Green chemicals for effective biofouling removal and preservation of reverse osmosis membranes

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Potential for nitrosamines formation during biofilm removal using free nitrous acid (FNA)

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Executive summary

The formation potential of five nitrosamines, including N-nitrosodimethylamine (NDMA), was investigated during the application of free nitrous acid (FNA) for reverse osmosis (RO) membrane cleaning and biofilm removal.

First, a nitrosamine formation potential test was developed at the laboratory scale for FNA application in order to quantify and compare the concentration of precursors in different matrices under controlled conditions (i.e. ideally different RO membrane cleaning solution). Based on the results, pH 5 was selected as the recommended pH values that can be used for the formation potential test without significant FNA degradation.

Preliminary experiments were performed with two model precursors. To this aim, dimethylamine (DMA) and doxylamine (DOX) were employed as surrogates for secondary and tertiary amines, respectively. Their nitrosamine formation potential when using FNA was compared with the one observed when using monochloramine, already well reported in the literature. In general, the formation of NDMA via nitrosation was found to be less than the NDMA formation during chloramination. Results indicate that NDMA formation from FNA depends on pH; NDMA formation potential increase up to pH 5 and then starts decreasing. The highest NDMA concentration after 7 days of contact time was generated at pH 4-4.5 regardless of the DMA/NO_2 ratio. Although the initial reaction rate for nitrosation of secondary amines reaches its maximum at pH 3-3.4 (pKa of HNO_2), the yield for prolonged periods is largest at pH 4-5 due to a faster decomposition of nitrous acid at lower pH values.

The nitrosamine formation potential of sewage was measured in two independent samples as a second test matrix to evaluate the testing protocol. As observed previously, the NDMA formation potential during chloramination was much higher than FNA for all tested pHs with the highest formation potential observed at pH 5. Interestingly, NDMA was the main species formed during chloramination while N-nitrosodiethylamine (NDEA), N-nitrosomorpholine (NMOR), N-nitrosopiperidine (NPIP) and N-nitrosodibutylamine (NDnBA) were measured during nitrosamine formation potential tests with FNA.

Finally, formation potential tests with both chloramine and FNA were conducted in simulated RO membrane cleaning solutions. Again, although the NDMA formation potential with FNA was much lower than during chloramination, the formation of other nitrosamines was observed. In particular, NDEA was measured up to 110 ng/L. High temperature (37^oC), high nitrite concentrations (500 mg/L as NO_2) and pH values of 4-5 were shown to enhance nitrosamine formation. However, little NDMA formation potential was measured with used membrane cleaning solutions at any condition applied (max 20 ng/L).

The data shows that small amounts of nitrosamines may be formed during the membrane cleaning process with FNA, but the quantity is not enough to accumulate and affect the environment. Based on these results, the application of FNA alone or in combination with hydrogen peroxide shows a low risk level for nitrosamine formation, including NDMA, NDEA, NMOR, NPip and NDnBA.

1. Objectives

With the number of reverse osmosis (RO) membrane plants rapidly increasing across Australia and worldwide for wastewater recycling and seawater desalination, optimisation for sustainable operation of the membranes is essential. One of the major remaining operational challenges is membrane (bio)fouling, which results in increased energy and chemical costs, loss of water production and quality and reduced membrane life. Chemical cleaning of the RO membranes is regularly required to restore their treatment capacity. However, the commonly used cleaning agents (alkalis and acids), used in large quantities, contribute significantly to operational costs, both economic and environmental, for their production, transport and disposal. Hence, there is a need to develop sustainable cleaning agents for RO membranes.

AWMC started a new project to investigate and demonstrate the effectiveness and benefits of a novel, low cost, non-oxidising cleaning agent, free nitrous acid (FNA), applied alone or in combination with hydrogen peroxide, for the prevention and removal of biofouling as well as for scale control in RO membranes.

FNA is the protonated form of nitrite, has been used as a biocide for biofilm control in sewer systems and is considered also for improving secondary sludge biodegradability [1-4]. FNA can be formed from the commonly available sodium nitrite and hydrochloric acid. It is readily biodegradable when diluted, which greatly facilitates its after-use disposal, and the technology is easily implementable using existing infrastructure. As such, it results in a low cost solution for biofouling removal. However, sodium nitrite, which will be used to form the FNA, is known to react with secondary amines under acidic conditions to produce carcinogenic nitrosamines [5]. Dimethylamine (DMA) is known to be present in wastewater from faeces (at fairly high levels ~ 1 ug/L) and would be one of the most common precursor.

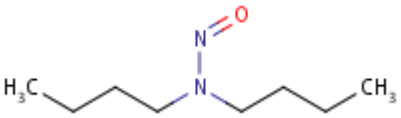
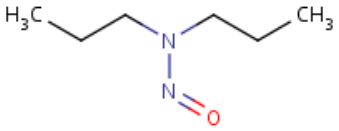
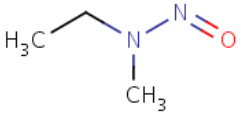
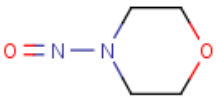
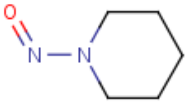
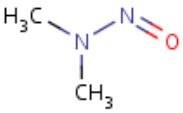
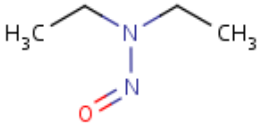
The aim of this project was to evaluate the potential for nitrosamines formation during biofilm removal using FNA. The following scientific approach was used in this project:

- ✓ Development of a nitrosamine formation potential test for FNA application;
- ✓ Validation of the nitrosamine formation potential test with model precursors and comparison with the results obtained during monochloramine formation potential test already well described in the literature;
- ✓ Evaluation of the FNA nitrosamine formation potential of sewage (i.e. complex matrix containing a large variety of precursors);
- ✓ Application of the FNA nitrosamine formation potential to RO membrane cleaning solutions.

