Synthesized compounds used as antimicrobial against the microorganisms in cooling fluids

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Abstract

In this work synthesized compounds have been used as antimicrobial against the microorganisms which have been found in the cooling fluid of industrial purposes ' by using media culture the results showed that sodium and potassium salts of 2-Mercaptobenzothiazole (2-MBT) are comparatively highly active in killing bacteria.

The pH of the cooling fluid samples had been measured after the addition of the media as well as the inhibitors 2-MBT , Sodium2-Mercaptobenzothiazole (Na2-MBT) , Potassium2-Mercaptobenzothiazole (K2-MBT) , and Benzothiazole disulfide (BT-disulfide). The results of PH measurement revealed that Na2-MBT and K2-MBT exhibit higher activity to inhibit the growth of bacteria compared with other synthesized compounds. The turbidity for such media and inhibitors has also been measured and the results indicated that Na2 -MBT, K2-MBT have given low turbidity compared to that of other synthesized compounds .The results indicate that the biological activities of the compounds Na2-MBT, K2-MBT are the best relative to the other synthesized compounds to inhibit the bacteria or the microorganisms that grow in the cooling fluid.

Key word : Cooling fluids , antimicrobial agent , 2-Mercaptobenzothiazole , Sodium 2-Mercaptobenzothiazole , Potassium 2-Mercaptobenzothiazole , antimicrobial agent .

الخلاصة

في هذا البحث حضرت مركبات استخدمت كعامل مضاد للكائنات الحية المجهرية التي تتواجد في سوائل التبريد المستخدمة للاغراض الصناعية . و ذلك باستخدام عملية الزرع التي اثبتت نتائجها بان املاح الصوديوم و البوتاسيوم للمركب 2-مركبتوبنزوثايزول (2-MBT) تكون ذات فعالية عالية لقتل الاحياء المجهرية . كما قيس ال PH لنماذج سوائل التبريد بعد اضافة المانعات الى الوسط و هي مركبتوبنزوثايزول و صوديوم 2-مركبتوبنزوثايزول (Na2-MBT) و بوتاسيوم 2- مركبتوبنزوثايزول (K2-MBT) و بنزوثايزول دايسلفيد (BT-disulfide) ,و اثبتت نتائج قياس ال PH ان لاملاح الصوديوم و البوتاسيوم فعالية كبيرة لمنع نمو البكتريا مقارنة مع بقية المركبات. كما قيست درجة العكورية للاوساط الملحية و اثبتت ان املاح الصوديوم و البوتاسيوم لها اقل قيمة للتعكر مقارنة مع بقية المركبات المحضرة . واثبتت النتائج ان

مركب ملح الصوديوم و مركب ملح البوتاسيوم هي الافضل من بقية المركبات المحضرة لمنع نمو البكتريا و الكائنات المجهرية الاخرى التي تنمو في سوائل التبريد .

Introduction

Cooling fluids are playing an important role in the industrial processes through metals cutting[(1)]. One of the problem which leads to lower efficiency is the deterioration in addition to huge loss in industry production. Many researches and conclusions have been placed to treat such problem during the last development of such researches. This study was based on preparation of some benzothiazole derivative figure (1) of the following structural formula:

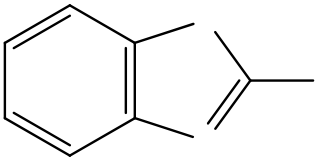

Figure (1) : 2-Mercaptobenzothiazole

Where X = H^+ , Na^+ , K^+ , 2-MBT

Using them as antimicrobial agents against some of microorganisms that cause the deteriorations. Cooling fluids are solutions or emulsions often prepared from mineral or artificial oils which are used in the mechanical works like cutting and smoothing, the first use of these kinds of oils turend back to 200 years ago, those oils were used alone at that time by obtained it with a brush on the cutting tool[(1)].

The used oils originally from animal fats such as pigs and sharks. These types of fats didn't fulfill the purpose when the cutting is acute, and the works were not on wide use as its up to date. These are called welting agent (meaning materials which help by wetting the surface for the cutting material) once again with original material, because of the high temperature. This phenomenon known as microscopic welding [(1)].

