ISSN No:-2456-2165

Bioremediation of N-Methylmorpholine-N-Oxide: A Scientific Evaluation

Abhismita Roy
Department of Botany, Kalinga University,
Naya Raipur, Chhattisgarh, India

Abstract:- N-Methylmorpholine-N-Oxide (NMMO) – a bulk solvent for cellulose in industrial fiber making processes, has high cellulose dissolving capacity and lesser toxicity than CS_2 which finds widespread use in the textile industry. In the NMMO process, more than 99% of the solvent is recycled within the process making the fabric eco-friendly. The small quantities released via waste water are readily degraded in the biological waste water treatment plants. As several scientific studies report, it is not mutagenic. Moreover, Amine Oxides find a wide application as active substances of personal hygiene (in hair conditioner, face wash, waxes and polishes), which normally undergo immense toxicological examination, therefore its non-toxicity can be assumed for all practical purposes.

However, its degradation and removal from the effluents discharged from industries is a major concern. A reliable study conducted by Meister and Wechsler determined the biodegradability of NMMO in the presence of non activated sewage sludge with 30 mg/L suspended solids. The test period was 28 days; after 28 days on average < 10% biodegradation was observed. The results showed that the substance is not readily biodegradable. However, significant biodegradation was observed when activated sludge was employed showing an ultimate biodegradability of 90 -100%) concluding that the substance is inherently biodegradable when treated with successfully adapted aged sludge involving slowly-reproducing (long-living) sludge organisms which seem to be more efficient degraders of the substance.

I. INTRODUCTION

The creation of man-made fibres contributes significantly to India's largest export sector, the textile industry. Overall apparel and textile demand is likely to expand in the upcoming years due to increased macroeconomic growth in major consumer areas like the U.S., EU, China, and India. As a result, an increase in fibre output is primarily predicted during this time. Lyocell is a type of rayon created by dry jet-wet spinning cellulose fibre from dissolving pulp (bleached wood pulp). It is used in numerous commonplace products and garments, including denim, chino, underwear, casual wear, and towels.

Items with a silkier appearance, including women's apparel and men's dress shirts, contain filament fibres, which are often longer and smoother than staple fibres. Conveyor belts, speciality papers, and medical dressings are more products that use lyocell. Additionally, a range of different fibres, including silk, cotton, rayon, polyester, linen, nylon, and wool, may be combined with it. Lyocell and other cellulosic fibres like cotton, linen, ramie, and viscose rayon have a lot in common in terms of characteristics.

Due to their ability to simulate a variety of textures, including suede, leather, and silk, plus their softness, absorbency, durability, and resistance to wrinkles, they are in high demand. Originally developed by American Enka in 1972, Lyocell burst into popularity in the latter decades of the 20th century, and it is still much popular around the world. Since it is primarily made from organic ingredients, this fabric is seen as a more sustainable alternative to completely artificial fibers like polyester.

Since its implementation 15 years ago, the Lyocell process has been used as an environmentally benign substitute for traditional fiber-making techniques (Chanzy et al. 1982; Firgo et al. 1994; Michels et al. 1994). The primary characteristics include the direct cellulose dissolution without chemical derivatization and the nearly perfect solvent recovery (N-Methylmorpholine-N-oxide Monohydrate) (NMMO). NMMO is used as a solvent in the creation of lyocells. Wood pulp is mixed with concentrated aqueous NMMO and dissolved while being subjected to strong shear pressures and contemporaneous water evaporation. The pulp that is used is a 96% cellulose, DP 750 industrial dissolving pulp. It uses a suspension of roughly 13% cellulose, 20% water, and 67% NMMO.

At 120 ^{o}C, cellulose in NMMO is disintegrated (Temperature more than 125-130 ^{o}c being unsafe for NMMO, resulting in an extremely viscous solution). After filtering, the solution is extruded into a water bath using tiny jets. As the solvent is removed, the fibres that have been transformed into tiny filaments are gathered as tow, from which staple fibre is made. The excess water is evaporated, and the process then reuses the concentrated NMMO.

II. BACKGROUND OF NMMO AND ITS APPLICATION

NMMO which stands for N-Methylmorpholine N-oxide, is a heterocyclic amine oxide and a Morpholine derivative. As per IUPAC‘s nomenclature, it is named 4-methyl-4-oxidomorpholin-4-ium. It finds widespread applications in the textile industry as a solvent for the synthesis of regenerated Cellulose. It is used as a 50:50 (w/w) mixture of solvent and water.