2. Literature review

Nitrosamines. Nitrosamines represent a group of organic compounds characterised by the N-nitroso functional group (–N–N=O). They are undesired contaminants, of rising concern due to their extensive occurrence in the environment (e.g., nitrite preserved foods, soil, drinking water, tobacco smoke, personal care products) as well as their high carcinogenic risk [6]. Table 1 presents six nitrosamines classified as probable B2 carcinogenic compounds to humans by either the United States Environmental Protection Agency (US EPA) [7] or the International Agency for Research on Cancer [8].

Table 1. Structure and cancer risk levels in drinking water for seven nitrosamines reported as possibly human carcinogenic compounds [7] and the standards for quality of recycled water supplied to augment a supply of drinking water [9].

Nitrosamine	**Structure**	**10^{-6} cancer risk level (ng/L) [7]**	**Guideline value for recycled water in Queensland (Australia) (ng/L)[9]**
N-nitrosodi-n-butylamine (NDnBA)-$C_8H_{18}N_2O$		6	
N-nitrosodi-n-propylamine (NDPA)-$C_6H_{14}N_2O$		5	
N-nitrosomethylethylamine (NMEA)-$C_3H_8N_2O$		2	
N-nitrosomorpholine (NMOR)-$C_4H_8N_2O_2$		0.8	1
N-nitrosopiperidine (NPIP)-$C_5H_{10}N_2O$		0.8	
N-nitrosodimethylamine (NDMA)-$C_2H_6N_2O$		0.7	10
N-nitrosodiethylamine (NDEA)-$C_4H_{10}N_2O$		0.2	10

Although N-nitrosodimethylamine (NDMA) has the simplest molecular structure of all the nitrosamines, it is one of the most concerning compounds with an estimated 10^{-6} cancer risk level in drinking water at a concentration as low as 0.7 ng/L [7]. Moreover, Public Health

Regulations in Queensland require that NDMA concentrations in recycled water used to augment a supply of drinking water are less than 10 ng/L [9]. NDMA is produced as a by-product of industrial processes that use nitrate and/or nitrites and amines in certain pH conditions, but it has become relatively well known in recycling water practices as it is directly formed from disinfection of wastewater with chloramines [10]. Also, NDMA may be present in industrial effluent discharges such as rubber manufacturing, pesticide manufacturing, food processing, dye manufacturing and in sewage treatment plant effluent. NDMA may be formed directly in sewage as a result of the biological and chemical transformation of alkylamines in the presence of nitrite or during drinking water production using chlorination processes [11, 12].
NDMA is a small (74.08 g/mol) uncharged and polar (Log Kow = -0.57) molecule resulting in a poor retention via RO processes [13]. A low rejection value of 54%, 61% and 70% has been already reported for ESPA-3 (Hydranautics, USA), BW-30 (Dow/FilmTec) and LFC-3 (Hydranautics, USA) membranes respectively [14]. Even lower rejection values between 10 and 25% have been cited elsewhere [15, 16].

Formation pathway. Two major pathways have been identified to contribute to the formation of nitrosamine:

- ✓ Nucleophile substitution reaction between inorganic chloramine and aliphatic amines (e.g. dimethylamine) to unsymmetrical dimethylhydrazine intermediate (UDMH) or chlorinated UDMH, and subsequent oxidation to nitrosamines by either monochloramine or dissolved oxygen,
- ✓ Nitrosation of nitrogen-containing compounds by nitrosating agents (e.g. nitrous acid).

While the first pathway is especially relevant for nitrosamine formation during disinfection of waste and drinking waters with chloramines [12, 17, 18], the second one involves a reaction between nitrite and organic nitrogen, mainly DMA and trimethylamine (TMA), and can occur almost everywhere especially in the living organism [5]. For example, sodium nitrite is known to react with secondary amines under acidic conditions to produce nitrosamines. This nitrosation reaction is due to the introduction of a nitroso group (-NO) into an organic compound causing the formation of nitroso compounds (see Figure 1). Nitrous acid (HNO_2), nitrogen oxides (N_2O_3 and N_2O_4), nitrosonium ion (NO^+), nitrous acidium ion ($H_2NO_2^+$) and nitrosyl halides (XNO, X = Cl, Br) are potential nitrosating agents [5, 19]. Although nitrates do not directly form nitrosamines, it might be transformed to nitrites by microorganisms in the environment [11, 20].

Nitrosation is catalyzed by acid, because for nitrosation to occur, nitrite is usually first converted to nitrous acid (pKa of 3.4). At a low pH, the kinetic of nitrosamine formation is usually determined by the formation of the nitrosating agent (i.e. slower step than the nitrosation of the amine). At a higher pH, the kinetic of nitrosamine formation is determined by the nitrosation of the amine due to the limited availability of the non-protonated amine [5, 21]. Fan and Tannenbaum (1973) reported than amines with weak basicity (e.g. morpholine) will also nitrosate faster than amines with strong basicity (e.g. dimethylamine) [22].