Cooling fluids has two main jobs[(2)]

(1) oiling

(2) cooling,

The oiling is the main operation in the field in which the friction of the cutting tool with the metal, which is used to cut would generate high amount of heat which is known as the external fiction, which, considered to be third of the total generated heat, and these is another heat which results also from the resistance of the cutting metal part and that active under the effect of the cutting tool, this called the internal friction , and the heat yield from this is about 2/3.

So the cooling fluids make to reduce from these yields heats by facilitate the slipping of the cutting tool . The second advantage is the cooling by reducing the yield heat in the metal piece. The rise in the temperature of the metal piece will reduce the work efficiency and accuracy. So the high temperature will lower the working to the cutting tool. These are several commercial types of the used cutting oils, and the most wide used oils are: oils that have the ability to mix with water in which these oils may by artificial or semi artificial and it goes by 90% from the uses[(1)].

There are two types of cooling fluids

1- Oil -based cutting fluids

Its petroleum or mineral oils which can be used readily or used with additives to improve some properties of it. These fluids are not necessary to be used for the light cutting for the metal. For the

S

SX

N

more acute cutting the fluids should have 20% fatty oils.

In the acute conditions, the additives should contain 20% of the sulfonated and chlorinated oils, which make as anti-welding. The oil based cutting fluid used for drilling for its high ability .To oiling, while the fluids that have the ability to miscible with water used in girding for its ability to absorb the heat [(2)].

2- Emulsions with conc. 60-90% petroleum oils: These fluids are called emulsions or the oils that have the ability to be emulsified, and sometimes as oils that have the ability to make emulsion in water and also contain petroleum oil and emulsifying additives agents between 60-90%.

Oils and water are mixed to form cooling fluid, which has fixed features. Emulsifing agent help to catch the cutting metal piece during the work these emulsifiers give the milky color of the fluid [(3)].

The purity of water is the most important factor that controls the success or the fail of cooling fluid .The water which would be used must be very pure, although any other types of water can be used for this purpose, So hard or salt water could be used but with unfavorable results because it [(2)]

1-Effects on the yield quantity in which it will lower from the production efficiency

2-Increases the conservation cost for machines, because it will expose to corrosions.

3- Precipitates the particles in the machine parts

4- Increases the growth of microorganisms.

5- Decreases in the life of the cooling fluid.

Cooling fluid does not deteriorate without any causing .The cause of deterioration for cooling fluid is the growth of many kinds of microorganisms such as: bacteria, fungi, yeast and other microorganisms[(4)].

Bacteria and fungi are found in the cooling fluid by the causes of [(4)] .

1-finding the bacteria and the other microorganisms naturally in the useful water that used in synthesis of cooling fluids.

2- Deterioration of cooling by microorganisms that comes from workers hands or this saliva.

3- Deterioration of the fluids from the air of the factory's, laboratory.

The changes that take place on the cooling fluids because of microorganism [(5)]:

1 - Change the PH as well as the bad smells because of the formation of H_2S gas.

2- Decreasing in cooling fluids efficiency as well as the additives specially the anti- corrosion compounds

3- The emulsion may be separated.

4- At these conditions the microorganisms will secret organic acids which help to increase the corrosion

5- The gases that result from the deterioration, the deterioration also hurts the works by respiration tract infection and the skin infection.

6- Decreasing in the quality of the yields metal as well as decreasing in the age of the cutting tool.

The main shapes of Bacteria are :

(1) spiral,
(2) cylindrical
(3) spherical or ellipsoidal .

Its dimensions go between (0.5-1) µm in the width and (2-5) µm in the high [6] and the percentage of water in it is (80-90)% from its weight, so that it consider to be the main nutrient for the cell [7]. Bacteria can be divided into two main groups.

1 - Aerobic bacteria.
2- Non-aerobic bacteria.

Pseudomonas is gram - negative, motile, aerobic some of which produce water-soluble pigment. Pseudomonas occurs widely in soil, waters, plants and animals, it has the following properties: odor, pigmentation, colonial morphology, gram reaction, somatic shape and sport formation. Production of hydrogen sulfide, arginine, hydrolase, indophenol oxidize, growth at 42C° and 40C°, oxidation of glucose xylose, lactose and maltose [8].

