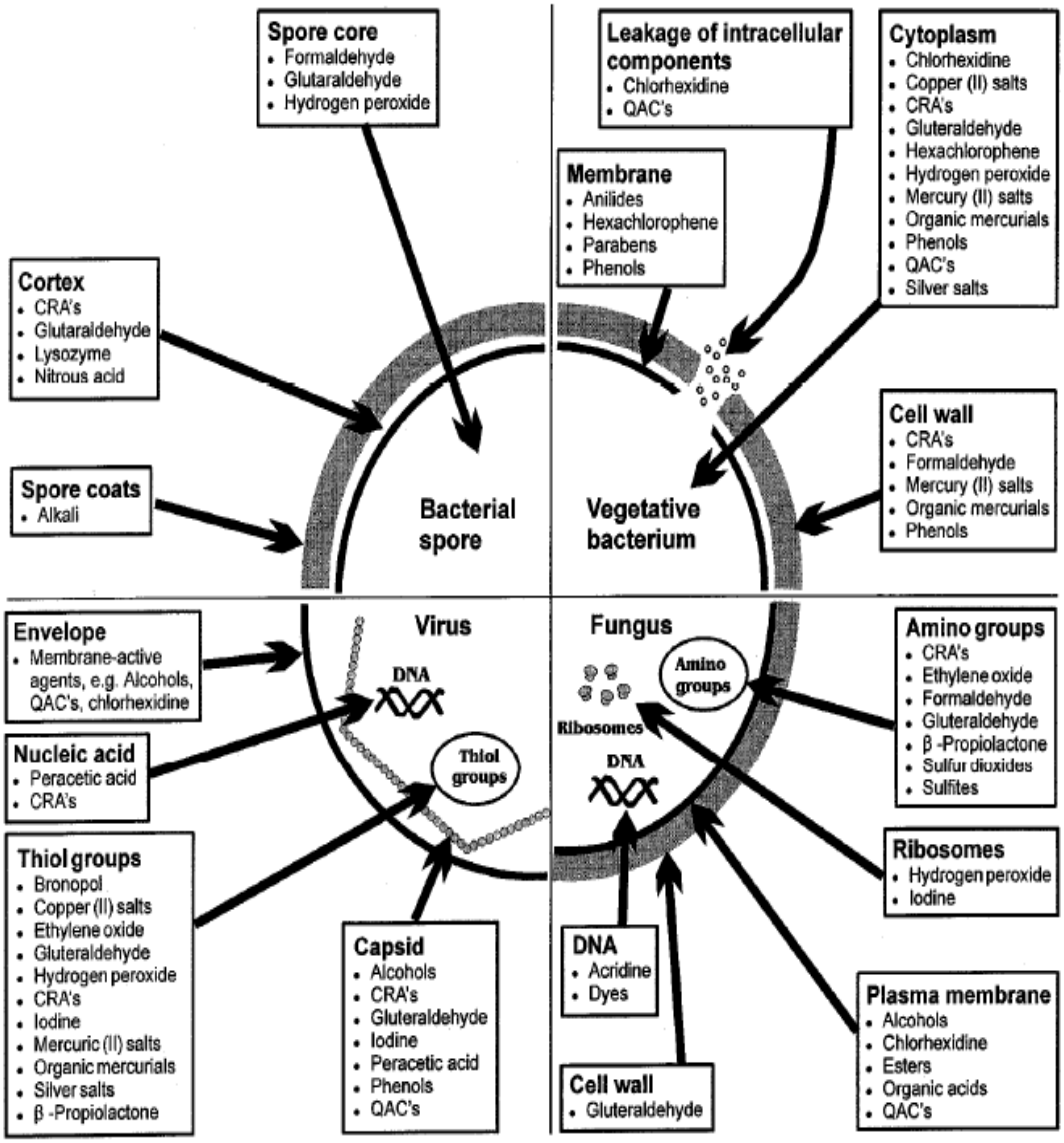
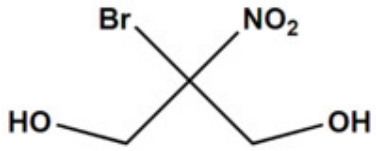


Figure 3. Mechanisms of microorganism inactivation by biocides CRA's = chlorine-releasing agents, QAC's = quaternary ammonium compounds /27/.

2 Chemical structures of biocides

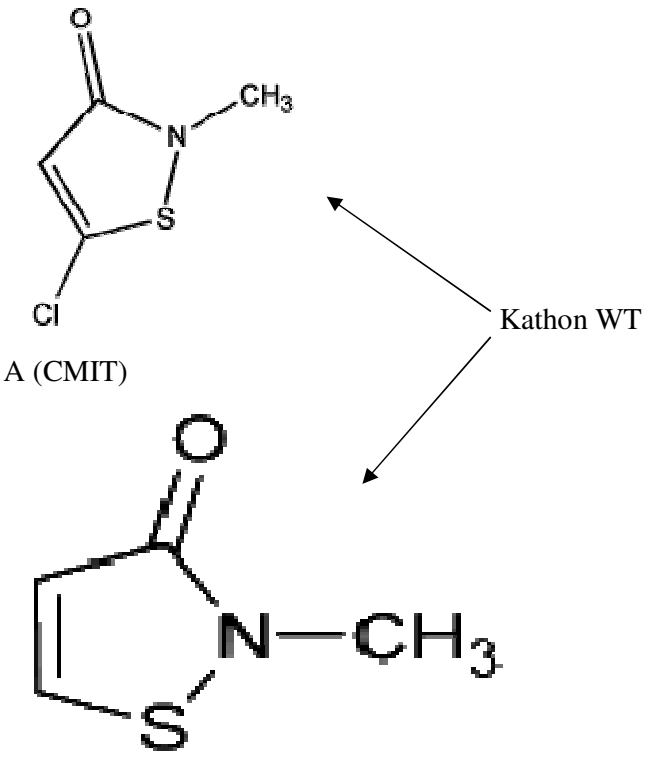
The chemical structures of the 13 most widely used biocides in papermaking are described as follows:

1)



2-Bromo-2-nitropropane-1,3-diol (Bronopol).

2)



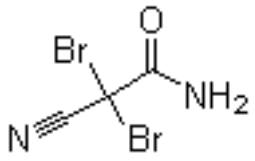
A (CMIT)

B (MIT)

A) 5-Chloro-2-methyl-4-isothiazolin-3-one (CMIT)

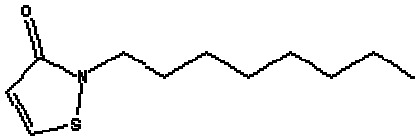
B) 2-Methyl-4-isothiazolin-3-one (MIT)

3)



2,2-Dibromo-3-nitrilopropionamide.(DBNPA)

4)

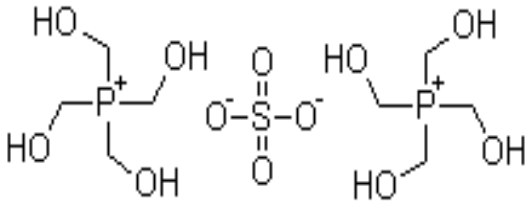


2-n-Octyl-4-Isothiazolin-3-One (OIT or Kathon 893)

5)

Methylene bisthiocynate (MBT)

6)

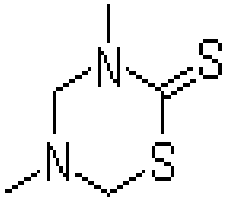


Tetrakis(hydroxymethyl)phosphonium sulfate (THPS)

7)

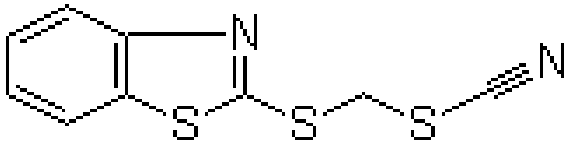
1,5-Pentanedial (Glutaraldehyde)

8)



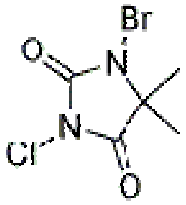
3,5-Dimethyltetrahydro-1,3,5-thiadiazine-2-thione (Dazomet)

9)



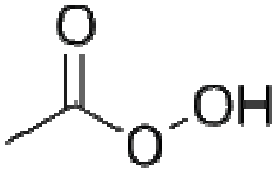
2-(Thiocyanomethylthio)benzothiazole (TCMTB)

10)



1-Bromo-3-chloro-5,5-dimethylhydantoin (BCDMH)

11)

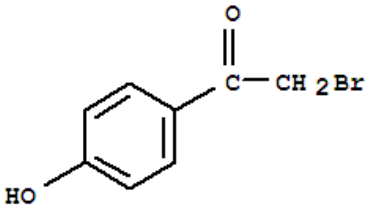


Ethaneperoxoic acid (Peracetic acid)

12)

Chlorine dioxide (Chlorine Peroxide)

13)



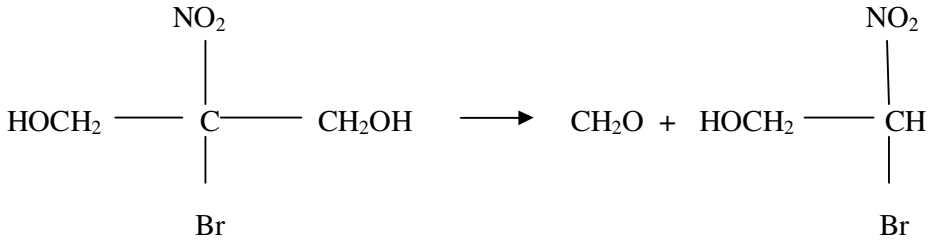
2-Bromo-4-hydroxyacetophenone (BHAP)

3 Physical and chemical properties

3.1 Bronopol (2-Bromo-2-nitropropane-1,3-diol)

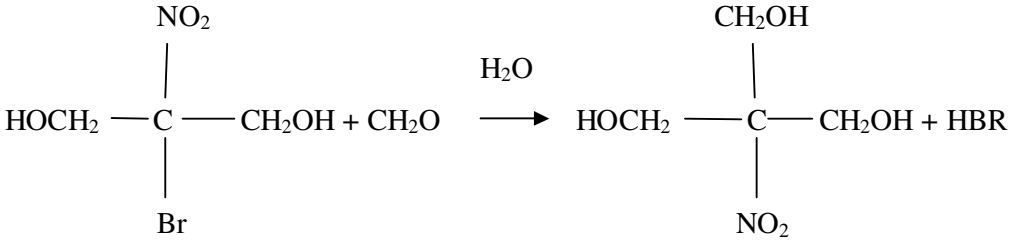
Bronopol is a white crystalline odorless substance melting at about 130 ºC. It is well soluble in water, lower alcohols, acetic acid, diethyl ether, and ethylacetate, but poorly soluble in chloroform and acetone, and virtually insoluble in hydrocarbon solvents /28/. Its solubility values in some of the solvents are as follows (wt./vol.% at 22- 25 ºC): water, 28; methyl alcohol, 89; ethyl alcohol, 56; isopropyl alcohol, 41; ethylene glycol, 61; methylcarbitol, 54; 1,2-propylene glycol, 52; dipropylene glycol, 48; polyethylene glycol, 300, diethyl sebacinate, 10; isopropyl myristate, mineral oil, and vegetable oils, less than 0.5. Aqueous solutions of the pure compound have pH 5.0-5.5, which is explained by the mobility of hydroxyl hydrogen atoms. The compound in the solid state can be stored for 3 years and longer, and it is not affected by factors such as daylight, air humidity (up to r.h. 90 %), and temperature (up to 45 ºC) /28,29/. However, aqueous solutions of Bronopol are stable only in the cold, provided that the acidity is sufficiently high. An increase in the pH and temperature leads to decomposition of the compound as a result of formaldehyde splitting. The initial process in the decomposition of Bronopol

appears to be a retroaldol reaction with the liberation of formaldehyde and the formation of bromonitroethanol as follows /29/.