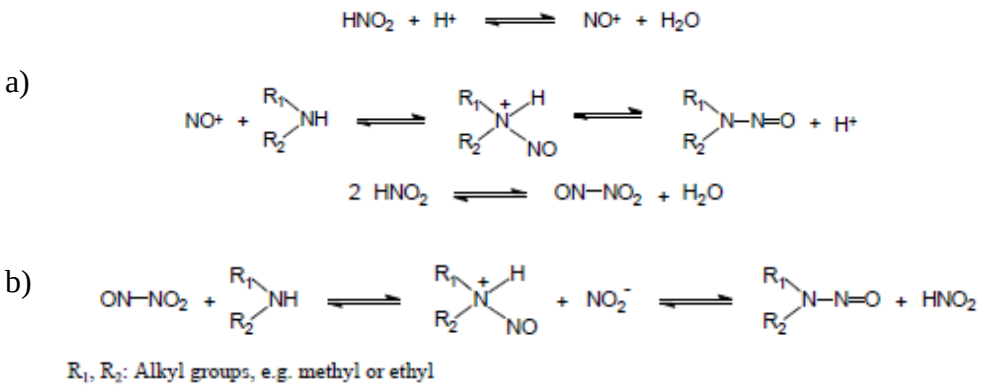


Figure 1. Formation of nitrosamines by N-nitrosation of secondary amines by (a) NO^+ and (b) N_2O_3. Under acidic conditions, nitrite is transformed to nitrous acid (pKa=3.37) which is not stable in aqueous solution but decompose either to the nitrosyl cation NO^+ or to dinitrogen trioxide N_2O_3, which are reacting with nitrogen-containing compounds to form nitrosamines [5].

Factors influencing nitrosamine formation. Nitrosamine formation can be impacted by different reaction factors such as pH, reaction time, temperature and the presence of catalysts. The nitrosation of DMA is fast at pH 3.4 (pKa of HNO_2) and low at neutral pH values [22, 23]. Similar results have been reported for morpholine (pH 3-3.36) [22, 23]. Although the initial reaction rate for nitrosation of secondary amines has its maximum at pH 3-3.4, the yield for prolonged periods is larger at pH 4-5 due to a faster decomposition of nitrous acid at lower pH values [21, 23].

Nitrosation is however also dependent on the amine not being protonated, and the decrease of the pH reduces the availability of the reactive amine species. At pH > pKa of HNO_2, the rate of nitrosation decrease rapidly because the concentration of active nitrosating species generated decrease. Nitrosation can be catalysed by metal salts (e.g., Cu, Fe, Zn), nucleophilic anions (e.g., SCN^-, I^-, Br^-, Cl^-), formaldehyde and chloral, phenols and fulvic acid [5, 22, 24]. Contradictory results have been reported regarding the role of microorganisms; while Ayanaba and Alexander (1974) observed that microorganisms play a role in the NDMA formation in water and soil, due to a nitrate reductase activity of the bacteria, Mills and Alexander (1976) did not notice impact [11, 25]. Reducing agents can also minimize the formation of nitrosamines by reduction of nitrite to nitric oxide [5, 26]. These substances, such as ascorbic acid, phenols sulphur compounds (e.g. bisulfite, sulfamate) compete with the amine for nitrosating species, resulting in the nitrosation inhibition [27].

Nitrosamines precursors. Organic nitrogen-containing compounds, especially aliphatic and aromatic amines, are key precursors in the formation of nitrosamines. Secondary amines are of most concern due to their rapid reactivity. Primary amines form very unstable nitrosamines, that quickly decompose, while tertiary and quaternary amines necessitate more complex mechanisms for nitrosamine formation (e.g. breakage of the C-N bond for the tertiary amines) [21]. At 25°C and pH 3.4 (pKa of HNO_2), nitrosation of tertiary amines is approximately 10,000 times slower than the homologous secondary amine [5]. Nitrosamine yields vary with steric accessibility of the nitrogen atom. Potential nitrosamine precursors are ubiquitously widespread and from both natural and anthropogenic origins. DMA (secondary

amine) has been frequently used as a model precursor to study nitrosamine formation from both nitrosation and chloramination [11, 12, 18, 25]. DMA is widely used in chemical industries (e.g. rubber, pesticide, dyes, pharmaceuticals industries) and its natural occurrence in the environment (e.g. human urine, plants, algae), as a metabolic product of proteins in animals and plants, makes it a key nitrosamine precursors. DMA is for instance known to be naturally present in wastewater from faeces (at fairly high levels ~ 1 ug/L) ([28]. This secondary amine can be formed by dimethylation of trimethylamine (TMA). TMA (tertiary amine), also considered as a potential precursor for nitrosamine formation, occurs also naturally in the environment as well(e.g. plant, fish, algae). A large number of anthropogenic chemicals containing DMA-like molecule fragments have also been identified as potential precursor for NDMA formation (see table 2).

Table 2. Examples of NDMA precursors from anthropogenic origins.