Experimental

1- Chemicals :

The chemicals used in this study are the following :

Chmicals	**Company**
Sodium hydroxide	BDH
Sulfur	Merk
Aniline	Fluka
Hydrochloric asid	Merk
Sodium carbonate	BDH
Hydrogen peroxide	Merk
Potassium hydroxide	Merk
Carbon disulfide	Riedel-DeHaeny

2- Methods :

A- Organic part

1- synthesis of (2-MBT)

To synthesis 2-MBT figure (1) the following quantities compounds should be prepare[12]

a- carbon disulfide 15 ml. b- aniline 22.8 ml. c- sulfur 8 gm.

1- These compounds are mixed in a beaker (150 ml).

2- The reaction carried out in a steel Autoclave (250 ml), which has been tightly closed to provide high pressure and temperature.

3- The Autoclave was heated to reach 250°C for 7 hours.

4- The reaction mixture was collected in a beaker, then a solution of NaOH (2.5M) was added to it.

5- Few milliliters of cone. HC1 were added to mixture, until it would become acidic.

6- The mixture was filtered through filter paper, then (0.94M) of Na_2CO_3 was added and mixed well.

7- The crude product is yellow precipitate and purified by using absolute ethanol

2. Synthesis of Sodium 2-Mercaptobenzothiazole (Na2-MBT) Figure (2):

The synthesis of Na2-MBT figure (2) was carried out as follows:

1- 2 gm of 2-MBT was dissolved into 25ml of absolute ethanol with continuous
stirring for 2 hrs.

2- 0.48 gm of NaOH was added with continuous stirring.

3- The mixture was left in the oven at 50 °C until the ethanol was completely evaporated.

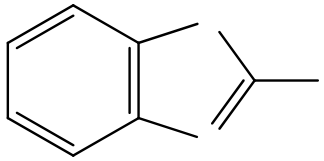

Figure (2) : Sodium 2-Mercaptobenzothiazole

3. Synthesis of Potassium 2-Mercaptobenzothiazole (K2-MBT) Figure (3):

The synthesis of K2-MBT was carried out as follows:

1- 2 gm of 2-MBT was dissolved into 25ml of absolute ethanol with
continues stirring for 2 hrs.

2- 0.67 gm of KOH was added with continues stirring.

3- The mixture was left in the oven at 50 °C until the ethanol was completely
evaporated.

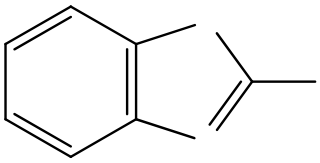

Figure (3) : Potassium 2-Mercaptobenzothiazole

4. Synthesis of benzothiazol Disulfide (BT-Disulfide) figure (4):

This compound was prepared by oxidation of compound 2-MBT using hydrogen peroxide. Hydrogen peroxide (31 ml, 3% w/v) was added drop wise to a solution of 2-MBT (45.8 m.mole, 6 gm) in ethanol (50 ml) with continuous stirring for 1 hour at room temperature, yellow precipitate was formed, and this was collected by filtration, washed with distilled water and dried in oven at 70°C to provide the compound

BT-Disulfide.

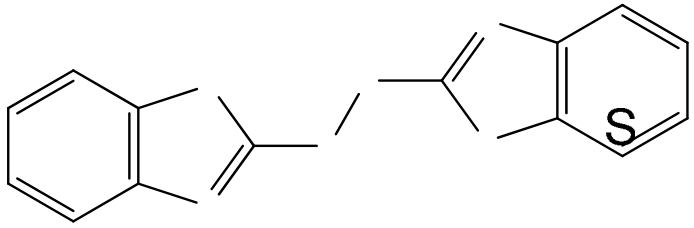

Figure (4) : benzothiazol Disulfide

B- Biological part :

1- Growth of bacteria strain :- Mineral salt medium was used to grow the bacteria and aeration of liquid culture was best achieved by incubating the flask in shaker incubator at 150 rpm for (6) days at 37C°.

100µl added from the emulsion samples in duration of 1- week, 2-weeks and 3- weeks, after using to the mineral salts . Then these conical

flasks incubated in the incubator for (6) day's .The (pH) and the turbidity are measured.