Bronopol **(Equation 1)**

Bromonitroethanol itself is considerably less stable than Bronopol and in the range of conditions investigated its maximal concentration did not exceed 0.5 % of the initial Bronopol concentrations. Simultaneously a second-order reaction involving Bronopol and formaldehyde occurs to give 2-hydroxymethyl-2-nitro-1,3-propanediol as follows:



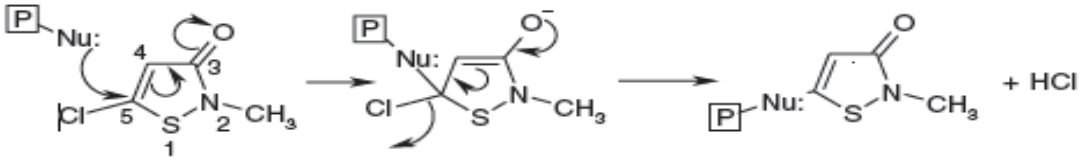
(Equation 2)

The antimicrobial activity of Bronopol is mostly due to the presence of electron-deficient bromine atoms in the molecules, which exhibit oxidation properties rather than the ability to liberate formaldehyde /28/. The mechanism of the antimicrobial effect of bronopol consists of the cross-linking of sulfohydride groups of dehydrogenase enzymes occurring on the surface of microbial cells. The disulfide bridges block microbial metabolism.

3.2 Kathon WT(5-Chloro-2-methyl-4-isothiazolin-3-one/2-Methyl-4-isothiazolin-3-one)

The active ingredient of Kathon WT biocides is a mixture of two isothiazolones identified by the IUPAC system of nomenclature as 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-Methyl-4-isothiazolin-3-one (MIT). Kathon WT has amber-gold color, is completely soluble in water having mild odor and specific gravity 1.32 at 20 ºC. Kathon is stable over a wide range of conditions found in cooling water and papermill applications. Kathon WT is an extremely effective, broad spectrum microbiocide which causes an immediate inhibition of growth on coming in contact with a microorganism. It is effective over a wide pH range and is therefore ideal for use in the alkaline conditions that exist in multi-cycle cooling towers and modern papermaking /30/.

Kathon WT rapidly interacts with proteins within the cell causing an inhibition of respiration and ATP synthesis, which results in an inability to synthesize biopolymers or catabolize substrate. Growth inhibition rapidly becomes irreversible and results in cell death when essential proteins are progressively oxidized. Even before cell death occurs, the Kathon-treated organisms are unable to synthesize either biodegradative enzymes or the exopolymers which facilitate microbial adhesion and biofilm formation. The proposed reaction of CMIT with nucleophiles and conjugate addition at the C-5 position followed by chlorine elimination; Nu, nucleophile; P, protein confirmed by ^{13}C NMR is shown in Eq. 3 below /30/.



(Equation 3)

3.3 DBNPA (2,2- Dibromo-2-cyanoacetamide)

DBNPA is a white crystalline powder having melting point of 124.5 ºC, water solubility 15000 mg/L at 20 ºC and vapour pressure 9.00 E-4 mm of Hg at 20 ºC /31/. DBNPA

has low vapour pressure and high water solubility which makes the compound to be retained mainly in the water phase. However, DBNPA has a short half-life and is rapidly degraded in water by hydrolysis. DBNPA is not an oxidizing biocide and bromine releasing. DBNPA does act similarly to the typical halogen biocides. The liquid formulated DBNPA is an oxidizer, and the solid formulated DBNPA as a tablet is not an oxidizer. The liquid formulation is oxidizing because of the hypobromous acid in the formulation. DBNPA readily degrades under alkaline conditions. It is sensitive to UV light and nucleophilic substances. It is uncharged and non surface active and it seems unlikely to interact with Wet-end additives /32/. DBNPA dissolves in water forming a relatively stable solution in an acid pH range. Its unusual solubility and stability in polyethylene glycol (average molecular weight, 200Da) make this glycol a preferred solvent. Aqueous solutions hydrolyze under alkaline conditions with the rate of decomposition increasing with the alkalinity. However, the rate of hydrolysis is not fast enough to interfere with the antimicrobial activity of fresh, alkaline (pH 7 to 9.5) solutions /33/.

DBNPA is a powerful biocide with two unique properties: it kills microorganisms immediately upon addition and it degrades rapidly /34/. Although DBNPA is compatible with many chemical classes, including oxidizing agents, it will react readily with nucleophilic agents and sulfur-containing reducing agents. The facile reaction of DBNPA with sulfur-containing nucleophiles common to microorganisms, such as glutathione or cysteine, is the basis of its mode of antimicrobial action. DBNPA is therefore not a typical oxidizing or halogen-releasing biocide. Unlike other thiol-reactive biocides, its action is such that thiol-based amino acids, like cysteine, are oxidized beyond the formation of disulfide species. This reaction irreversibly disrupts the function of cell-surface components, interrupting transport across cell membranes, and inhibiting key biological functions. DBNPA degrades rapidly by both nucleophilic and hydrolytic pathways to relatively nontoxic products. The rate of hydrolysis of DBNPA is strongly pH-dependent: at pH 6.0 and 25 ºC, the DBNPA molecule has a half-life of 155 hours (about 6.5 days), but at pH 8.0 and 25 ºC, its half-life is about two hours. The ultimate degradation products of DBNPA are ammonia, carbon dioxide, and bromide ion.

The mechanism for the environmental degradation of a DBNPA has been described by Exner *et al*. /34/. An overall scheme based on their work is given in Fig. 4. This

illustrates the two competing pathways: (1) DBNPA~DBAM (dibromoacetamide) and (2) DBNPA~ CAM (cyanoacetamide). For the first pathway, they provide data on hydrolysis rate measurements for DBNPA~DBAN (dibromoacetonitrile) ~ DBAM. The second pathway was defined as occurring in the presence of nucleophiles or sunlight.

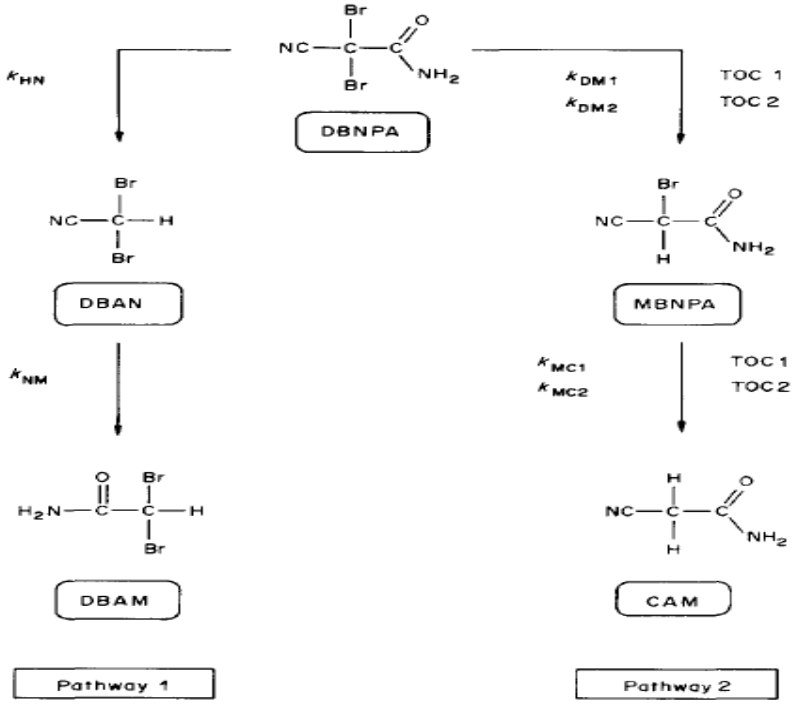


Figure 4. Chemical degradation model for DBNPA with rate constants indicated, DBNPA (dibromonitrilopropionamide), DBAN (dibromoacetonitrile), DBAM (dibromoacetamide), MBNPA (monobromonitrilopropionamide), CAM (cyanoacetamide) /35/.

3.4 OIT or Kathon 893(2-n-Octyl-4-Isothiazolin-3-One)

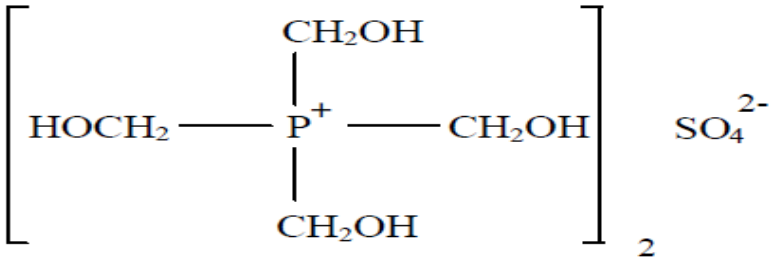
OIT is a yellow liquid miscible in water and oil and stable in light and pH~9.5. 2-N-octyl-4-isothiazolin-3-one is effective against mildew, bacteria and fungi. OIT also belongs to the isothiazolinone group which in general all are electrophilic molecules containing an activated N-S bond that enables them to react with nucleophilic cell entities thus exerting an antimicrobial action /36/.

3.5 MBT (Methylene bisthiocynate)

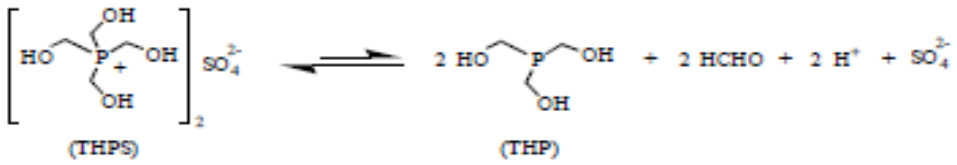
Methylene bis(thiocyanate) is a yellow to light orange solid that melts at 105-107 ºC. It has limited solubility in water (< 1 mg/mL) but is soluble in organic solvents and therefore it is usually formulated with dispersants or supplied as a water slurry /37/. The toxic mechanism of MBT responsible for biocidal activity is not well understood. However, a suggested mechanism is competitive inhibition of cell respiration. Since it is a competitive microbiocide, it inactivates the electron transfer cytochromes of the microorganisms. The thiocyanate fragment, NCS-CH_2-SCN, of the methylene ester of the thiocyanic acid reacts to blocking the transfer of electrons in the microorganism, resulting in cell death. Methylene bisthiocyanate is pH sensitive and rapidly hydrolyses in the pH range above 8.0. For this reason it is not recommended for use in systems where the recirculating water pH generally exceeds 8.0 /38/.

3.6 THPS (Tetrakis (hydroxymethyl) phosphonium sulfate)

THPS is fully water soluble and a clear colorless liquid. It is highly effective against sulfate reducing bacteria (SRB) having an odor that resembles aldehydes and is stable for 14 days at temperatures 21-54 ºC /39/. THPS is a quaternary phosphonium salt having the following structure /40/:

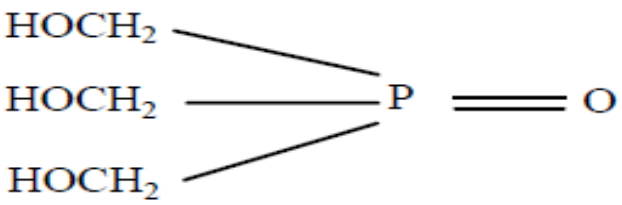


Aqueous solutions of THPS are acidic (pH~ 3,2) due to the small dissociation of THPS to tris(hydroxymethyl)phosphine(THP).P(CH2OH)3, formaldehyde, HCHO, and sulfuric acid (Eq 4)(pk_a= 12.20) /41/.