Compounds family	Examples
solvents	trimethylamine, *N*,*N*-dimethylformamide
pesticides	diuron, isoproturon, tolylfluanid
pharmaceuticals	ranitidine, erythromycin
dyes	methylene blue
surfactants and emulsifiers	*N*,*N*-dimethylamino fatty acid amides
corrosion inhibitors	*N*,*N*-dimethylaminoethanol

Occurrence of nitrosamine and precursors in wastewater and drinking water treatment plant. Previous studies have reported the formation of NDMA from DMA and nitrite in sewage samples [11, 25]. Apart from the occurrence as a disinfection by-product, NDMA and other nitrosamines can be formed unintentionally from industrial processes in which amines get into contact with nitrogen oxides, nitrous acid or nitrite and may be discharged with wastewater. As a result, wastewater often contains significant concentrations of NDMA and other nitrosamines (see Table 3).

The presence of nitrosamines in WWTPs has been reported and shown to be highly dependent on the degree of industrial contribution [29]. Indeed, concentrations in urine samples and domestic wastewater indicated that human excretion accounted for levels of <5 ng/L of NDMA and <1 ng/L of the other nitrosamines in municipal wastewater [29]. Furthermore, algae, bacteria, amino acids, proteins, and natural organic matter have rather low NDMA formation potentials and no strong relationships have been observed with bulk wastewater parameters such as DON concentration, indicating that NDMA precursors in water are mainly of anthropogenic origin [30]. Secondary amines (such as DMA) are well-known nitrosamine precursors and can produce nitrosamines via N-nitrosation at different stages of water and wastewater treatment [31]. Although the presence of a DMA functional groups in tertiary amines is crucial for NDMA formation, this precursor cannot quantitatively explain NDMA formation [30]. Many of these anthropogenic chemicals are predominantly present as dissolved, low molecular weight compounds and have been detected in wastewater or human-impacted surface water. Several of these are only partly degradable during wastewater treatment.

Table 3. Examples of nitrosamines and precursors concentrations in wastewater treatment plant.

References	Matrix	Nitrosamines	Formation potential
Sedlak and Kavanaugh (2005)	Influent samples (USA)	NDMA	7–790 ng/L
Krauss et al. (2009)	Primary effluents (Switzerland)	NDMA	5–20 ng/L (max 1 ug/L.)
	Influents	NDMA	< 5 ng/L
	Municipal sewage,	NDEA, NPYR, NPIP, and NDnBA	<1 ng/L
Mitch and Sedlak (2004)	Primary effluent (USA)	DMA	30 to 80 μg/L,
	Secondary effluent	DMA	< 15 μg/L
Padhye et al. (2009)	Primary sludge (USA)	NDMA	678 ± 302 ng/L
		Secondary amines*	1280 ± 689 μg/L
	Waste-activated sludge	NDMA	394 ± 322 ng/L
		Secondary amines*	210 ± 266 μg/L
	Anaerobic digester mixed liquor	NDMA	271 ± 100 ng/L
		Secondary amines*	6.2 ± 3.9 μg/L

***** mostly DMA and pyrrolidine. (NDEA, N-Nitrosodiethylamine; NOYR, N-Nitrosopyrrolidine ; NPIP, N-Nitrosopiperidine ; NDnBA, N-Nitrosodi-n-butylamine)

Nitrosamines and precursors are present in wastewater but can also be generated by the wastewater treatment itself. N-nitrosation can produce nitrosamines during sludge thickening in the second clarifier, when DMA-based synthetic polymers are used in primary and waste-activated sludge thickening processes. These polymers are important particle-associated nitrosamine precursors in sludge systems[32]. Significant nitrite levels can also be produced in the clarifier during nitrification-denitrification processes for N removal due to the reduction of nitrate or oxidation of ammonium. Although N-nitrosation is most favourable at acidic pH, the reaction can also be catalysed by carbonyl compounds such as formaldehyde, which is abundantly detected in industrial and municipal wastewater at near neutral pH conditions [24, 33]. Keefer and Roller (1973) suggested that formaldehyde catalyzes N-nitrosation by interacting with a secondary amine to form an adduct that is highly reactive toward nucleophilic attack by nitrite [24]. Primary and waste-activated sludge are further transfer to anaerobic digesters for sludge stabilization. The particle-associated nitrosamine precursors, the residual nitrosamines and nitrite may then either be deactivated or additional ones can be formed from larger biomolecules. N-nitrosation of precursors in anaerobic digesters may be particularly important due to the formation by fermentation of carbonyl catalysts and possible low pH conditions in not well mixed digesters [20].

Fate of nitrosamines and precursors in wastewater treatment plant. Since NDMA is miscible in water, has a low vapour pressure and a low octanol/water partition coefficient (log Kow of -0.57), it is not likely to bioaccumulate, adsorb to particulates, or volatilize to any significant extent. Consequently, the removal of NDMA during biological treatment is attributed to microbial degradation rather than adsorption [20]. Studies have shown that

nitrosamines can be biodegraded in aqueous and soil system under both aerobic and anaerobic conditions [34, 35]. However, Sedlak et al. (2005) showed high variability in the removal of NDMA by secondary biological treatment [36]. Mitch and Sedlak, (2004) showed that secondary biological treatment effectively also removed DMA, lowering its concentration to levels that could not produce significant quantities of NDMA upon chlorine disinfection [32]. However biological treatment was less effective at removing NDMA precursors in secondary effluent other than dimethylamine [32].