2- Preparation different concentrations of 2-MBT in the mineral salt media:-

After the growth of bacteria, the solution of (0.1gm/10ml ethanol) from 2-MBT used to prepare the following concentrations that added to the conical flaks which contains 25ml of mineral salts media with 100 µl of the emulsion sample ,Table (1) show the volume of added solution per ml., against the concentrations in µg per ml.

Table (1): the volume of added solution per ml. against the concentrations in µg per ml.

Conical flask	Vol. of added solution /ml	Concentration µg/ml
0	0	0
1	0.5	100
2	1	200
3	1.5	300
4	2	400
5	2.5	500

The following experimental work had been done step by step

1- pH standardization has been made for each contain of conical flask.
2- Measurement the PH of the content of conical flasks before addition different concentrations of 2-MBT.
3- After addition of the inhibitor the conical flasks were kept in the incubator at constant temperature 37C° to 48 hours.
4- The PH was measured to the 6 conical flasks after incubation period.
5- Turbidity meter to the 6 conical flaks measured the turbidity.
6- 10 µl of control (sample oil) has been taken and 90 µl of sterilized normal saline was added in epindrof tube (stock solution).
7- Another 10 µl has been taken from the above tube, and 90 µl of sterilized normal saline was added in epindorf tube. This process has been made for each of the (6) conical flasks.
8- 10 µl from each epindorf tube had been taken and added to (12) petridshes that contain the nutrient agar and spreader carefully by sterilized spreader.
9- The (12) Petridishes had been kept in an incubator at 37C° for 48 hours.
10-The above procedure was repeated for each inhibitor [9, 10, 11] which had been used.

Results and Discussion:

A- Organic part

1- Synthesis of 2-mercaptobenzothiazole (2-MBT):

2-MBT was synthesized from the reaction of aniline with carbon disulfide and sulfur according to a reported procedure[12]. It is noteworthy that this method was

found to give good yield with highest purity.

The structure of 2-MBT was evidenced by its melting point 182°C and by spectroscopic method (F.T.I.R) figure (5).

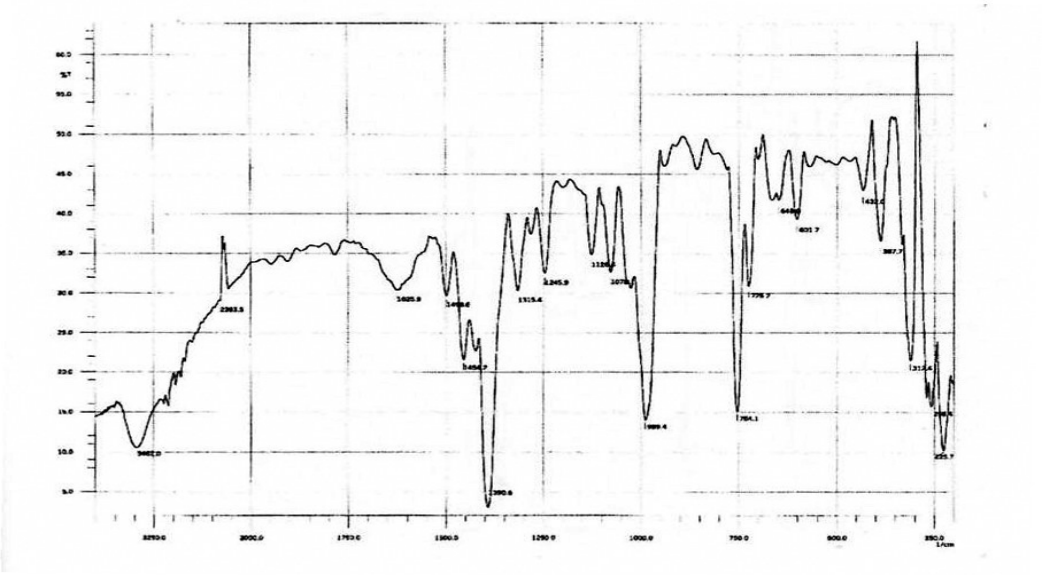

Figure (5) : The FTIR spectrum for the Synthesized 2-MBT.