(Equation 4)

THPS is also readily biodegradable and has no potential to bioaccumulate. A further environmental benefit is that THPS is rapidly oxidized in the environment to THPO (trishydroxymethylphosphine oxide) which has a very low aquatic toxicity and is not considered to present an environmental hazard. THPO has the following chemical structure /40/:



The low toxicity of THPO is illustrated by the data: Rainbow Trout, 96 hour LC50: > 5000 mg/L; Daphnia Magna, 48 hour EC50: > 1000 mg/L and *Skeletonema Costatum* EC50: 2090 mg/L. Both THPS and THPO will also photodegrade in the environment. Based on these and other data, compared to conventional biocides, THPS offers real environmental benefits.

3.7 Glutaraldehyde (1,5-Pentanedial)

Glutaraldehyde was first synthesized by Harries and Tank in 1908 /42/. The saturated 5-carbon dialdehyde is an amber coloured liquid usually supplied in solutions of acidic pH. As with other aldehydes, the two aldehyde groups react readily under suitable

conditions, particularly with proteins /43/. It is miscible with water and having melting and boiling points -14 ºC and 187 ºC respectively. The ratio of monomer to polymer and type of polymer present has been the subjects of numerous publications. The dialdehyde existed as a monomer (25%, Fig. 5a) in equilibrium with the cyclic hemiacetal (Fig. 5b). It has been stated that the presence of free aldehyde groups is necessary for biocidal activity. From an H-NMR study on commercial (aqueous) acid glutaraldehyde it has been proposed that the protein cross-linking reactions are possible due to the presence of α, β - unsaturated aldehydes (Fig. 5a) /43/. The pure acid glutaraldehyde underwent very rapid hydration on dissolution in water to give three hydrates in equilibrium (Fig. 5 a, b, d, e). An acetal-like polymer (Fig. 5c) similar to that suggested by Aso and Aito was also shown to exist in acid solution /44/.

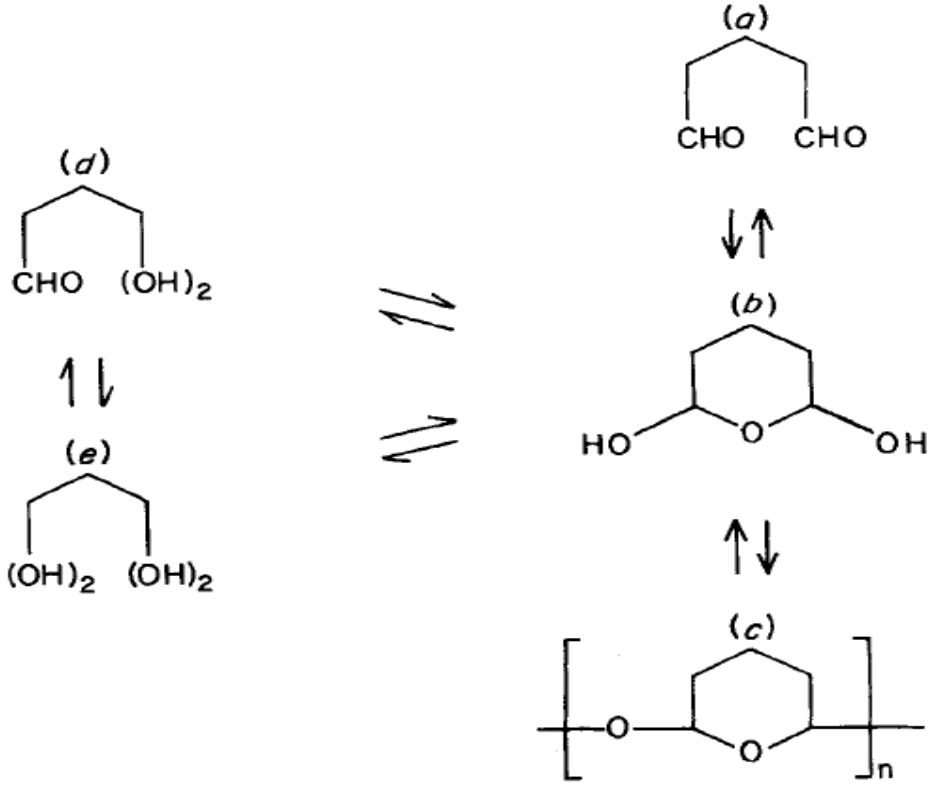


Figure 5. Scheme for glutaraldehyde polymerization in aqueous acid media. (a) Monomer, (b) cyclic hemiacetal, (c) acetal-like polymer, (d) monohydrate, (e) dihydrate /42/.

A scheme depicting glutaraldehyde polymerization in acid and alkaline solutions is outlined in Fig. 6 /42/. An increase in temperature produces more free aldehyde in acid solution whereas in alkaline solution loss of reactive aldehyde groups is possible. Progression to the higher polymeric form (Fig. 6b) could occur with increased time and

pH because it has been shown that there is an extensive loss of aldehyde groups from polymerization in alkaline solution /45/. Therefore loss of reactive aldehyde groups could be responsible for the rapid loss of biocidal activity of alkaline solutions in storage. Increased biocidal activity in heated acid solutions can also be explained by displacement of equilibrium towards the monomer.

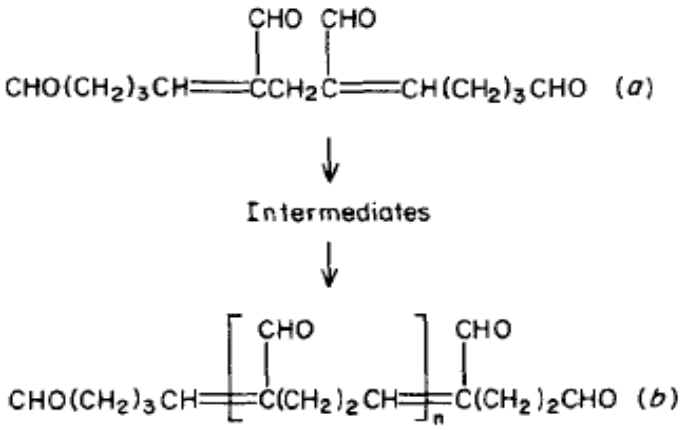


Figure 6. Scheme for glutaraldehyde polymerization in aqueous alkaline media. Aldol-type polymer (a) progression to higher polymeric form (b) with time and increased pH /42/.

Glutaraldehyde is an agent which acts as a protein cross-linker and is used as a biocide. It is able to bridge aminoacids or H- bonds, thereby modifying the folding of the proteins and stopping its activity as described in Fig. 7 /42/. It is thus likely to react and be consumed by wet-end additives that carry an amine function /33/.

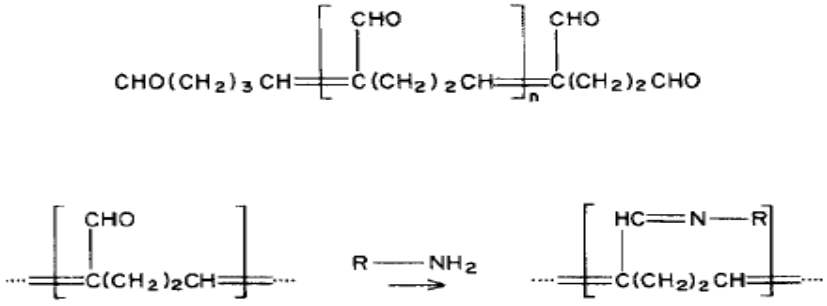


Figure 7. Reaction of aldol-type polymers of glutaraldehyde with amino groups /43/.

The dialdehyde reacted with 30-50% of the E-NH2 groups in the isolated peptidoglycan and it was proposed that two tripeptide side-chains could be joined when free and amino

groups are available (Fig. 8) /46/. Cell wall peptidoglycan (murein, glycopeptide, mucopeptide) contains many chemical groupings capable of reaction with glutaraldehyde. The effect of lysozyme on the isolated wall peptidoglycan of *B. subtilis*, was studied and it was found that although splitting of the lysozyme-sensitive bond occurred, glutaraldehyde-treated peptidoglycan was less sensitive than the untreated polymer to lysis by lysozyme /46/.

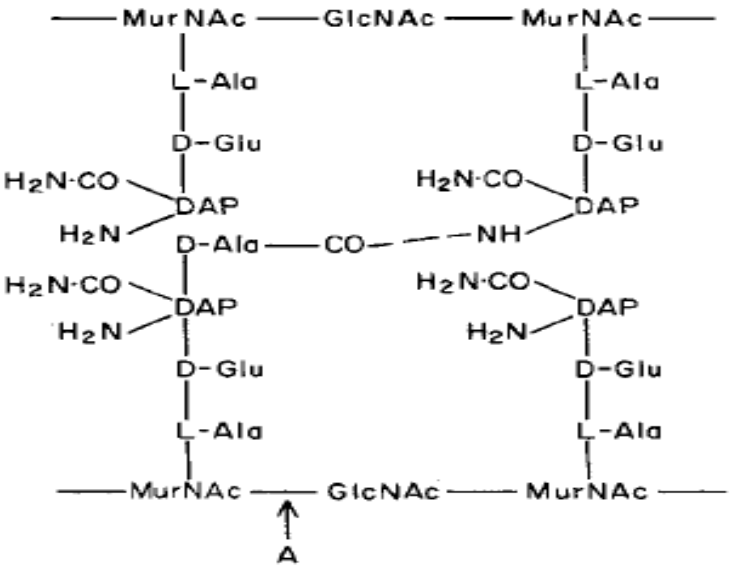


Figure 8. Peptide side-chains in Bacillus subtilis peptidoglycan showing availability of amino groups for interaction with glutaraldehyde /46/.

3.8 Dazomet (3,5-Dimethyltetrahydro-1,3,5-thiadiazine-2-thione)

Dazomet is used as a slimicide in paper mills. It has a melting point of 104-105 ºC, Flash point 156 ºC, water solubility < 0.1 g/100 mL at 18ºC and storage temperature 0-6 ºC. Dazomet is degraded by hydrolysis in an aqueous solution within a time period of days /47/. Dazomet, a biocide with good activity in principle, suffers in particular in products adjusted to an alkaline pH (for example talcum or calcium carbonate slurries) rapid degradation with release of toxic and highly odorous gases /48,49/. Dazomet is rapidly degraded in aqueous media to carbon disulfide, formaldehyde, and methylamine, with half-lives in the order of 3–20 days. However, in moist soil it degrades mainly to Methylisothiocyanate. It is unstable and decomposes to methylamine in water, probably via thiocarbamic acid. Multiple products are observed including methyl isocyanide, sulfur dioxide, hydrogen sulfide, N-methyl formamide,

methylamine, and carbonyl sulfide. Methyl isocyanide in turn degrades to methyl isocyanate as described in Fig. 9 /50/.

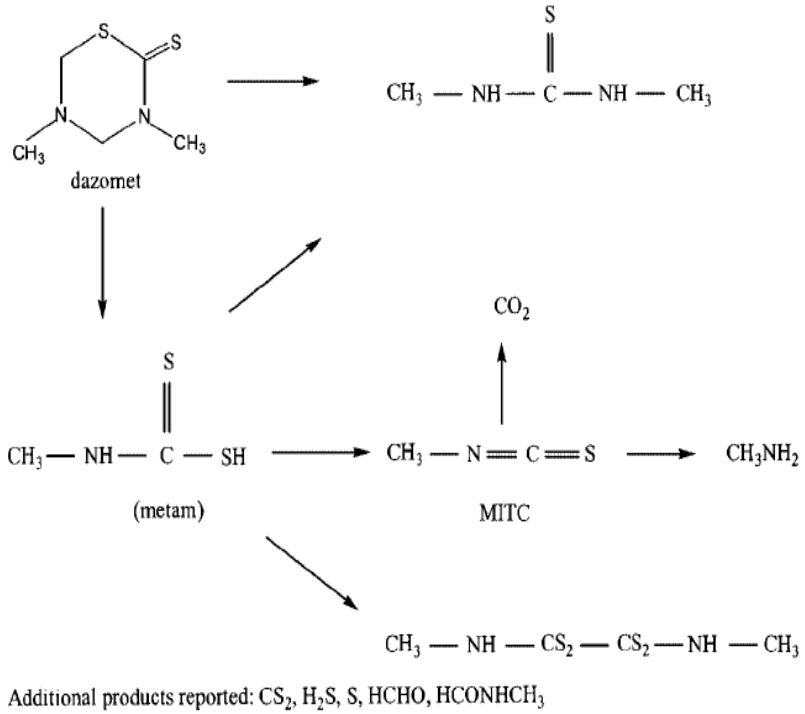


Figure 9. Degradation of dazomet, metam and MITC in water and soil /50/.