During activated sludge treatment, Krauss et al. (2010) observed that NDMA and other small polar and charged nitrosamines precursors were removed to about 80%, while less polar ones tend to be more recalcitrant [30], even after extended biological treatment. In aerobic conditions, NDMA biotransformation takes place either through the cleavage of the N—N bond (denitrosation, i.e. the reduction of the nitroso group), which results in the formation of DMA and ammonia, or by α-hydroxylation of the methyl group followed by a demethylation [31]. NDMA biodegradation under anaerobic conditions has also been reported for indigenous soil microorganisms and biofilm reactors; however, the rate and extent of biodegradation under anaerobic conditions were significantly lower than under aerobic conditions. Padhye (2009) conducted bioassay experiments with secondary amines and showed that the lower molecular weight secondary amines had the highest rate of biodegradation under anaerobic conditions and, hence, are expected to be removed relatively fast [20]. Over time, nitrosamines with relatively high logP values, containing symmetric, long-chain alkyl groups (e.g. NDEA, NDPA, and NDnBA) were more resistant to biodegradation than nitrosamines with relatively low logP values and shorter or asymmetrical alkyl chains (e.g. NDMA, NMEA, and NPYR) due to their higher hydrophobicity, lower bioavailability, and steric hindrance resulting from the longer chain, bulkier alkyl groups present in these molecules.

Photodegradation is the main process for removing NDMA from the aquatic environment. However, the efficiency of removal of NDMA depends on the characteristics of the particular water environment. Typically, photodegradation of NDMA is much slower in waters with high concentrations of organic substances and suspended solids [37].

3. Materials and Methods

3.1. Matrix

Model precursors. Dimethylamine (DMA) hydrochloride (≤99%) and Doxylamine (DOX) succinate salt (>98%) were purchased from Sigma Aldrich and dissolved in Milli-Q water at 20 mg/L for further experiments. These compounds were used as model precursors of nitrosation and referred to secondary and tertiary amines, respectively.

Sewage. Domestic wastewater was collected from a local wet well (Brisbane, Australia), stored at 4 °C and heated up to 20 °C before being used for the experiment. Two independent samples were tested.

Cleaning solutions. To simulate cleaning solutions, biofouling was collected by scraping a known surface area of a RO module. Subsequently, the foulant was mixed with FNA cleaning solutions. Two different fouled RO membranes from full-scale wastewater treatment plant were used to carry out this test:

- ✓ ROFNA-1 was an ESPA2-LD RO membrane (Hydranautics, USA) taken out from the Beenyup ground water replenishment plant (Water Corporation, Perth).
- ✓ ROFNA-2 was an SWC5-LD RO membrane (Hydranautics, USA) collected from an industrial water recycling plant.

Based on the results of the autopsies, the fouling layer for these two membranes consisted of biological, organic and inorganic material (see technical report 1). However, the analysis suggested that the RO modules have been exposed to mostly organic and biological foulants.

3.2. Reagents

Free nitrous acid (FNA). FNA is related to the total nitrite concentration, the pH and the temperature and is calculated as follows [38]:

$$FNA = NO_2^-\text{-}N / (K_a \times 10^{pH})$$

where K_a is the ionization constant of the nitrous acid ($K_a = e^{-2300/(T+273)}$) and T is the temperature (°C).

The required FNA concentration was achieved by varying the nitrite concentration and pH. Sodium nitrite (≤99%) from Sigma Aldrich was used. The pH was adjusted with hydrochloric acid (analytical reagent, 32%, Univar) and measured using a SevenEasy^{TM} (Mettler Toledo, USA). Nitrite concentrations were analysed using a Lachat QuikChem8000 (Lachat Instrument, Milwaukee, Wisconsin) flow injection analyser (FIA). To quench the reaction, the samples were adjusted to pH 7, using sodium hydroxide solution (analytical reagent, pellets, Univar).

Hydrogen peroxide (H_2O_2). H_2O_2 (30 wt %, Merck) was purchased from Sigma Aldrich. The H_2O_2 concentration was determined by means of a spectrophotometric method utilising ammonium metavanadate (NH_4VO_3) [39]. UV_{450nm} was measured using a Cary 50 bio UV–vis absorption spectrophotometer (Varian, Australia). The H_2O_2 residual was eliminated by

adding a specific amount of catalase (catalase from bovine liver solution C-100 mg, Sigma Aldrich) before applying routine analysis [40].

Monochloramine (NH_2Cl). Ammonium chloride (TraceSELECT, 99.9% purity), sodium hydroxide (SigmaUltra, 98%, pellets), and sodium hypochlorite solution (reagent grade, available chlorine 4%) were used to generate NH_2Cl. NH_2Cl was freshly prepared before each experiment because of its ability to autodecompose at high concentrations. Prior to the preparation of the chloramine solution, the free chlorine concentration in the hypochlorite stock solution was determined using commercial DPD test kits (Hach, USA). Based on the free chlorine concentration in the hypochlorite solution, the volume of hypochlorite stock solution to be added was calculated to achieve molar ratio ammonia to free chlorine of 1.2:1. The respective volume of hypochlorite stock solution was added dropwise to the ammonium chloride solution at pH 8. Monochloramine and dichloramine have different absorption bands with maximum at 254 nm and 295 nm respectively. Then, chloramines speciation was spectrophotometrically tested before initiating the experiment. To quench the chloramines solution, sodium sulfite (Fluka, puriss. p.a., 98.0%) was employed.

3.3. Nitrosamines analysis

The nitrosamines were analyses using either High Performance Liquid Chromatography coupled to a photodiode array detector (HPLC-DAD) or Gas Chromatography coupled to a Mass Spectrometry(GC-MS) depending of the concentration expected (Table 4).