This spectrum showed the following characteristic bands (cm^1)[13].

1.	3080 cm^{-1}		C-H aromatic
2.	3462 cm'^1	N-H secondary	
3.	2750 cm'^1		S-H
4.	1625 cm'^1	C=N aromatic	
5.	1425, 1498 cm'^1	C=C aromatic	
6.	1315 cm'^1		C=S

It may be conclude from these data that this compound exists in the following tautomeric [14]forms figure (6).

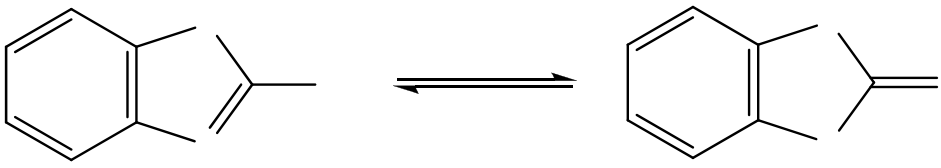

Figure (6) : tautomerism of 2-MBT

2 -Synthesize of (Na2-MBT) and (K2-MBT):

The 2-mercaptobenzothiazole undergoes many reactions typical of thiol group, 2-MBT is a weak acid, and therefore it's soluble in aqueous alkalis. With metals, such as magnesium, zinc, lead, silver and mercury they formed salts [15].

Sodium and potassium 2-MBT was prepared by the reaction of 2-MBT with ethanolic solution of alkali hydroxides.

The structure of this salt was identified by its melting point 265°C of (Na2-MBT) and 242^0C of (K2MBT).

3- Synthesize of benzothiazole disulfide(BT-disulfide):

Selective oxidation of 2-MBT using hydrogen peroxide afforded the title compound. The structure of this compound was confirmed on the basis of its melting point 178°C and its spectral data that shown in figure (7)

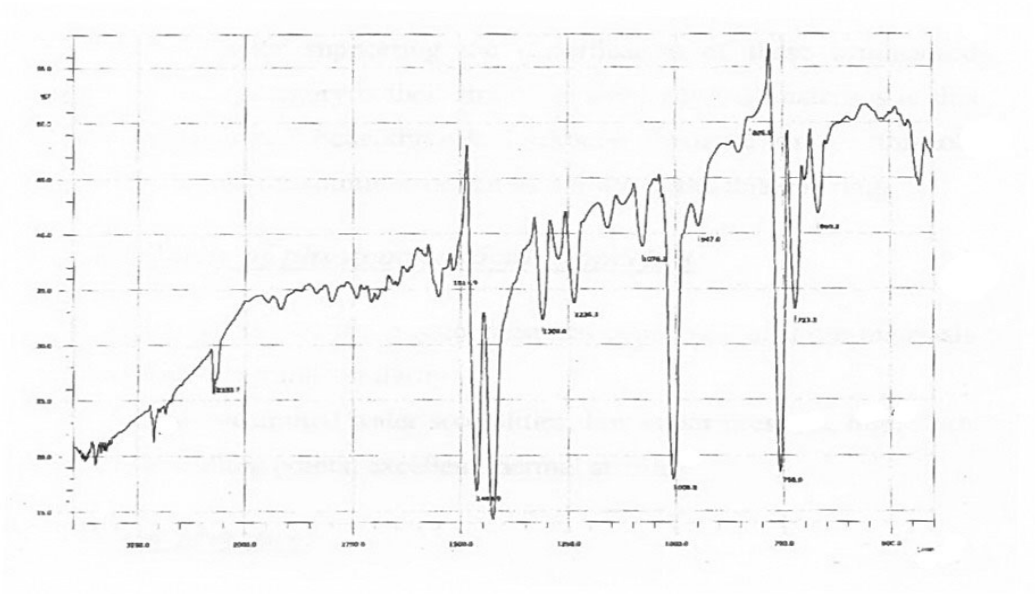

Figure (5) : The FTIR spectrum for the Synthesized BT-disulfide

the following absorption bands were observed:

1.	3060 cm'^{1}	C-H aromatic
2.	1650 cm'^{1}	C=N aromatic
3.	1425, 1468 cm^{-1}	C=C aromatic
4.	669cm'^{1}	C-S
5.	440 cm'^{1}	S-S

B- Biological part :

Three types of emulsions (samples) were taken from the laboratory of the industry at different time uses as follows in table (2).