3.9 TCMTB (2-(Thiocyanomethylthio)benzothiazole)

In its pure form, TCMTB is a white crystalline while technical grade TCMTB is a black viscous liquid. It forms the active ingredient of the commercial fungicide Woodstate 30WBR. It has low aqueous solubility having boiling point: >120 ºC, Melting point: <-10 ºC, Relative density (water = 1): 1.4, Solubility in water, 100 g/mL: 0.0033 /51/. The substance decomposes on heating in the presence of sulfide or when exposed to sunlight producing toxic fumes including hydrogen cyanide, nitrogen oxides and sulfur oxides /52/. Hydrolysis and/or photolysis of TCMTB results in 2-MBT, which can photolyze to benzothiazole and 2-Hydroxybenzothiazole or undergo biomethylation to 2-(Methylthio)benzothiazole. At pH 5 TCMTB is stable; at pH 7 TCMTB is slowly hydrolyzed; and at pH 9 the hydrolysis is rapid. The major breakdown pathway of TCMTB results from photolysis and is described in Fig.10 /53/.

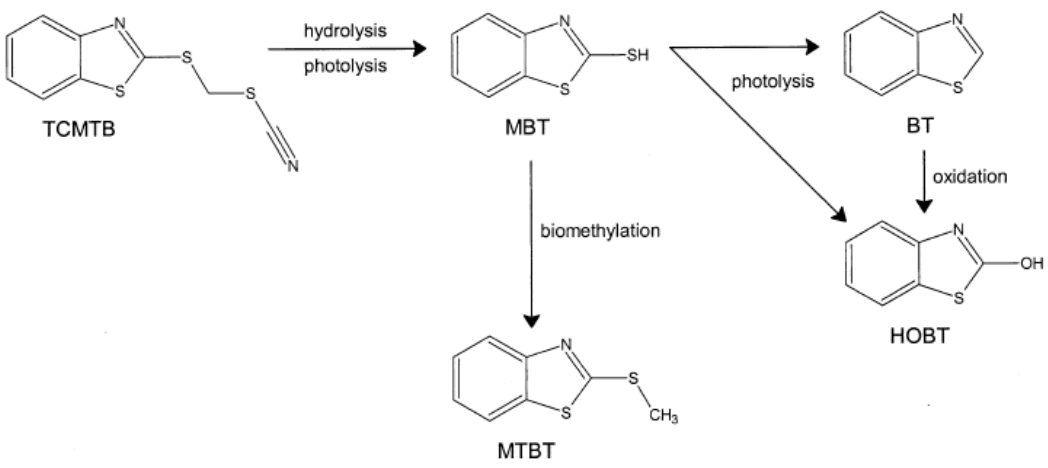
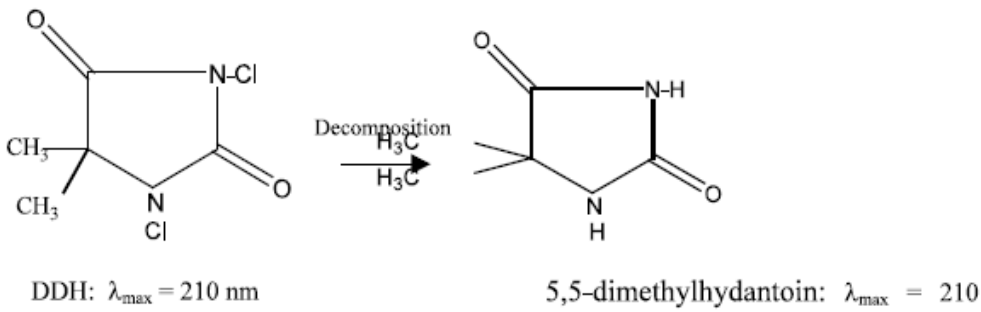


Figure 10. Proposed degradation and transformation pathway of TCMTB in the aquatic environment, TCMTB = 2-(thiocyanomethylthio)benzothiazole /53/.

3.10 BCDMH (1-Bromo-3-chloro-5,5-dimethylhydantoin)

It is a white crystalline compound slightly soluble in water having a melting point of 159 – 163 ºC. 1,3-Dichloro-5,5-dimethylhydantoin (DDH) belongs to the family of imidazolone compounds /54/. This compound shows low solubility in water, but ppm levels are enough to serve as a good disinfectant and bactericide because it slowly decomposes to produce free chlorine in water /54/. After this process, the remaining compound (5,5-dimethylhydantoin) can be rapidly decomposed into ammonia and carbon dioxide by light, oxygen and microorganisms without leaving any environmentally polluting residues. The decomposition reaction of DDH into 5,5-dimethyhydantoin is described below in Eq. 5 /54/.



(Equation 5)

3.11 Peracetic acid (Ethaneperoxoic acid)

PAA is a clear, colorless liquid with no foaming capability. It has a strong pungent acetic acid odor (acetic acid is the principal component of vinegar) and has an acidic pH of less than 2. It is soluble in water in all proportions and in polar organic solvents. However, it is slightly soluble in aromatic solvents. Peracetic acid or peroxyacetic acid (PAA) is the peroxide of acetic acid (AA). PAA is a strong oxidant and disinfectant. Its oxidation potential is larger than that of chlorine or chlorine dioxide. PAA is commercially available in the form of a quaternary equilibrium mixture containing AA, hydrogen peroxide (HP), PAA, and water as shown by the following equation /55/.

$CH_3CO_2 + H_2O_2 \rightarrow CH_3CO_3H + H_2O$ **(Equation 6)**

Where

CH_3CO_2H = acetic acid

CH_3CO_3H = peracetic acid

H_2O_2 = hydrogen peroxide

The decomposition products of PAA are acetic acid, hydrogen peroxide, oxygen, and water. There are three reactions in which PAA is consumed in an aqueous solution: spontaneous decomposition, hydrolysis, and transition-metalcatalyzed decomposition. In Finland, the PAA mixture Desirox has been successfully used to control microbial growth in waters of the paper mill process /56/.

3.12 Chlorine dioxide (Chlorine peroxide)

Chlorine dioxide (ClO_2) is a powerful selective oxidizing agent. It is a greenish yellow gas at room temperature that is very soluble in water and having melting and boiling points of - 59.5 ºC and 11 ºC, respectively. Unlike chlorine, chlorine dioxide is not a chlorinating agent and pure chlorine dioxide does not form THMs, and it does not chlorinate organics. It also does not react with water to form free chlorine or react with ammonia to form chloramine. Additionally, ClO_2 is soluble in water and has effectiveness across a broad pH range /57/.

ClO_2 retains its distinct chemical structure and properties after being dissolved, just like carbon dioxide does when dissolved in a can of soda pop. This property has important

implications for both treatment effectiveness and safety. First, with regard to effectiveness, ClO_2 remains just as potent a biocide when dissolved in solution as it was in its pure gaseous form. In the laboratory, ClO_2 is prepared by oxidation of sodium chlorite as follows /58/.

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2\ NaCl$$

(Equation 7)

When a bacterial cell comes into contact with chlorine dioxide it donates an electron from its cell wall, thereby creating a breach in the cell wall through which cell contents pass in an attempt to bring the concentrations on either side of the cell membrane to equilibrium. The cell dies through lysis /59/. ClO_2 has a lower oxidation strength and it is more selective in its reactions. Typically ClO_2 will react with compounds that have activated carbon bonds such as phenols, or with other active compounds like sulfides, cyanides, and reduced iron and manganese compounds. Because chlorine is a more powerful oxidant than ClO_2 it will react with a wider variety of chemicals, including ammonia. This property limits the overall effectiveness of chlorine as a biocide.

3.13 BHAP (2-Bromo-4-hydroxyacetophenone)

BHAP is an organo-bromine compound, A good biocide for bacterial slimes, it is a reddish brown, viscous, odorless liquid, having boiling point 139.1 ± 0.7 ºC at 737.9 mm Hg and solubility 0.248 ± 0.07 g/100mL water. It is stable under all storage conditions /60/. BHAP has a hydrolytic half-life of 272, 250, and 173 hours at pH 5, 7, and 9, respectively. It is useful for situations requiring continuous or semi-continuous dosing at low level, such as once-through cooling systems, where the dose rate is only 1 to 3 mg/L. It is supplied as a 30% active biocide. The dose rate for recirculating cooling systems is typically 10 to 20 mg/L, but could possibly reach up to 80 mg/L in fouled systems. Considerably higher dose rates are required for algal and fungal slimes. Because BHAP is not pH dependent, it is effective at high pH levels. However, BHAP has a relatively long half-life, typically 175 to 250 hours, which may affect its potential for cooling system bleed discharge. Other organo-bromine group products with similar biocidal mechanisms include: bisbromo acetyl butene (BBAB) and β-bromo-β-nitro-styrene (BNS). Example of BHAP is BRM®10 from Buckman Laboratories, Inc. /61/.

BHAP photo degrades in water with a half-life of less than 2 days. Also, BHAP's half-life in aerobic aquatic environments is 2.5 days.

4.1 Analysis methods

New methods are developed for the determination of biocides in the paper industry as the research advances in this field. Some of the conventional and modern analysis methods are described below.

4.1 Paper chromatography

In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action */62/*. The components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent. The paper is composed of cellulose to which polar water molecules are adsorbed, while the solvent is less polar, usually consisting of a mixture of water and an organic liquid. The paper is called the stationary phase while the solvent is referred to as the mobile phase. Performing a chromatographic experiment is basically a three-step process: 1) application of the sample, 2) "developing" the chromatogram by allowing the mobile phase to move up the paper, and 3) calculating R_f values and making conclusions. In order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, we can calculate an "R_f value" for each separated component in the developed chromatogram. An R_f value is a number that is defined as */64/*:

$$R_f = \frac{\text{distance traveled by component from application point}}{\text{distance traveled by solvent from application point}}$$

The distance traveled by the spot is measured to the middle of the spot.

4.2 Thin layer chromatography

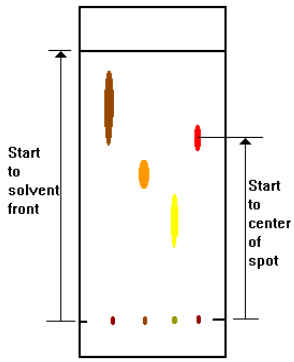
Thin layer chromatography (TLC) is a sensitive, fast, simple, and inexpensive analytical technique that will be used repeatedly in carrying out organic experiments. It is a true micro technique; as little as 10^{-9} g of material can be detected, although the usual sample size is from 1 to 100×10^{-6} g. TLC involves spotting the sample to be analyzed near one end of a sheet of glass, plastic, or aluminum coated with a thin layer of an adsorbent. The sheet, which can be the size of a microscope slide, is placed on end in a covered jar containing a shallow layer of solvent. As the solvent rises by capillary action up though the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent and the stationary adsorbent phase. The particle size, surface structure, and adhesiveness of the medium must he quite carefully controlled if reproducible results are to be obtained /63/.