Table 4. Comparison of the two methods used in this project to quantify nitrosamine formation.

Parameters	**HPLC-DAD**	**GC-MS**
Matrix	Model precursors (i.e. compounds which do not absorb at 228nm)	Sewage Cleaning solutions
Sample preparation	Filtration (0.45 µm nylon filter)	Solid Phase Extraction
Nitrosamines	NDMA	NDMA. NDEA, NMOR, NPip, NDnBA
Limit of quantification	NDMA (10 µg/L)	NDMA (5 ng/L), NDEA (10 ng/L), NMOR (10 ng/L), NPip (20 ng/L), NDnBA (20 ng/L)

HPLC-DAD. NDMA was analysed by reversed phase HPLC-DAD using a Shimadzu LC-20 AT Prominence LC coupled to a SIL-20A HT Prominence auto sampler plus a SPD-M20 A Prominence diode array detector (Shimadzu, Japan). Isocratic elution of NDMA was used in a 10 cm LiChrospher RP-select B (Merck, U.S.A) column with 5 µm pore size, using a mobile phase consisting of 5% methanol and 95% phosphate buffer (26.9 mM) at pH 6.85, at 1 mL/ min flow rate. The limit of detection was 10 µg/L. A calibration curve was prepared with NDMA obtained from Supelco (5000 µg/mL in methanol, purity of >99.9%) and the analytical method showed linearity up to 1000 µg/L (see appendix A). NDMA exhibits two absorption bands, with one maximum at 228 nm and one at 332 nm with molar adsorption coefficient of 7378 $M^{-1}.cm^{-1}$ and 109 $M^{-1}.cm^{-1}$ respectively [41]. As a consequence, the NDMA was quantified using HPLC with UV detection at 228 nm.

SPE-GC/MS. Solid phase extraction (SPE) was performed based on EPA Method 251 using commercial EPA charcoal cartridges optimized for NDMA analysis (Restek). HPLC grade dichloromethane, methanol and water were used for conditioning and cleaning of the SPE cartridges. The cartridge was allowed to dry during dichloromethane and methanol cleaning but not during the last cleaning with HPLC water. The flow rate for sample loading was adjusted to 10 mL/min. Anhydrous sodium sulphate, granular 10–60 mesh from Mallinckrodt was used to remove water from the extracts. Finally 99% decane (Sigma-Aldrich) was used as keeper in the final concentration step. In that analysis, water is passed through a carbon SPE cartridge and the N-nitrosamines are eluted off with dichloromethane. The extracts were concentrated by evaporation under nitrogen to 1 mL and analysed by capillary GC-MS in Positive Chemical Ionisation (PCI) mode with anhydrous ammonia as the chemical ionisation gas (Finnigan Trace G.C. Ultra and Finnigan Trace DSQ Mass Spectrometer with Ammonia Chemical Ionization). 2 mL sample were splitless injected into the gas chromatograph at 250^{o}C. The column used was ZB-5 MS. The initial temperature of the oven was 40^{o}C for 1 min. Then a ramp was programmed at 40^{o}C/min to 265^{o}C. Final temperature was held during 5.4 min. Helium gas at 1 mL/min was used as carrier gas. Anhydrous ammonia gas 99.99% pure was used in the mass spectrometer. Ammonia inlet pressure was 100 kPa and the reagent gas flow for CI was 3.5 mL/min. The quantification limit for the technique was 5 ng/L. Samples were analysed at Queensland Health Forensic and Scientific Services (QHFSS). Deuterated d6-NDMA was used as surrogate (1,000 mg/mL in dichloromethane, Accustandard). Fifty microlitres of a 0.5 mg/L deuterated d6-NDMA stock solution in methanol solvent (25 ng/L) were added as surrogate to each 1 L sample to evaluate the percentage of recovery of the extraction. 50 ng/L of internal standard was added to the final 1 mL. Every batch was accompanied by a standard spiked solution and a blank to determine the performance of the extraction and the possible NDMA contamination in the water.

3.4. Other analysis

Non purgeable organic carbon (NPOC). Dissolved organic carbon was measured with a TOC-multi N/C 2100S (Analytik Jena, Australia) using the non-purgeable organic carbon method.

Conductivity. Conductivity was measured using a SevenEasy^{TM} (Mettler Toledo, USA).

4. Results and Discussion

4.1. Development of the Nitrosamine formation potential test for FNA application

The NDMA formation potential test was introduced to characterise the maximum formation of NDMA that could be formed in a water sample disinfected with chloramines [10]. The test maximizes nitrosamine formation using high monochloramine dose (140 mg/L as Cl_2) during 10 days of contact time to ensure that all potential precursors reacted. Then, this test is an indicator for nitrosamine precursor concentration.

An NDMA formation potential test, closely closely the procedure described by Mitch et al. (2003), has been adapted and applied in various research projects related to water recycling [15, 42]. This test is performed at pH 6.8 using a 10 mM phosphate buffer (addition of 700 mg/L KH_2PO_4 and 880 mg/L Na_2HPO_4, $2H_2O$ to the sample). The experiments are performed in head-space free amber glass bottles stored at room temperature (23 ± 2 °C) in the dark for seven days. On the seventh day, the residual chloramine concentration is quenched with sodium sulphite to prevent further NDMA formation and the samples are filtered through 0.45 µm nylon filters and analysed for NDMA quantification.