N-Methylmorpholine-N-Oxide (NMMO) is employed as a bulk solvent for cellulose in industrial fiber making processes. Furthermore, it is widely utilized in organic synthesis for direct or transition metal-catalyzed oxidation of organic compounds. NMMO being an organic cyclic, polar solvent, has high cellulose dissolving capacity than alternative polar solvents like DMSO, DMY, DMAC etc., comes across as a better option as compared to CS_2 which has its own ill effects on the environment as well as human health.

➢ *Need of biological treatment*

It has long been known that NMMO is resistant to biological deterioration. Due to this, numerous efforts have been undertaken to create physical-chemical degradation procedures, such as the TiO2 photocatalytic degradation process (Doherty et al. 1995) or the ozonation of wastewater containing NMMO (Stockinger 1995; Stockinger et al. 1996). But because they are expensive and therefore uneconomical for use in commercial applications, these and other physical and chemical methods of NMMO removal, such as membrane polyelectrolyte filtration and ion exchange resins, are not widely used(6) As a result, there is a lot of interest in NMMO's biodegradability. The main goal is to use helpful and efficient microorganisms for a sustainable treatment that will allow industrial effluents to be disposed of without causing unacceptable harm to the environment.

Meister and Wechsler's research from 1985 indicates that NMMO-containing wastewaters may be treated in conventional effluent treatment facilities. The results of the trials indicated that the sludge can adapt in between 15 and 20 days. Adapted sludge has the ability to breakdown the drug and its main metabolites to quantities below detection and can maintain this ability even for short periods of time without solvent being present in the wastewater. A high sludge age is the primary condition for a successful adaption. There are numerous stages to the degradation. First, N-Methylmorpholine is created from NMMO. Demethylation of N-Methylmorpholine to Morpholine is the following step. This stage of the adaption process is essential.

This study is of prime importance because results from conventional biodegradation tests using non-adapted activated sludge, the solvent is generally considered being persistent [(7)]

Once Morpholine has been formed, the adaptation proceeds very quickly until none of the substances in question can be detected any longer. So the next step must be the cleavage of the Morpholine ring structure.

The acclimation of the microorganisms is the initial phase in a wastewater treatment plant's biodegradation of harmful chemicals. When bacteria are exposed to harmful substances in a chemically stressful environment, adaptation to those substances may take place (Aelion et al., 1989). Wiggings et al. (1987) discussed various mechanisms to explain the acclimation phase and proposed that during this phase, specialised microorganisms are selected and multiplied, and physiological transformations take place in the microorganisms' metabolic system through changes to the enzymatic level, its regulation, production, and ultimately mutations.

Further also stated that in aerobic microbial communities, the acclimation periods range from several hours to several days. NMMO is among the 1500 chemicals which are released each day into environment and commonly found in the industrial effluents of textile, rubber, plate, pharmaceutical, personal care and fungicide industry**.**

Moreno-Andrade, I. and G. Buitrón 2004 analyzed that the acclimation can be performed using two strategies, the first one fixing the reaction time, independent of the degradation removal efficiency (fixed time) and the second one fixing a removal efficiency/complete removal (variable time), independent of the reaction time. The second strategy (variable time) results a microbial community with higher specific activity compared with that obtained for the fixed time strategy. This second strategy can be implemented for near complete removal of Morpholine in left out spent media.

III. METHODS

This paper forms a meta-analysis on the Bioremediation aspects of NMMO. Systematic review of the theme is not restricted either to any qualitative or to quantitative method, since this review process has analyzed methods from both the data bases. [14,15].

IV. LITERATURE REVIEW AND DATA ANALYSIS

A keyword centric search was conducted employing online databases such as Scopus, Pub Med, Elsevier, Springer and Google Scholar, using the keywords ‘ NMMO ’, ‘NMMO Bioremediation’, ‘ NMMO degradation path’ etc. Papers with both a qualitative as well as a quantitative approach were thoroughly reviewed to find the collect information on the following points.

➢ *Chemistry of NMMO*

The Chemical formula of NMMO is $C_5H_{11}NO_2$. It has a Molar mass of 117.15 g/mol and its melting point at 180 to 184 °C (356 to 363 °F; 453 to 457 K) NMMO, as an N-oxide, is an oxidant It is typically utilised as a secondary oxidant (a co-oxidant) in stoichiometric levels to renew a primary (catalytic) oxidant after the latter has been reduced by the substrate. It exhibits distinctive reactivity with different transition metals, which are oxidised by this reagent.