Excellent results are obtained when silica gel thin layer supported on glass plate is used. A 4-cm wide chromatoplate can hold 5-10 spots (diameter, 1 mm). To obtain the separation of spots the plate is placed in a jar covered with a lid for a few minutes. A polar solvent will carry along with it polar substrates, and nonpolar solvents will do the same with nonpolar compounds, another example of the generalization “like dissolves like” /63/.

The R_f value is the ratio of the distance the spot travels from the point of origin to the distance the solvent travels. The best separations are achieved when the R_f value falls between 0.3 and 0.7 without units.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

It indicates that sample cannot be mobile if the R_f value is zero. The more different the R_f values between species of the sample, the better the separation of the mixture.



4.3 Gas chromatography

Gas-liquid chromatography (GLC), or simply gas chromatography (GC), is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition */64/*. In gas chromatography, the moving phase (or "mobile phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator").

In the operation of GC analysis process a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a micro syringe (or solid phase micro extraction fibers or a gas source switching system) */64/*. As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.

Two types of columns are generally used in GC analysis, packed columns and capillary columns. Packed columns are 1.5 - 10 m in length and have an internal diameter of 2 - 4 mm. The tubing is usually made of stainless steel or glass and contains a packing of finely divided, inert, solid support material (e.g. diatomaceous earth) that is coated with a liquid or solid stationary phase. Capillary columns have a very small internal diameter, on the order of a few tenths of millimeters 0.10 to 0.53 mm, and lengths between 25 - 60 meters are common */65/*. The inner column walls are coated with the

active materials (WCOT columns; liquid stationary phase on the inside wall of the column), some columns are quasi solid filled with many parallel micropores (PLOT columns; solid stationary phase on the inside wall of the column). Most capillary columns are made of fused-silica with a polyimide outer coating. These columns are flexible, so a very long column can be wound into a small coil. Compared with packed columns open tubular columns offer higher resolution, shorter analysis time, greater sensitivity and lower sample capacity and that is why they are commonly used nowadays.

Most used detectors for quantitative analyses in gas chromatography are a flame ionization detector (FID) and an electron capture detector (ECD); for qualitative analysis detectors are a mass spectrometer detector (MSD) and a Fourier transform infrared detector (FTIRD) */64,65/*. Quantitative analysis is based on the area of chromatographic peak, which is proportional to the quantity of the compound. In FID the elute is burned in a mixture of H_2 and air. When organic compounds are burned in an oxidising flame, CH radicals are formed and they are thought to produce CHO^+ ions in a flame. Flame´s electrical conductivity is linearly dependent on the molarity of organic compounds. The FID is insensitive to non-hydrocarbons.

ECD involves electrons from a radioactive emitter in an electric field /65/. As the analyte leaves the GC column, it is passed over this emitter which typically consists of nickel-63 or tritium. The electrons from the emitter ionize the nitrogen carrier gas and cause it to release electrons. In the absence of organic compounds, a constant standing current is maintained between two electrodes. With the addition of organic compounds with electronegative functional groups, the current decreases significantly as the functional groups capture the electrons.

4.4 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is basically an improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres that makes the process much faster. Equipment for HPLC contains eluant containers, pump system, injector, column and detector. In analytical HPLC the mobile phase is pumped

through the column with a known flow rate of 1-5 cm^3/min /66/. During injection the sample is transported with the eluant to the column. Sample particles interact with the eluant and stationary phase, which causes separation and it is detected at an inline detector. There are several different techniques to use in liquid chromatography (LC) but generally the normal phase technique, in which the stationary phase is more polar than the mobile phase, and the reverse phase technique in which the eluant polarity is much bigger than stationary phase polarity are used. Reverse phase chromatography is the most used technique in LC. Other techniques used are ion exchange, ion pair, ion, size exclusion, and affinity chromatography. The pump system must be able to pump with different flow rates and pressures /*67*/. The flow must be controlled because pulses may disturb the function of the detector. A typical flow rate in analysis is 0.1-3 mL/min. When capillary columns are used the flow rate may be under 0.1 μL/min. Sample injection is done with an injection valve. The injection valve usually has interchangeable sample loops with well-defined volumes.

HPLC columns are usually made of steel or plastic and their lengths are 5-30 cm /*65,66*/. Inner diameters of the colums are 1-5 mm and they are packed with the stationary phase having a particle size of 3-10 μm. In preparative chromatography columns with a longer inner length and a particle size of 10-40 μm are used. A short guard column normally containing the same stationary phase as the main column to prevent degradation by dust or small particles and sample impurities protects the entrance to the main column. The stationary phase material can be nonporous, pellicular, porous or perfusion. The shape of particles can be spherical, lump or block. The most common stationary phase contains highly pure, spherical, microporous particles of silica that are wetted by the solvent and have a surface area of several hundred square meters per gram. Bonded stationary phases can be covalently attached to the surface of the silica particles by chemical reactions.

HPLC detection is based on chemical and physical properties of the compounds /67/. HPLC detectors can be divided into spectrophotometric, electrochemical and other detectors. Spectrophotometric detectors are a UV/VIS detector or a diode array which give a response to compounds, or derivatives that can absorb ultraviolet or visible light combined with a fluorescence detector that gives response to the compounds or derivatives that absorb fluorescence light. The UV/VIS detector is one of the most used detectors because it is relatively sensitive, its linear response area is large and changes

the temperature of samples and the composition of eluants does not much interfere with the detection. Electrochemical detectors give a response to compounds that oxidise or reduce easily. The function of these detectors is based on conductance, conductivity, potential or carrying current. The most used electrochemical detector is an amperometric detector. A conductivity detector is used in ion chromatography. Other detectors are refractive index detectors, MS detectors and evaporative light-scattering detectors. The refractive index detector is thought to be a general detector in HPLC because it gives a response to all compounds. This response is based on changes in the index of refraction caused by eluant sample molecules. Light-scattering detectors are based on interactions between light and molecules. When light hits molecules, it induces momentary dipoles to the molecules and makes them oscillate at the frequency of the original light. Nowadays HPLC-MS and HPLC-MS/MS are used for the analysis of biocides /68/.

4.5 Supercritical fluid chromatography

Chromatographers are taking renewed interest in supercritical fluid chromatography (SFC). SFC refers to a mode of chromatography in which a fluid above its critical temperature and critical pressure is used as the mobile phase. This technique is appicable for the separation of components in complex samples that exhibit low volatility or thermal instability */69/*. Supercritical fluids possess properties that are intermediate between those of liquids and gases. The higher diffusion coefficients and lower viscosities of supercritical fluids as compared with liquids make the analysis time for SFC separations shorter and the chromatographic efficiencies greater than those obtained in HPLC. The higher solvating power of supercritical fluids makes SFC ideal for the separation of higher molecular weight and polyfunctional compounds either not volatile enough or too nonpolar for GC. The successful combination of HPLC and MS does not provide all the features desired of a truly universal separation/ identification system. Most of the various approaches to direct liquid introduction (DLI) LC/MS */68/* produce only chemical ionization (CI) mass spectra of separated compounds because of the excess eluent vapor present in the mass spectrometer ion source. In addition, there are very few instances of LC/MS accurate mass measurements or reports of molecular weight detection beyond 1000 daltons.

The higher volatility of SFC mobile phases makes it an alternative to HPLC for coupling with mass spectrometry. Reports of combined supercritical fluid chromatography/mass spectrometry (SFC/MS) have appeared by using both conventional packed and open tubular HPLC columnn /*70*/. For packed-column applications higher liquid flow volumes are used and for capillary SFC/MS electron ionization may be achieved because the mass spectrometer vacuum system can more readily maintain the required low pressure for this mode of ionization.

4.6 Micellar electrokinetic chromatography

Micellar electrokinetic chromatography (MEKC) was introduced in 1984 by Terabe et al. to solve the problem of separating neutral analytes in an electric field. MEKC is a chromatography technique which is a modification of capillary electrophoresis (CE), where the samples are separated by differential partitioning between micelles (pseudo-stationary phase) and a surrounding aqueous buffer solution (mobile phase) /71/. The basic set-up and detection methods used for MEKC are the same as those used in CE. The difference is that the solution contains a surfactant at a concentration that is greater than the critical micelle concentration (CMC). Above this concentration, surfactant monomers are in equilibrium with micelles.

Typically, the ionic or hydrophilic head groups of surfactants (*e.g.* sodium dodecyl sulfate, SDS) form the surface of the micelles, while the hydrophobic tails occupy the core /72/. SDS is the most commonly used surfactant in MEKC. When an electric field is applied, the analytes are separated according to the differences in their partition between the bulk aqueous solvent and the micelles. The separation process involves hydrophobic as well as other non-specific interactions. Recent applications of MEKC include the analysis of uncharged pesticides essential and branched-chain amino acids in nutraceutical products, hydrocarbon and alcohol contents of the marjoram herb.

4.7 Mass spectroscopy

In mass spectrometry (MS), gaseous molecules are ionized, accelerated by an electric field, and then separated according to their mass /65,66/. Analyzing can be proceeded

either directly with MS or it can be connected to some analytic chromatographic or electrophoresis instrument that acts as a sampling system. Mass spectrometers contain a source to produce the ions from the analyte, a mass analyzer to separate ions based on mass, and a detector to convert a beam of ions into an electrical signal for recording or processing. For analysis, the desired species must be charged and in the gas phase. Most conventional mass analyzers use electric and/or magnetic fields to control the path of the ions to a detector and to disperse the ions according to mass to charge (m/z) ratios. Two types of mass analyzers were used extensively: sector analyzers and quadrupole analyzers. Both of these are still used today. The ionization sources used in mass spectroscopy were electron impact (EI) and chemical ionization (CI). In EI-MS the sample is thermally vaporized and sample gas molecules enter the ionization source /73/. This ionizes molecules ($M + e^- \rightarrow M^+ + 2e^-$) and breaks them into smaller fragments. The ion formation process occurs with controlled internal energy deposition, therefore molecules ionized with CI generally exhibit less fragmentation than EI. Both EI– and CI–MS may be coupled with separation techniques such as gas chromatography. For the determination of the mass/charge (m/z) ratio, using two of these separation methods is enough.

The most used analyzator is a quadrupole mass spectrometer, the composition of which is presented in Fig. 11. In a quadrupole mass spectrometer, eluate passes through a heated connector into the electron impact ionization chamber, which is pumped rapidly to maintain a good vacuum. Ions are accelerated through a potential of 5-15 V before they enters the quadrupole mass separator. The separator consists of four parallel metal rods, which are applied with a constant voltage and a radio-frequency oscillating voltage. The electric field deflects ions and allows only an ion with one particular m/z-ratio to enter the detector /67/. By varying rapidly the applied voltages, the ions of different masses reach the detector. Quadrupole mass spectrometer is best for the quantification of analytes. Other much used ion separation techniques include a time-of-flight MS and an ion trap MS. The time-of-flight MS is based on velocities of the ions caused by their same kinetic energies and different m/z values. The ion-trap MS has a cavity that is controlled by a gate electrode that takes care that ions are entering to the detector one by one.

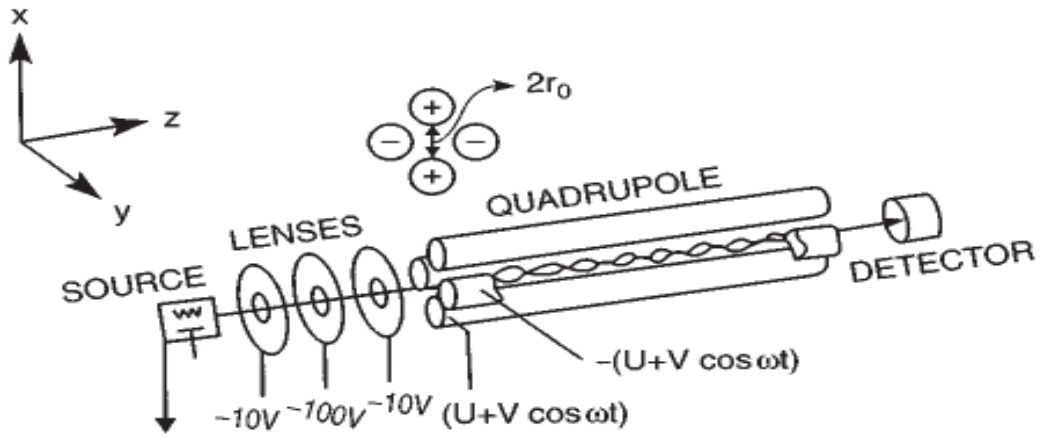


Figure 11. Quadrupole mass spectrometer consisting of a source, ion focusing lenses, a quadrupole, and an ion detector /73/.