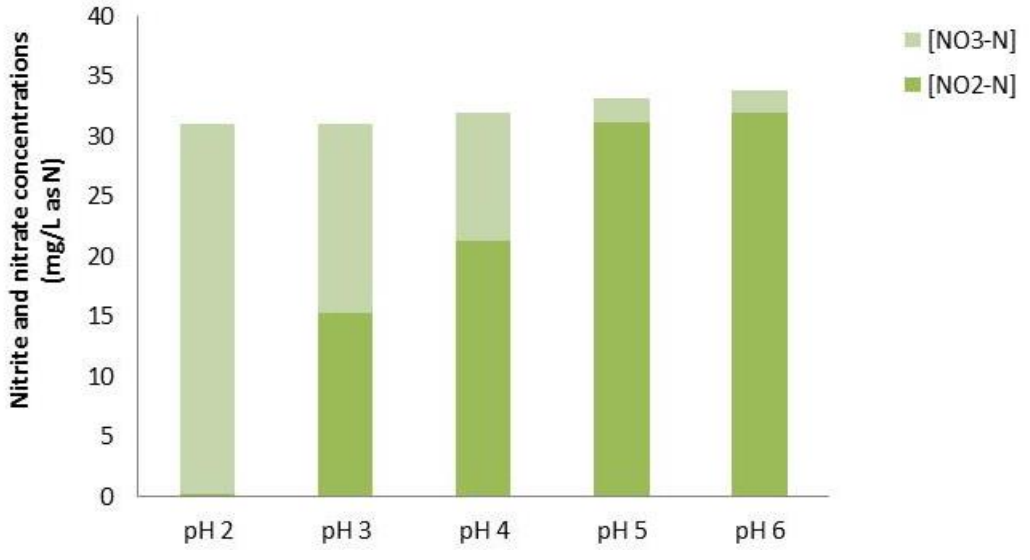
A similar test was developed in this project in order to measure the concentration of nitrosamines generated during FNA application and compare the nitrosamine formation potential of different matrices. The adapted formation potential test involves applying a high dose of FNA (e.g. 30 mg/L as NO_2-N) to a pH-buffered water sample during seven days of contact period to produce the maximum amount of nitrosamines. On the seventh day, the residual FNA concentration is quenched by adjusting the pH to 7. To optimize the test, the impact of pH value, the buffer solution and effect of head-space on FNA stability was first investigated (see appendix B). The following conclusions were drawn:

- ✓ Impact of the pH.

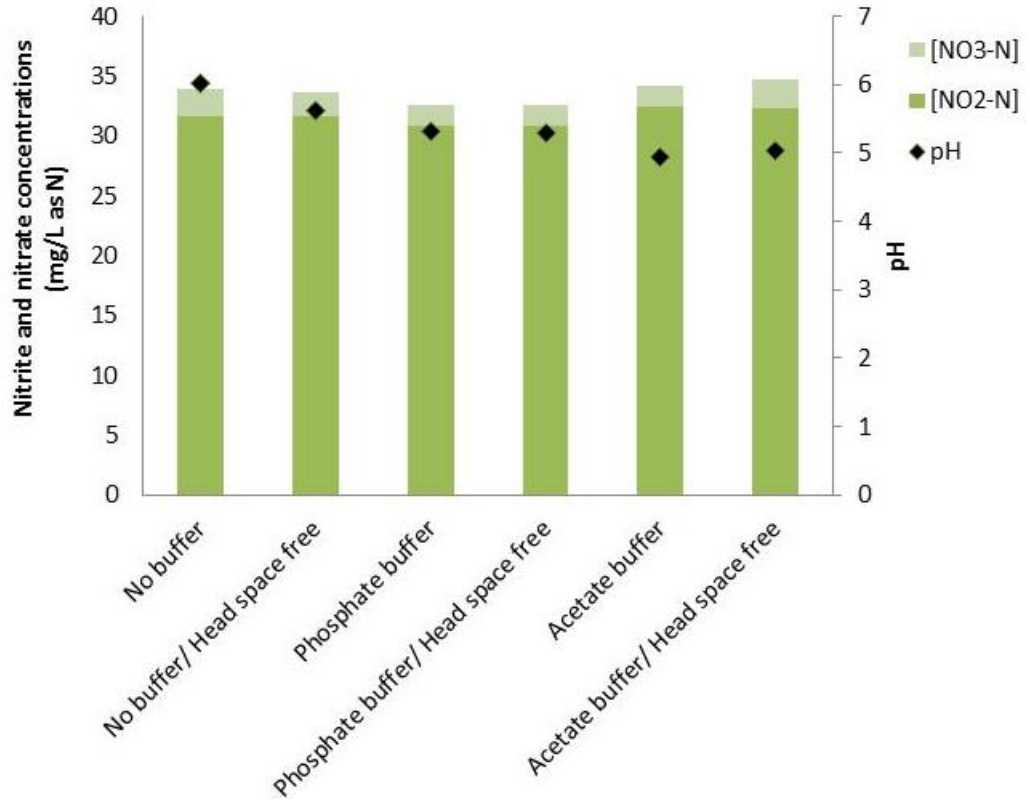
The stability of FNA solutions was strongly pH dependant. FNA solutions were stable at pH 5 and above after 7 days. While the pH values were stable over the time (i.e., pH variation below 0.15 pH unit, n=6), the total nitrite concentration decreased resulting in a decrease of FNA concentration at pH 4 and below (Figure 2.a and 3.a). The lower the pH, the higher total nitrite concentration decreased. Experiments conducted at pH 4, 3 and 2 showed a total nitrite concentration decreasing of 30, 50 and 95 %, respectively, together with nitrate formation. Both pH 5 and 6 did not demonstrate FNA degradation after 7 days. When pH is decreased by one unit, the amount of nitrite required to make up a certain FNA concentration can be reduced by 10 times. Therefore, pH 5 is the recommended pH values that can be used for the formation potential test without FNA degradation.