➢ *Importance in the textile industry*

Since cellulose has a robust and highly organised intermolecular hydrogen bonding network that resists common solvents, it is frequently utilised in the textile industry and stays impervious to most solvents. The hydrogen bonding network that keeps cellulose insoluble in water and other solvents can be broken by NMMO.

For a very long time, CS 2 was used to make viscose rayon and cellophane film, accounting for 75% of the total yearly production. Alkali cellulose, which has the approximate formula [C6H9O4-ONa] n, is created by treating dissolving pulp with aqueous sodium hydroxide (usually 16–19% w/w), and this "alkali cellulose" is then allowed to partially depolymerize to create viscose. After that, carbon disulfide is used to transform the alkali cellulose into sodium cellulose xanthate.

$[C_6H_9O_4\text{-}ONa]_n + n\,CS_2 \rightarrow [C_6H_9O_4\text{-}OCS_2Na]_n$

The biggest flaw in this method is that carbon disulfide is not bonded to the final product when making viscose fibres and cellophane film. Therefore, the majority of the carbon disulfide used in these processes is likely to wind up in the atmosphere unless suitable safeguards are implemented. There would be an annual emission of about 700 kilotonnes if these two processes accounted for roughly 75% of the world's production of about 1 million tonnes. Only about 50% of the CS 2 used in the process is recovered, and it is impossible to recover all the sulphur used in it.

Sulphur introduced in this process gets dispersed as Sulphur compounds (CS, H_2S, COS, SO_2) in the exhaust gases, process baths, solid wastes and the product itself. Moreover it can cause some severe health effects ranging from cardiovascular disease, particularly strokes, neurophysiological impairment, priapism, psychosis, keratosis and even death by respiratory failure. On the other hand, in the NMMO process, more than 99% of the solvent is recycled within the process making the fabric eco-friendly. The small quantities released via waste water are readily degraded in the biological waste water treatment plants.

As several scientific studies report, it is not mutagenic. Moreover, Amine Oxides find a wide application as active substances of personal hygiene (in hair conditioner, face wash, waxes and polishes), which normally undergo immense toxicological examination, therefore its non-toxicity can be assumed for all practical purposes.

➢ *Toxicological studies of NMMO*

NMMO cannot be completely separated from Ionic liquids, which are frequently used in the textile industry.Its secondary amine functionality causes nitrosation in the natural environment, resulting in the formation of the well-known carcinogen N-nitrosomorpholine. Therefore, the 25,000 tonnes of morpholine used annually on a big scale and its possible carcinogenic consequences have environmental significance for biodegradation. Even in normal environmental conditions, NMOR is produced by the reaction of aqueous solutions of nitrite with morpholin [15] or by the reaction of gaseous nitrogen oxides, such as N 2, O 3, N 2 O 4, and NOx in aqueous solutions of morpholin [15] [16] [17]. Additionally, NMOR has been demonstrated to cause harm and malignancies in the liver and kidneys.

Many studies assessing the carcinogenic activities of nitroso compounds have shown that this compound could enhance, even at low doses, the development of early stages of hepato-carcinogenesis in rats (Enzmann et al., 1995). As Morpholine is a precursor of carcinogenic nitrosamine, so, its removal from industrial wastewaters is in the interest of protecting the environment and demands the development of biological approaches of remediation treatment.

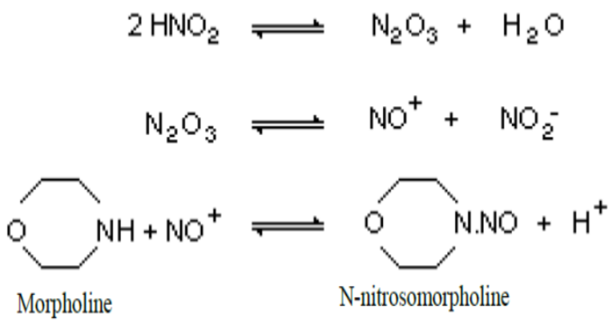

Fig. 1, Reaction mechanisms of the formation of NMOR [15] [16]

Morpholine being toxic and carcinogenic, affecting almost all organs in the body explains the concerns over the need for its effective removal from the biome. Other symptoms observed were diarrhea, spasms, gastrointestinal haemorrhage, dyspnoea and haemorrhage of the nose, mouth, eyes and lungs. [4]