A mass spectrometer records the total current from all ions of all masses over a wide, selected range /65/. Another, selected ion monitoring (SIM) can also be used. SIM is used to look for one particular compound or class of compounds. A mass spectrum is a graph of peaks that shows the relative abundance of each fragment that strikes the detector of MS. Analytes are then identified from spectrum by comparison with spectral library of known compounds /67/.

4.8 Infrared spectroscopy

IR spectroscopy is one of the most common spectroscopic techniques used by chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. At temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other /74/. When the frequency of a specific vibration is equal to the frequency of the IR radiation directed on the molecule, the molecule absorbs the radiation. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The infrared area of an electric-magnetic spectrum having wavenumbers from roughly 13 000-10 cm^{-1}, or wavelengths from 0.78 to 1000 µm. It is bound by the

red end of the visible region at high frequencies and the microwave region at low frequencies.

Its common uses are: Identification of compounds by matching spectrum of unknown compound with reference spectrum (fingerprinting), identification of functional groups in unknown substances, identification of reaction components and kinetic studies of reactions, identification of molecular orientation in polymer films, detection of molecular impurities or additives present in amounts of 1% and in some cases as low as 0.01%, identification of polymers, plastics, and resins, analysis of formulations such as insecticides and copolymers. Most samples can be prepared for infrared (IR) analysis in approximately 1 to 5 min /74/. Recently Fourier transform spectrometers have replaced dispersive instruments for most applications due to their superior speed and sensitivity. They have greatly extended the capabilities of infrared spectroscopy and have been applied to many areas that are very difficult or nearly impossible to analyze by dispersive instruments. Instead of viewing each component frequency sequentially, as in a dispersive IR spectrometer, all frequencies are examined simultaneously in Fourier transform infrared (FTIR) spectroscopy. There are three basic spectrometer components in an FT system: radiation source, interferometer, and detector. A simplified optical layout of a typical FTIR spectrometer is illustrated in Fig. 12.

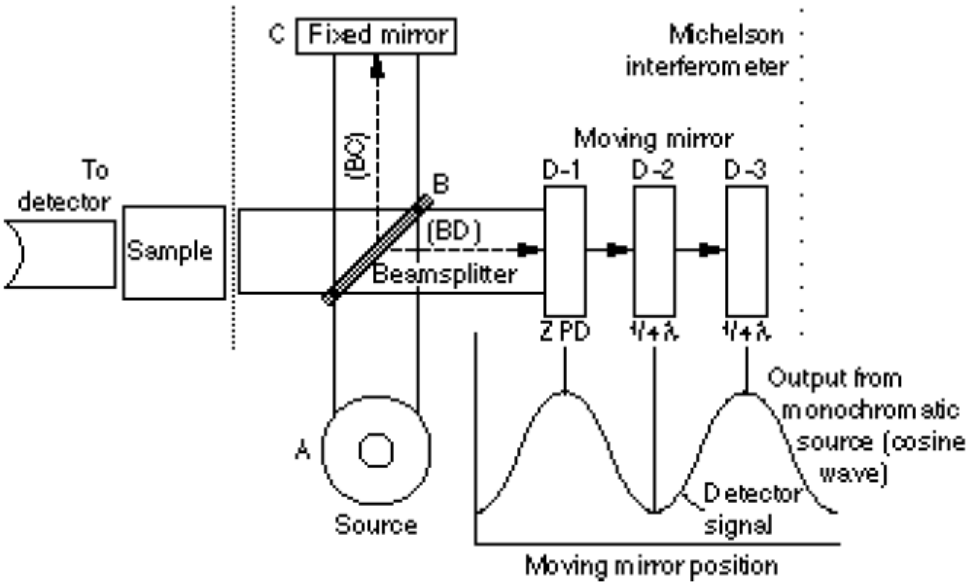


Figure 12. Simplified optical layout of a typical FTIR spectrometer /74/.

A background spectrum is first obtained by collecting an interferogram (raw data), followed by processing the data by Fourier transform conversion. This is a response curve of the spectrometer and takes account of the combined performance of source, interferometer, and detector /67/. Then a single-beam sample spectrum is collected. It contains absorption bands from the sample and the background (air or solvent). The ratio of the single-beam sample spectrum against the single beam background spectrum results in a "double-beam" spectrum of the sample. Advantages of FTIR are better speed and sensitivity, increased optical throughput, internal laser reference, simpler mechanical design, elimination of stray light and emission contributions, and powerful computerized data system. It can perform a wide variety of data processing tasks such as Fourier transformation, interactive spectral subtraction, baseline correction, smoothing, integration, and library searching.

4.9 Extraction methods

4.9.1 Shake-flask extraction

In shake-flask method a solvent is added to the sample followed by agitation. It works well when the analyte is highly soluble in the extraction solvent and the sample is quite porous. For fast extraction the sample should be finely divided /75/. Shake-flask extraction requires minimal glassware, smaller volumes of organic solvent and is relatively fast (10-30 min). However it is unlikely to produce a quantitative extract. For these reasons alternative extraction strategies have been developed.

4.9.2 Soxhlet extraction

Soxhlet extraction has been the most widely used method for the extraction of solids. In this procedure the solid sample is placed in a Soxhlet thimble (a disposable porous container made of stiffened filter paper) and the thimble is placed in the Soxhlet apparatus. The extraction solvent is refluxed; it condenses into the thimble and extracts the soluble analytes /*76*/. The apparatus is designed to siphon the extract each time the chamber holding the thimble fills with solution. The siphoned solution containing the dissolved analytes returns to the boiling flask, and the process is repeated until the analyte has been transferred from the solid sample to the flask. Because the sample is contained in the boiling extraction solvent, it must be stable at the elevated temperature.

Only clean warm solvent is used to extract the solid in the thimble. This increases the efficiency of extraction compared to the simple shake-flask method Soxhlet extractions are usually slow (12-24 hr, or more) but modern Soxhlet extractors can speed this up 8 to 10 fold. The analyte concentration, the necessary mass to obtain a representative sample, and the chromatographic detector sensitivity, together determine the required sample size /*76*/.

4.9.3 Microwave assisted extraction

The first use of microwave assisted extraction (MAE) for the extraction of analytes with organic solvents appeared in 1986 /*75*/. In MAE organic solvent and sample are subjected to radiation from a magnetron in either a sealed vessel (pressurized MAE) or an open vessel (atmospheric MAE). In the former the sample is heated by microwaves to 150 ºC. This temperature is 50 ºC to 100 ºC higher than the boiling points of the individual solvents in an open vessel at atmospheric pressure. Pressurized MAE allows upto 14 samples to be extracted simultaneously. Atmospheric MAE is capable of extracting a single sample per experimental arrangement. The major limitation of MAE is that the solvent needs to be physically removed from the sample matrix upon completion of the extraction prior to analysis /*75*/.

4.9.4 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is an important method for the analytical scale extraction of solid samples. In SFE a pressurized solvent, a supercritical fluid, is pumped through a heated extraction vessel /*65*/. After the vessel the fluid flows through a capillary tube to release the pressure. Extracted analytes go through the capillary tube to the collection vessel where the fluid is evaporated, or analytes can be dissolved from the collection vessel to obtain a solution of analytes. Supercritical fluid possesses distinctive properties as an extraction fluid. It is gas-like in terms of viscosity, diffusion and surface tension. At the same time, its high density gives it dissolving properties similar to those of liquid. Carbon dioxide, CO_2, is the most commonly used supercritical fluid because it has relatively low critical values (31.1 ºC and 72.9 bar); it is non-toxic, non-flammable and non-explosive, and is available in high purity. If a second solvent such as methanol, CH_3OH, is added, the solubility of polar analytes increases. SFE can

also dissolve non-volatile compounds. SFE can also be coupled to the chromatographic instrument /*65,67*/. This technique is between GC and HPLC, because of the solvent properties that lie between those of gas and liquid. SFE chromatography (SFC) allows analysis for a wide scale of compounds, because also relatively polar compounds can be separated contrary to GC. SFC can be coupled to a GC, HPLC or another SFC device.

4.9.5 Solid-phase extraction

There is a growing realization that faster and more efficient methods for sample pretreatment are essential. One study showed that more than 60% of analysis time was spent in sample preparation compared to only about 7% for actual measurement of the sample constituents. Solid phase extraction (SFE) is a particularly attractive technique for the isolation and preconcentration of target analytes. SPE is fast and effective, and can provide concentration factors of 100-fold or more. Very little chemical waste is produced. SPE is rapidly replacing older liquid–liquid extraction procedures of chemical analysis /*77*/. SPE and HPLC are both based on differential migration processes in which compounds are adsorbed and eluted as they are swept through a porous medium by a mobile-phase flow, which is dependent on the differential affinities between the sorbent material and the mobile phase. Retention is achieved with strong but reversible interactions between the analyte and the surface of the sorbent. Typical interactions are hydrophobic (van der Waals forces), polar (hydrogen bonding and dipole-dipole forces) or ion exchange interactions. To date, typical SPE materials are modified silicas with C8, C18, CN and other groups, carbon blacks and styrene-divinylbenzene copolymers (PS-DVB) /*78*/.

The most frequently used design in SPE is the cartridge. Many different types and amounts of sorbent are contained between two polyethylene or stainless steel frits in glass or polypropylene cartridges which have different column volumes. Another design which has become available in the last few years is the disk. Two different kinds of disk are now available: particle loaded membranes (PLMs) and particle-embedded glass fiber disks (PEGFDs). The former is the most common /78/. PLMs consist of a web of polytetrafluoroethylene (PTFE) microfibrils in which sorbent particles with a diameter of about 8 µm are suspended, and the membranes have a homogeneous flexible structure. The PEGFDs contain particles embedded in a glass fiber supporting matrix.

4.10 Microbiological methods

In the paper industry microbiological methods have been used for bacterial studies for several years. These methods have created a scientific basis for scientific studies and they are still used as a part of reliable studies. However their role has somewhat changed from being the only method in use, to be more in a supporting role when using present methods e.g. molecular biology.

4.10.1 Gram stain

Gram-stain is a method used to classify bacteria into two main groups, gram-positive or gram-negative, on the base of their cell wall morphology. Bacteria stain either gram-positive or gram-negative on the basis of differences in their cell wall compositions and architectures /79/. Those which retain the primary dye are called gram-positive and those that take the color of the counterstain gram-negative. The primary dye is crystal violet and the secondary dye is usually either safranin O or basic fuchsin. Some of the more common formulations include: saturated crystal violet (approximately 1%), Hucker's crystal violet, and 2% alcoholic crystal violet. Gram-negative bacteria possess a lipid-rich outer membrane as well as a plasma membrane and a thin peptidoglycan layer. The alcohol decolorizing step of gram staining washes the primary stain (crystal violet) from the cells and the secondary stain (carbol fuchsin or saffranin) colors the bacteria red. Gram-positive bacteria are covered with thicker and more resilient cell walls which do not allow the crystal violet to be removed and, accordingly, remain purple although the vast majority of bacteria adhere to the color /80/.