Table. (2) : Types of emulsions (samples) at different time

Sample No.	Used time	Emulsion type
1	1 day	White
2	7 days	Semi-turbid
3	14 days	Turbid

Now, to estimate which one of the three Samples has most deteriorated a standardization process the turbidity and the PH after adding it to the mineral samples to measure the turbidity and the PH after adding it to the mineral salts media with incubation time, the results are shown in Table (3) and Table (4).

Table (3): Effect of the using emulsion time on the (PH).

Sample No.	pH	Used Time	Emulsion Type
1	7.17	1 Day	White
2	6.84	7 Days	Semi-Turbid
3	6.68	14 Days	Turbid

Table (4) : The effect of the using emulsion time an the turbidity.

Sample No.	Turbidity / NTU	Used Time	Emulsion Type
1	475	1 Day	White
2	125	7 Days	Semi-Turbid
3	50	14 Days	Turbid

Conclusion

From the above data, that the sample no.(3) is more deteriorated than the others, so sample (3) was used in measuring the biological activity of the synthesized compounds .

MBT and its derivatives have been tested as anti-bacteria in the cooling fluids. After the addition of 2-MBT and its derivatives to the mineral salts media with the sample of the two weeks, which has been deteriorated, in different concentrations, then PH values and the turbidity were measured as been shown in table (5) and table (6).

Table (5) : The PH value After the addition of 2-MBT and its derivatives to the mineral salts media with the sample

Sample No.	Cone./ ppm	pH of 2-MBT	pH of Na2-MBT	pH of K2-MBT	pH of BT-disulfide
1	0	6.50	6.62	6.58	6.78
2	100	6.67	6.77	6.67	6.85
3	200	6.78	6.97	6.74	6.91
4	300	6.95	7.15	6.96	7.08
5	400	7.11	7.20	7.09	7.15
6	500	7.18	7.24	7.26	7.21

The results obtained indicated that as long as the concentration of inhibitors in mineral salts media increased, the PH values would be in versed which leads to decrease the formation of the gas.

Table (6) : The turbidity value After the addition of 2-MBT and its derivatives to the mineral salts media with the sample

Sample No.	Cone./ ppm	Turbidity of 2-MBT NTU	Turbidity of Na2-MBT NTU	Turbidity of K2-MBT NTU	Turbidity of BT-disulfide NTU
1	0	85	55	55	105
2	100	215	235	235	245
3	200	325	440	440	430
4	300	550	655	655	635
5	400	785	710	710	735
6	500	833	825	825	810

From the results of table (6) indicate that as long as the concentrations increased, as the turbidity would be decreased, because that the growth of these bacteria would be decreased which leads to increased the NTU values.

In addition to the present work the biological activity was measured.

Table (7) give the biological activity for each sample to kill bacteria found in the cooling fluid in different concentrations.

Table (7) : Percentage of killing for the microorganisms by some of 2-MBT compounds in different concentration

Compound	*Control (10^{-1}) No. of surviving microorganisms*	*300 ppm No. of surviving microorganisms*	*Percentage of killing in 300 ppm*	*500 ppm No. of surviving microorganisms*	*Percentage of killing in 500 ppm*
2-MBT	*102*	*21*	*79.4*	*2*	*98*
Na 2-MBT	*94*	*3*	*96.8*	*0*	*100*
K 2-MBT	*121*	*1 1*	*89.2*	*0*	*100*
BT-disulfide	*108*	*60*	*44.44*	*5*	*95.4*

We can see from the results above, that the number of surviving microorganisms for each compound in different concentrations as well as the Percentage of killing for the microorganisms by using each synthesized compound, whatever the concentration in creased for each inhibitor compound, the number of surviving microorganisms decreased and the percentage of killing microorganisms for each synthesized compound increased, whatever the concentration of these compounds in creased.

So we can conclude the best compounds of killing for the microorganisms are sodium and potassium 2- mercaptobenzothiazole.

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