Table.1 Various Hazards posed by NMMO and its discharge in waste water

Type of Hazards	**Acute Hazards / Symptoms**
Fire	Flammable, produces toxic fumes in air
Explosion	Explosive vapour mixture formation above 35*c
Inhalation	Burning sensation, cough, troubled breathing, shortness of breath
Skin	Redness, Pain, Skin burns, Blisters
Eyes	Blurred vision, burning sensation, redness
Ingestion	Abdominal pain, Diarrhoea, Shock
Carcinogenic	Nitrosated to the carcinogenic N-nitrosomorpholine by reactions outside, or within, the human body

➢ *Reason for environmental interest*

N-methylmorpholine and certain of its related chemicals can harm the kidneys and liver over time. These substances have been shown to cause cancer in animals.[4]21][22] After exposure to the substance has stopped, symptoms similar to asthma may last for months or even years. This might be because to reactive airways dysfunction syndrome (RADS), a non-allergic disorder that can develop after exposure to a lot of a very irritating substance. There aren't many studies on its persistence in air, although it is known to have high persistence in soil, little bioaccumulation, and extremely high mobility. [4] [22] However, a literature search turned up no relevant acute toxicological data.

It is a problem that needs attention since bioremediation can be used as a remedy to lessen the toxicity or contamination of NMMO and its breakdown products. However, there is currently no method available for the direct detection of intermediates or even the product(s) of morpholino breakdown. Because morpholin does not have a chromophore and is very soluble in water, easy extraction is not possible, the metabolic route involved in morpholin's biodegradation has been exceedingly challenging to identify. As a result, there hasn't been a method available for the direct detection of intermediates or even morphine. There have only been indirect tactics established, such as COD, optical density, and ammonia (NH_3) measurements, growth on intermediates, and in-vitro enzymatic assays based on which studies have been carried out.

➢ *Regulatory guidelines for NMMO (India and global)*

The NIOSH suggests the following concentrations (US health exposure limits)

- PEL (Permissible) **-** TWA (Time weighted average) **-** 20 ppm (70 mg/m3)
- REL (Recommended) **-** TWA 20 ppm (70 mg/m3) ST 30 ppm (105 mg/m3)
- IDLH (Immediate danger) **-** 1400 ppm

Based on available acute aquatic toxicity data, the lowest valid effect concentration for freshwater aquatic organisms is > 100 mg/L. One chronic data point is available, a 72h-NOErC for algae > 100 mg/L but this value is not used for the Predicted No Effect Concentration derivation.

➢ *Degradation of NMMO using Biological Systems –*

The biodegradation of morphine by sludge-derived *Mycobacterium* or *Arthrobacter* strains is described by a number of authors and is thought to be the most appropriate among the options of numerous bacteria. However, employing *Mycobacterium* for this purpose has its own drawbacks, including pathogenicity and a slower rate of growth. It is crucial to investigate the potential for NMMO biodegradation in additional microorganisms from other backgrounds.

It has been quite challenging to identify the metabolic process that takes place during NMMO biodegradation. Employing biological systems, NMMO can be degraded to N Methylmorpholine (NMM) by the action of the enzyme Trimethylamine – N- oxide reductase. This NMM undergoes further degradation by the action of Trimethylamine dehydrogenase which breaks it down to formaldehyde and Morpholine.

Meister and Wechsler used non adapted sludge inoculum from Lenzing AG's wastewater treatment plant, anaerobic sludge from the sludge digester of a Waste Water Treatment plant which treats municipal waste water (situated in Lenzing, Upper Austria). In addition to this, they employed *Saccharomyces cerevisiae* , taken from a commercially available baker's yeast cube (Ottakringer, Austria) and *Saccharomyces carlbergensis* was obtained from a brewery (Zipf, Austria). [7] The 2 yeast strains were cultivated at room temperature in batch cultures with 500 and 1,000 mg/l of NMMO, respectively, without any addition of other nutrients. The cells were incubated in closed flasks under anaerobic conditions.