4.10.2 Catalase test

This test is performed to detect the catalase enzyme present in most aerobic and anaerobic bacteria. The catalase presence is detected by the decomposition of hydrogen peroxide to release water and oxygen. Hydrogen peroxide is an oxidative end product of the aerobic breakdown of sugars. The test is performed by adding 3-4 drops of hydrogen peroxide to an overnight growth on an agar slant and the cap is replaced. Catalase-enzymes residing intracellularly convert hydrogen peroxide to oxygen thus resulting in

visible immediate "bubbling" of the added solution. Vigorous bubbling within 10 seconds indicates a positive test and no bubbling indicates a negative test /81/.

4.10.3 KOH test

Gram-stain is still a useful method to classify bacteria into Gram-positive and Gram-negative but the procedure is still relatively time consuming, costly and reagents are needed to be replaced periodically /82/. In the middle of the 20th century a rapid non staining KOH method was developed for the determination of the Gram reaction. To perform the test a drop of 3% aqueous KOH is placed on a slide. A visible amount of bacterial growth from an agar culture is transferred via a sterile loop to the slide. The cells and KOH are mixed thoroughly on the slide. If the bacterium-KOH suspension becomes markedly viscid or gels within 5 to 60 s, the isolate is gram negative. If no gelling is observed, the isolate is gram positive. The gels are due to potassium hydroxide (KOH) which will degrade the cell wall of gram-negative bacteria and probably the liberation of DNA which is a highly viscous compound. The reaction is visible with a naked eye.

4.10.4 Optical density measurements

Optical density measured in a spectrophotometer is an important technique for the determination of bacterial concentration with results that are reliable. The result of the test is based on the relationship between the number of bacterial cells in a growth solution and its optical density measurement. Light is emitted from the light source and the scattered light is collected in the collector. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. The amount of light scatter can be measured in a spectrophotometer. Light is absorbed when it is hit by particles in the solution, in this case bacteria. Emitted light is compared to original light to calculate the absorbance in the solution /83/.

4.10.5 Epifluorescence microscopy

Epifluorescence microscopy is a method of fluorescence microscopy that is widely used in life sciences. Epi' is a prefix that means 'above', referring to the source of the light

that is passed from above to illuminate and allow electrons to emit light. In its most basic design, the epifluorescent microscope is a light microscope. However, it also has additional features that allow it to work differently /84/.

Epifluorescence microscopy utilizes light to excite the atoms of the specimen but light coming from the source is first passed through the instrument's objective lens before it strikes the specimen as described in Fig. 13. In other forms of light microscopy, light is transmitted to pass through the specimen. With epifluorescent microscopy, transmitted light is filtered out, allowing it to produce highly detailed and sharper images /84/.

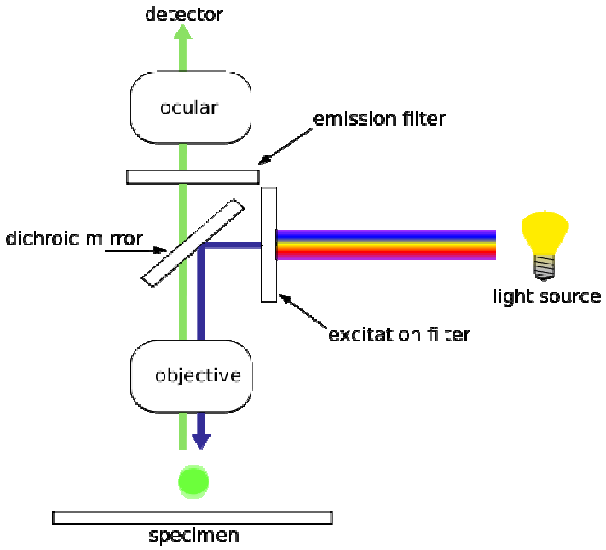


Figure 13. Structure of a fluorescence microscope /82/.

4.10.6 Scanning electron microscopy

Scanning electron microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today /85/.

A beam of electrons is generated in the electron gun located at the top of the column. The electron beam comes from a filament, made of various types of materials. The most common is a tungsten hairpin gun. This filament is a loop of tungsten which functions as the cathode. A voltage is applied to the loop, causing it to heat up. The anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate toward the anode. Some accelerate right by the anode and on down the column, to the sample. Other examples of electron sources are lanthanum hexaboride filaments and field emission guns.

This beam is attracted through the anode, condensed by a condenser lens, and focused as a very fine point on the sample by the objective lens. The scan coils are energized (by varying the voltage produced by the scan generator) and create a magnetic field which deflects the beam back and forth in a controlled pattern /85/. The varying voltage is also applied to the coils around the neck of the Cathode ray tube (CRT) which produces a pattern of light deflected back and forth on the surface of the CRT. The pattern of deflection of the electron beam is the same as the pattern of deflection of the spot of light on the CRT. The image may be captured by photography from a high resolution cathode ray tube, but in modern machines it is digitally captured and displayed on a computer monitor and saved to a computer's hard disk. For sample preparation these measures should be taken: The sample should be dried by removing all the water, solvents or other material which is volatile in vacuum and firmly mount all the samples to the machine. Non-metallic samples, such as bugs, plants, fingernails, and ceramics, should be coated so that they are electrically conductive. Metallic samples can be placed directly into the SEM.

5 Environmental effects

Biocidal products are necessary for the control of organisms that are harmful to human or animal health and for the control of organisms that cause damage to natural or manufactured products, On the other hand, biocidal products can pose risks to humans, animals and the environment in a variety of ways due to their intrinsic properties and associated use patterns /86/. To decrease the problems associated with microbial, fungal and algal growth, it is common to dose biocides for wood preservation and during papermaking. This means the undesirable handling of toxic chemicals and the risk of negative effects in receiving waters as biocides are discharged with the wastewater. Furthermore, high concentrations of biocides in whitewater may diminish the efficiency

of secondary treatment that is available in some paper mills. Biocides used in paper mills can be of different types. Once in the paper mill, biocides are periodically dosed (e.g. every 3~6 hours) to control bacteria and fungi that can produce slimes on machine surfaces. TCMTB and DBNPA are added at doses of 5-25 L/day. However, the size and the type of the paper mill (recycle Kraft, pulp, etc.) and open/closed circuits are crucial to determine the types of biocide to be used and their doses. Biocides used in paper making are generally highly soluble polar compounds. The fate of biocides is as follows: a fraction of biocide will degrade (chemically or biologically), a fraction will remain in circulating waters and finally a fraction will be present in the effluent or remain in the solid matter. An additional problem is that, because of their physico-chemical properties, some biocides may remain in fibers that can accumulate in the final paper product. Little information is available on biocides in pulp and paper-mill whitewaters and effluents because of the complexity of the water matrix /87/.

Paper mill whitewaters and effluents cause many environmental problems. Among these environmental hazards marine pollution is perhaps the most dangerous in the long run. The sea, which covers about 70 percent of the planet, plays a vital role in maintaining the fundamental biological and ecological balance by supplying much of the oxygen on which life ultimately depends. Various organic compounds present in the industrial wastes that join the sea water cause water pollution, e.g., affecting the precarious ecological balance of the Baltic environment. Contamination by harmful and toxic substances, such as chlorinated hydrocarbon pesticides (DDT, dieldrin, endrin), polychlorinated biphenyls (PCBs), and mercury and other heavy metals, seems to be an exclusively human source of threat for the Baltic Sea /86/. Mercury compounds are used in Finland and Sweden as fungicides and slimicides in the paper and pulp industry and high mercury contents have been found in fish off the coast of Denmark, Sweden, and Finland.

Biocides may be added to the papermaking system periodically (e.g., every 3-6 h) and thus the concentration will peak and trough. Biocides can remain in the paper simply because not all the water drains from the paper and it leaves a residue after drying. Additionally, some biocides may be fiber retentive. Virtually every paper manufacturer uses biocides at some time, including the manufacturers of papers for hot filtration (e.g., coffee filters and tea bag tissue). The tea bags are commonly used in every house so it can also effect the human health if it contains hazardous chemicals in biocides /89/. The

health of the workers in the industry is also vulnerable to the biocides risks. A group of 28 priority biocides and their aquatic environmental risk assessment is collected in Table 5 /90/.

Table 5. The priority biocides and their (presumed) hazards /90/

	Priority biocides	**EU product type**	**Hazard Classification**				
			Environmentally very hazardous application				
			A	B	**C**	**D**	**E**
1	1,2-benzisothiazolin-3-one	Slimicide	▼				
2	2-phenyl-phenol	textile preservative	withdrawn from the market				
3	2-thio(cyanomethyl thio)benzothiazole	textile preservative	withdrawn from the market				
4	2,2-dibromo-3-nitrilopropionamide	Slimicide	▼				
		liquid-cooling preservative	▼				
5	2-bromo-4-hydroxyacetophenone	Slimicide	▼				
6	2-methyl-4-isothiazolin-3-one	Slimicide		▼			
		liquid-cooling preservative	▼				
		wood preservative		▼			
7	bromonitrostyrene	Slimicide	withdrawn from the market				
		liquid-cooling preservative					
8	dichlorofluanide	in-can preservative					▼
		wood preservative				▼	
		antifouling					▼
9	formaldehyde	public health area (medical equipment)			▼		
		(accommodation man)				▼	
		(chemical toilets					▼
		Other			▼		

Table 5. (Continued also on next page)

	Priority biocides	**EU product type**	**Hazard classification**				
			Environmentally very hazardous application				
			A	B	C	D	E
10	sulfofuron	textile preservative	withdrawn from the market				
11	Zinc oxide	antifouling					▼
12	2,2-dithio-bis-benzamide	in-can preservative					▼
		slimicide					▼
13	4-chloro-3-methyl-phenolate sodium	in-can preservative					▼
14	5-chloro-2-methyl-4-isothiazolin-3-one	slimicide		▼			
		liquid-cooling preservative	▼				
		wood preservative		▼			
15	5-oxo-3,4-dichloro-1,2-dithiol	slimicide	▼	withdrawn from the market			
16	arsenic pentoxide	industrial wood preservative				▼	▼
17	bromoacetic acide	food and feed area					▼
18	cupric carbonate hydroxide	wood preservative				▼	▼
19	disodium cyanodithioimido-carbonate	slimicide	▼				
20	glutaric aldehyde	slimicide	▼				
		liquid-cooling preservative	▼				
		food and feed area					▼
		public health area (medical equipment)			▼		
		public health area (accommodations man)				▼	

		public health area (other)				▼	
21	potassiumbifluoride	wood preservative				▼	
22	potassiumhydroxide	public health area (medical equipment)				▼	
23	potassium-N methyldithiocarbamate	slimicide	▼				
24	sodium dichloroisocyanurate	public health area (swimming pool)	▼				
		public health area (accommodations man)	▼				
		public health area (other)	▼				
		food and feed area					▼
25	sodium hypochlorite	public health area	▼				
		public health area		▼			
		food and feed area					▼
		liquid cooling preservative	▼				
26	sodium-p-toluenesulfonchloramide	public health area (accommodations man)			▼		
		public health area (other)			▼		
		food and feed area					▼
27	zinc borate	public health area (accommodations man)	withdrawn from the market				
		antifouling					
28	zinc oxalate	public health area (accommodations man)	withdrawn from the market				
		antifouling					
Total number of substance - specific applications			16	5	5	8	16

In Table 5, A is an environmentally very hazardous application, B is an environmentally hazardous application, C is an environmentally hazardous application with discussion, D is an environmentally non-hazardous application, E has an application with discussion as its hazards are unknown. It can be deduced from Table 5 that among the prioritized biocides there are 26 problematic substance-specific applications (A, B and C classifications), 8 non-problematic substance –specific applications (D classification), 16 substance-specific applications without sufficient data or models for initial or refined risk assessment (E classification) /90/.