V. TESTS WITH MICROORGANISMS OTHER THAN ACTIVATED SLUDGE

➢ *Anaerobic wastewater treatment*

Under anaerobic conditions NMMO was completely converted to NMM, but at this point the reaction stopped and no further metabolization took place. Addition of glucose didn't influence the capability of anaerobic sludge to metabolize NMMO. [7]

➢ *Yeasts*

The experiments with anaerobic sludge and *Saccharomyces carlsbergensis* showed a quantitative reduction of NMMO to NMM, but no further degradation could be achieved. The two yeast strains considered for the study behaved differently, but none was capable of degrading NMMO within 14 days. While *Saccharomyces carlbergensis* could reduce NMMO to NMM (completely), *Sachharomyces cerevisiae* was not able to do even this. [7]

The addition of Saccharose did not alter the metabolic capabilities of the yeast strains, but had an influence on the kinetics of the formation of NMM by *S. carlbergensis*. Saccharose concentrations of 10 g/l significantly decreased the reduction rate of NMMO to NMM, it took about one week until NMMO concentrations began to decrease and NMM concentrations started to increase. On the contrary, without Saccharose the reduction process started after 2 days. The addition of Saccharose in equal concentrations as NMMO didn't affect the reduction kinetics. [7]

VI. TESTS WITH ACTIVATED SLUDGE

➢ *Biodegradability*

When treated with non-adapted sludge (in 1989, with sludge from the first erection state of the WWTP) NMMO was to be classified as a persistent substance according to the OECD guidelines. [7]

In 28 days, less than 20% of the initial COD had been degraded. The study also revealed that NMM was almost completely degraded in 28 days, but showed a long lag period of about 20 days until the degradation started emphasizing on the need of an adaptation phase. [7] Thus, Meister and Wechlser's studies suggest that a complete biodegradation of NMM can be achieved when the sludge is allowed to adapt and the sludge age is high enough to ensure that the adapted organisms cannot be washed out.

As a consequence the complete biodegradation of NMMO seems to be possible, because NMMO can be reduced to NMM on one hand, and NMM again can be completely biodegraded on the other, both steps being carried out biologically. [7]

Meister and Welscher substantiated their hypothesis by laboratory-scale experiments with small activated sludge plants, in which the sludge age was ensured not to be shorter than 15 days. After an adaptation period of about 14 days, the NMMO, NMM and M in the treatment plant effluent were

ISSN No:-2456-2165

reduced to concentrations below their detection levels. (7). Similar observations were made by Watson (1993), who studied the effect of two different acclimation procedures on the biodegradation of substances known not to be degraded rapidly or easily (8). The enrichment procedure, which is a standard procedure for acclimating pure cultures of microorganisms to problematic substances, produced sludges much worse in their biodegradation capacities than the single-flask method. One reason for this phenomenon is that, due to repeated dilutions every 2 to 3 days, slowly growing microbes are eliminated in the enrichment procedure. (8)

The second set of experiments by Meister and Wechsler were designed to examine the ability of the sludge to retain their adaptation over a period of several weeks without any NMMO or its metabolites being present in the wastewater, thus simulating a short-term cessation of the fiber production (for repair, service, etc.). (7) Having been adapted, the sludge organisms retain their ability to degrade NMMO for several weeks, even if no NMMO is present in the wastewater to be treated. (7)

To determine the degradation of NMMO in the presence of other nitrogen containing compounds, nitrate and nitrite were added to the influent of one line. As the wastewater itself, which was used as influent, contained practically no nitrogen the second line could be used as a reference.

The existence of nitrogen salts in the influent also had an influence on the degradation rates of NMMO.

The most important prerequisite for a successful adaptation is a high enough sludge age to ensure that also slowly growing microorganisms can establish themselves in the biocoenosis.

- *Degradation path*

The first step is in the degradation of NMMO is its reduction. The reduction of NMMO could easily be achieved with anaerobic bacteria and some yeast strains. Yet, they stopped at NMM and no further biodegradation was obtained under anaerobic conditions in several studies.

The second step in the biodegradation process i.e., Demethylation of NMM turns out to be the most critical step. Post demethylation, rest of the biodegradation takes place without further problems.

Static biodegradation tests, supported by pilot-plant experiments suggest that the demethylation step require a long adaptation phase. [7]

Once N – Methylmorpholine has been methylated to Morpholine the further biodegradation takes place rather rapidly. The biodegradability of Morpholine has been shown by various authors.(4) The monooxygenase enzyme is in charge of ring cleavage during the degradation of morphine as observed in Mycobacterium aurum MO1. This ring cleavage most likely starts when a bond between the hetero atom and a neighbouring carbon atom is broken.[4] [23]. An essential enzyme in the breakdown of morpholin is morpholine monooxygenase (Sielaff et al., 2001).