Biocides from the paper mills are mostly water soluble and may be highly likely to bio-accumulate in the fatty tissues of aquatic organisms. These biocides have been detected in environmental samples at several locations worldwide, and at levels which could be damaging to phytoplanktonic, algal and/or seagrass communities. Some biocides act as an endocrine modulating chemical to wildlife species. Some chlorinated biocides upon excretion from industry can react with other chemical compounds in water and some gases can be formed that may be harmful for the ozone layer. From this discussion it is concluded that further research is needed to study the harmful effects of biocides available in the market and find out ways for more environmentally friendly biocides, e.g., green biocides.

6 Green biocides

Any substance, especially a bactericide or fungicide, that kills or retards the growth of microorganisms and is considered to inflict minimal or no harm on the environment is called a green biocide or eco-friendly biocide. Until now the most significant requirement of the industries for a biocide is that it must be effective against a broad spectrum of microorganisms, cost effective and compatible with the product application environment (including no interference with the other additives). However, the current interest in environmental responsibility (i.e., greener chemicals) means that microbiocides must meet new demands, such as a broad spectrum of activity consistent with the application, no interference with other paper mill additives, very low toxicity, environmental acceptability, safety and ease of use in handling and storage, efficiency, fast action and cost effectiveness /91/.

Recent concerns over the presence of toxic compounds in the environment have been widely publicized. The concern about biocides used in the paper industry start from the knowledge that some toxic compounds present in the biocides are resistant to biodegradation and have the potential of bioaccumulation. For these reasons, there is a growing concern that the currently used slimicides may not be acceptable to regulatory agencies in the near future /92/.

The operation of most paper mills would be virtually impossible without the use of chemicals to control slime formation. The very characteristics which make desirable slimicides, high toxicity and slow biodegradability, are the characteristics which attract regulatory attention. There would, obviously, be less regulatory agency concern with a slimicide derived from a natural product that would be easily biodegradable /92/.

6.1 New Strategies for replacing traditional biocide use

New approaches in controlling biofouling and MIC are based on what is believed to be the mechanism for biofilm formation outlined earlier. These new approaches focus on chemical treatment programs that do not function by killing the microorganisms, but by keeping the microorganisms from attaching to surfaces, or by dispersing them from surfaces if they do become attached. The dispersed microorganisms then can be killed with an oxidizing biocide such as chlorine or the more environmentally acceptable bromine, chlorine dioxide or ozone. Such chemical treatment programs are commonly referred to as biodispersants or hydrolytic enzymes. Considering the complexity of the problem, completely replacing traditional biocides is not a possibility in the foreseeable future, but today's thinking is changing on how new biocides are developed /93/.

6.2 Biodispersants

Bio-dispersants are non-biocidal surface-active agents which break up biofilms and cause them to “slough off” of surfaces to which they attach. They can be continuously applied or periodically “slug-dosed” /94/. Various dispersant products are classified as inorganic or organic. Phosphates are the dominant family of inorganic dispersants, whereas acrylate polymers are the dominant family of organic ones. The mechanism by which conventional dispersant additives function in papermaking systems is by

contributing a high negative ionic charge to surfaces upon which they adsorb. The increased negative charge of the particle surfaces increases the electrostatic potential energy barrier, inhibiting contact between the surfaces as the bacteria are negatively charged. To be effective, the amount of dispersant needs to be enough to overpower any coagulants which may be present, e.g. aluminum sulfate or high charge density cationic polymers. The use of dispersants has sometimes been criticized on the grounds that these products effectively mobilize solids, they can cause clogging in downstream and down-gradient locations, and this potential must be taken into account when designing proper application that it should not lead to uncontrolled buildup of dispersed materials in a paper machine system. Cationic retention aids, in particular, tend to be deactivated by dispersants. In some cases, however, dispersants have helped papermakers overcome specific deposit problems /94/.

6.3 Enzyme additions

Another approach receiving considerable attention is the use of enzymes to control biofilms. An enzyme is a protein molecule having both catalytic activity and specificity for the substrate. There are three approaches being investigated, enhancing the removal of biofilm, preventing the formation of biofilm and improving the efficacy of biocides. Enzyme use for biofilming control in whitewater circuits has long been in the focus of discussions. The goal of this strategy is to partly or completely replace organic microbicides that are currently being used. Since the selection of enzymes or enzyme systems is decisively dependent on the nature of the extracellular polymeric substances (EPS) in a biofilm, these substances had to be analysed in order to identify the principal components of a biofilm matrix. Since April 1995, trials have been conducted on a paper machine for the production of wood free writing and printing paper in a neutral process. These trials are aimed at reducing both the microbicide dosages and the specific treatment costs. The experiences gained with enzymes for biofim control on a laboratory and mill scale have encouraged the authors to continue their work in this field. However, it seems doubtful whether enzymes are the sole answer to the complex problem of biofilming in PM whitewater circuits. A combined use of microbicides, biodispersants and enzymes appears most promising when it comes to developing an environmentally compatible and low-cost method of treatment /95/.

6.4 Ozone (as a green biocide)

Ozone is a water-soluble gas which is naturally produced by the ultraviolet rays from the sun. From previous uses of ozone in drinking water and waste water treatment, it is known that ozone acts as a powerful oxidizer and a strong disinfectant. The biocide action of ozone is a result of its reaction with the double bonds of fatty acids of the bacterial cell wall and membrane. Ozone application results in a change in bacterial cell permeability and a leakage of cells contents into solution. The action of ozone in water is instantaneous and after performing its action it reverts back to oxygen. The main advantage of ozone use consists of its superiority compared to chlorine for three capital reasons; first, it has been reported to be 1.5 times stronger than chlorine and it is acting 3,000 times faster than chlorine without producing harmful decomposition products. In 1995, ozone was declared as Generally Recognized as Safe (GRAS) by FDA for treatment of bottle of drinking water. Finally, its application was extended as GRAS to the food processing by experts some years later /96/. Another positive effect for ozone application is that ozone decomposes back to oxygen very quickly depending on the temperature and relative humidity. Moreover, it does not leave any harmful by-products.

Ozone has been tested as a biocide to control microbial growth in a printing paper machine white water system from Stora Enso newsprint Varkaus Mills in Finland. Two samples, a cloudy discharge from a disc-filter saveall and a clear filterate from the same saveall were treated by ozone dose. The test showed good results of destroying about 80% of the aerobic heterotrophic bacteria in the disc saveall cloudy discharge and 90% removal of the aerobic heterotrophic bacteria in the clear filtrate /97/. Recently, the use of ultrasound with ozone is of great interest because the use of ultrasound in conjunction with biocides offers a greener alternative. Ultrasound is currently employed in a range of industries such as surface cleaning, medical scanning ultrasonic therapy, food and beverage technology, materials science nanosynthesis (nanotechnology), mineral processing, industrial welding, non-destructive testing and environmental applications (water, land and air remediation). Although the energies required for ultrasonic disinfection alone are high there is now commercial equipment available using lower powers that is often combined with ozone /98/.

6.5 Electrochemically generated biocides (as green alternatives)

Electrochemically generated biocides can be a green alternative for organic biocides in use today. Electrochemically generated biocides are created by electrolysis of diluted salt solutions in an electrolysis cell. Most of the available information is dedicated to biocides electrochemically generated from a brine (NaCl) solution. Salts such as KCl and $MgCl_2$ can also be used. Electrochemical biocides have been studied intensively during last decade in terms of using them in food industry, drinking water and waste water sanitation and hospitals. It is generally accepted that chlorine is the primary oxidant in the electrolyzed brine solution /99/. Bromine has been recognized as an effective biological control agent for many years. Due to its rapid degradation to harmless bromide, is not a persistent pollutant. Bromine is produced at the point of use by electrolysis from a nonhazardous aqueous solution of sodium bromide and chloride. Produced electrolytic bromine is used to totally replace the use of hazardous/toxic chemical biocides for the control of microorganisms in cooling towers /100/. Chlorine is generally accepted as the primary oxidant in the electrolyzed brine solution. The oxidation-reduction potential was shown to be an important factor that defines the biocidal activity of electrolyzed anode water. In paper and board machines broke towers are usually the places where anaerobic conditions develop. Anaerobic bacteria are more sensitive to oxidizing compounds than aerobic ones and hence electrochemically generated biocides are powerful against anaerobes. Bacterial spores are highly resistant to many traditional organic biocides. Oxidizing biocides such as hypochlorite are proven to be more effective against spores. Electrochemically generated hypochlorite had a bleaching effect and that was apparent as an ISO brightness increase of approximately 1 unit.

Electrochemically generated biocides showed an effective way to control microbial problems at a paper mill. They can be added to water or pulp and they have hardly any negative effect on the process or end-product. For paper makers this study is of great interest because onsite-generated biocides are low cost solutions based on actual biocide need. Onsite-generated biocides also eliminate the storage and transportation of biocides and provides a basis for building a new control program /101/. These electrochemically generated biocides are environmentally friendly, nontoxic, not required to have special handling, hypoallergenic, safely disposed in municipal sewage systems, fast acting,

powerful biocide agents, used during all stages of disinfection and cleaning, applied in liquid, aerosol or frozen forms, chemical residue free, generated on-site or in concentrated amounts for imminent use, eliminating handling and storage issues and produced from municipal tap water and salt /102/.

7 Biocide analysis

The methods used for biocide analysis are described in Table 6 as follows.

Table 6. Methods for analysis of biocides

Compounds	**Matrix**	**Method**			**References**
		Extraction	**Separation**	**Detection**	
Bronopol	Food packaging and board	Hot water extraction	MEKC	UV	89
MIT	Food packaging and board	Hot water extraction	MEKC	UV	89
CMIT	Food packaging and board	Hot water extraction	MEKC	UV	89
DBNPA	Paper food packaging	Hot water	MEKC	UV	87
	Paper recycling water	SPE	LC	ESI-MS	103
	Food packaging and board	Hot water extraction	MEKC	UV	89
OIT	Water-miscible metalworking fluids	Methanol extraction	LC	UV	104
MBT	Food packaging and board	Hot water extraction	MEKC	UV	89

Table.6. (continued)

Compound	**Matrix**	**Method**			**References**
		Extraction	**Separation**	**Detection**	
THPS	Industrial cooling tower water	Making Solution	HPLC	UV	105
Glutaraldehyde	Food packaging and board	Hot water extraction	MEKC	UV	89
Dazomet	Solid sample from company	Making solution	HPLC GC FAB	UV-VIS MS MS	106
TCMTB	Paper recycling process water	SPE	LC	MS	87
	Surface treated lumber	Extraction with ACN	LC	UV	87
	Paper recycling water	SPE	LC	ESI-MS	103
BCDMH	Swimming pool water	SPME	GC	MS	107
Peracetic acid	Peroxide solution from company	Derivatiza-tion	HPLC	UV-VIS	108
Chlorine dioxide	From reaction of sodiumchlor-ite and hydrochloric acid	Concentration Determinati-on	HPLC	UV-VIS	109

8 Summary

Biocides are of utmost importance both technically and economically for the paper industry in the control of the slime formation in paper mills, on which about 200 million Euros are spent annually in Europe. There is a long list of biocide types but in this Thesis the 13 most important and most widely used biocides in papermaking and their function mechanism have been focused on. Biocides are dosed to prevent the problems associated with microbial, fungal and algal growth in papermaking, but on the other hand, a study of the negative effects of these toxic chemicals towards human beings and environment is also important. Among the 13 biocides studied it was concluded that 9 biocides are most environmental friendly: bronopol, kathon WT, DBNPA, THPS, glutaraldehyde, dazomet, BCDMH, peracetic acid and BHAP. In this Thesis 28 additional priority biocides and their aquatic environmental risk assessment were also collected in a table. Nowadays research work is going on to reduce the traditional biocide use or replace it with more environmental friendly biocides. Currently the research on the reduction of biocides with enzymes and biodispersants is in progress. Future research is needed to replace the currently used biocides in papermaking with green biocides which will be eco-friendly and will have no negative effects on the environment and human beings.

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