This enzyme has a cytochrome P450 catalytic subunit and catalyses the biotransformation of morphine to 2-(2-aminoethoxy) acetic acid (Shaikh et al., 2009, Poupin et al., 1999). Direct methods have been used to assess the degrading reaction mechanism and characterise these reactions, although little is known about the enzymes involved (Combourieu et al., 1998). Furthermore, good bioremediation can be determined by the byproducts of microbial activity.

Table 2 Potential isolates/microbes for degradation and possible end product [7][4]

Sl No	Substance to be degraded	Degraders	Concentration	Duration	End products	References
01.	NMMO	*Saccharomyces carlbergensis*	-	14 days	NMM	Meister& Wechsler, 1998
02.	NMMO	*Saccharomyces cerevisae*	high NMMO (1G / L) and high sucrose (10g/l)	Only a slight degradation	NMM	Meister& Wechsler, 1998
03.	Morpholine	*Mycobacterium gilvum.* strain HE5	10-15mM	10 hr	Ammonia	Schrader et al., 2000
04.	Morpholine	*Mycobacterium. Strain MorD and MorG*	10 mM	60 hr	Ammonia	Knapp et al., 1982
05.	Morpholine	*Mycobacterium aurum MO1*	10 mM	10 hr	Ammonia	Cech et al 1988 Combourieu et al.,1998, 2000, Mazure and Truffaut 1994
06.	Morpholine	*Pseudomonas fluorescens*	1.26 mM	10 days	Ammonia	Magda M. and Aly 2011

➢ *Gap in research*

For the simultaneous determination and quantification of NMMO, NMM, and M, no straightforward and widely applicable approach has yet been established. This is mostly caused by built-in issues with how the potent oxidant NMMO is handled in analytical techniques and machinery. For example, the GC's heat lability and the oxidative stress placed on the columns make detection impossible.

Direct measurement of NMMO is also a challenge and only indirect strategies have been used till date. These strategies include Chemical Oxygen Demand, Optical Density, NH3 measurements and NMR/GC/MS monitoring to identify the intermediates or degradation products/efficacy, etc. Thus, there is scope for optimizing an individual or a consortium of microbe which would effectively remove NMMO and its derivatives from waste water and may be used for the effluent treatment by the industry.

VII. CONCLUSION

Despite its 99% recoverability, eco friendly and sustainable production approach, the use of NMMO in the textile industry poses several threats in the form of potential health concerns. With the advancements in detection and quantitative estimation technologies and better studies on the biochemical characteristics of microbes and their metabolic capacities to alter xenobiotics into relatively lesser toxic compounds or entirely harmless compounds, Bioremediation has a promising scope when it comes to treating toxic compunds**.** Morpholine was earlier thought to be recalcitrant to biodegradation but later several studies have established that many microbes are metabolically able to carry out biodegradation of morpholine. With the majority of physical and chemical methods for NMMO degradation and removal, such as membrane polyelectrolyte filtration, ion exchange resins, the TiO2 photocatalytic process, and ozonation of NMMO-containing water, are expensive and therefore uneconomical for use in commercial applications. Bioremediation using microbes like *Saccharomyces carlbergensis*, *Mycobacterium* strains, and *Pseudomonas* strains appears to be the most viable option.

ACKNOWLEDGMENT

The author would like to express her gratitude to Dr Deepa Biswas, Head of the Department, Department of Botany, Kalinga University for her valuable and constructive suggestions that helped in the framing and shaping of this work. Her willingness to give her time so generously has been very much appreciated.

REFERENCES

[1]. Shaokai Zhangab XunKea Tong Zenga Yonghao Nib XiaolinLuoa Hui Chao Hua Lihui Chena Liulian Huanga , Determination of N-Methylmorpholine in biomass pretreatment solutions by the ammonia-assisted headspace gas chromatography , Renewable Energy , Volume 145, January 2020, Pages 2380-2386
[2]. Zeng, T., Ke, X., Li, L. et al. Quantification of N-methyl morpholine N-oxide in biorefinery process solution by headspace gas chromatography. Cellulose 27, 6861–6870 (2020). https://doi.org/10.1007/s10570-020-03259-7
[3]. A General method for the Quantification of NMMO and its main degradation products by Capillary Electrophoresis, T. Rosenau, A. Potthast, P. Kosma, Christian-Doppler-Laboratory, University ol'Agricultura1 Sciences Vienna, Muthgasse 18, A - 1190 Vienna, Austria
[4]. Biological Approaches for Treating Industrial Effluents Containing "Morpholine", Dr Rupak Kumar
[5]. NMMO treatment: An effective pretreatment for improvement of lignocelluloses by increasing enzyme accessibility, Amir Goshadrou, Keikhosro Karimi
[6]. Poupin, P., Truffaut, N., Combourieu, B., Besse, P., Sancelme, M., Veschambre, H., & Delort, A. M. (1998). Degradation of morpholine by an environmental Mycobacterium strain involves a cytochrome P-450. Applied and environmental microbiology, 64(1), 159–165. https://doi.org/10.1128/AEM.64.1.159-165.1998
[7]. Meister G, Wechsler M. Biodegradation of N-Methylmorpholine-N-oxide. Biodegradation. 1998;9(2):91-102. Doi:10.1023/a:1008264908921
[8]. Watson H M (1993) A comparison of the effects of 2methods of acclimation on aerobic biodegradability. Environ. Toxicol. Chem. 12(11): 2023–2030
[9]. Brown VR (1988) The microbiology of an activated sludge plant involved in the treatment of xenobiotic compounds. PhD Thesis, The University of Leeds, Department of Microbiology
[10]. Brown VR & Knapp JS (1990) The effect of withdrawal of Morpholine from the influent and its reinstatement on the performance and microbial ecology of a model activated sludge plant treating a morpholine-containing influent. J. Appl. Bacteriol. 69: 43–53
[11]. Calamari D, da Gasso R, Galassi S, Provini A & Vighi M (1980) Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere 9: 753–762
[12]. Chudoba J, Albokov á J & Cech JS (1989) Determination of kinetic constants of activated sludge microorganisms responsible for degradation of xenobiotics. Wat. Res. 23: 1431–1438
[13]. Harden A. Mixed-methods systematic reviews: integrating quantitative and qualitative findings. In Technical Brief of Focus: A Publication of the National center for the Dissemination of Disability Research [NCDDR]. 2010; 25.

[14]. Joanna Briggs Institute reviewers' manual 2014. The Joanna Briggs Institute. Available at http:// ww.joannabriggs.org/assets/docs/sumari/ReviewersManual_Mixed-Methods- Review-Methods- 2014-ch1.pdf.

[15]. Mirvish SS. Blocking the formation of N-nitroso compounds with ascorbic acid in vitro and in vivo. Ann N Y Acad Sci. 1975 Sep 30;258:175-80. doi: 10.1111/j.1749-6632.1975.tb29277.x. PMID: 1106296.

[16]. Challis BC, Edwards A, Hunma RR, Kyrtopolous SA, Outram JR. Rapid formation of N-nitrosamines from nitrogen oxides under neutral and alkaline conditions. In: Walker EA, Castegnaro M, Griciute L, 17. 17. Lyle, RE, Editors. Environmental Aspects of N- Nitroso Compounds, IARC Publications No. 19, Lyon, IARC; 1978, pp 127-142.

[17]. Mirvish, SS. Formation of N-nitroso compounds: Chemistry, kinetics and in vivo occurrence,Toxicol. Appl.Pharmacol. 1975: 31: 325–51.

[18]. Mirvish, SS, Ramm, MD, Sams, JP and Babcock, DM. Nitrosamine formation from amines applied to the skin of mice after and before exposure to nitrogen dioxide, Cancer Res., 1988: 48: 1095–99

[19]. Challis, BC and Kyrtopoulos, SA. The chemistry of nitroso-compounds. Nitrosation of amines by the two-phase interaction of amines in solution with gaseous oxides of nitrogen. J. Chem. Soc. Perkin Trans., 1979: I: 299–304.

[20]. Schuster, RH, Nabholz, F and Gmünder, M. The inhibition of the formation of N-nitrosamines: Part 1. The situation and the effect of alpha-tocopherol. KautschukGummiKunstst, 1990: 43:95–106.

[21]. Rupak Kumar and Suman Kapur. Morpholine: A Glazing Agent for Fruits andVegetables Coating/Waxing.International Journal of Science Technology & Engineering, 2016: 2 (11) 694-697

[22]. Mazure N & Truffaut N (1994) Degradation of morpholine by Mycobacterium aurum MO1. Can. J. Microbiol. 40: 761- 765