- ✓ Impact of the buffer solution and conditioning.

Figure 2.b presents the pH and nitrite/nitrate concentration after 7 days and Figure 3.b shows the evolution of FNA concentrations for different buffer solutions and conditioning. FNA concentration was similar for all the different conditions, demonstrating that using head-space free bottle and buffer solutions were not necessary for the test with FNA.

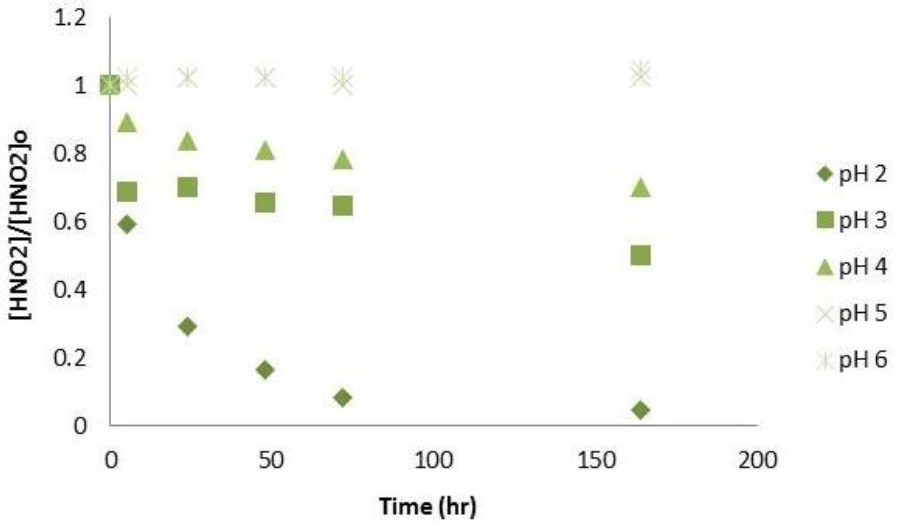


a)

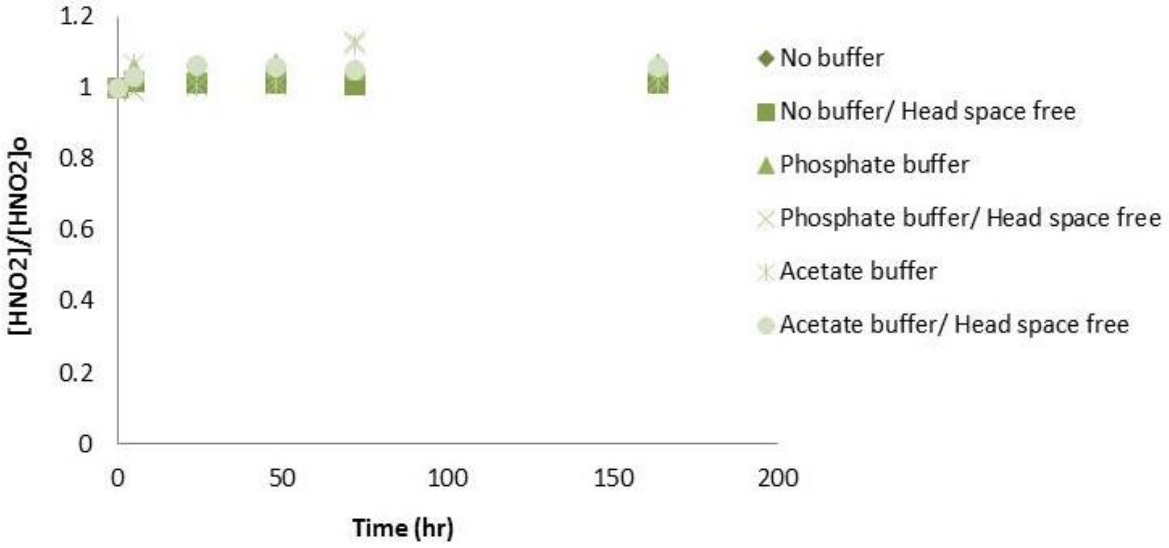


b)

Figure 2. Impact of (a) pH ([NO_2-N]o=30 mg/L), (b) buffer solution and bottle conditioning on pH, nitrite and nitrate concentration after 7 days (pHo=5, [NO_2-N]o=30 mg/L).



a)



b)

Figure 3. Impact of (a) pH ([NO_2-N]o=30 mg/L), (b) buffer solution and bottle conditioning (pHo=5, [NO_2-N]o=30 mg/L) on FNA concentration after 7 days.

Table 5 summarise the final conditions applied for the nitrosamine formation potential test with FNA.

Table 5. Conditions applied for the nitrosamine formation potential tests conducted with monochloramine and FNA.

Parameters	Monochloramine application	FNA application
Reagent dose	140 mg/L as Cl_2	100 mg/L as NO_2
Contact time	7 days	7 days
pH	7	5
Buffer solution	Phosphate buffer	No buffer solution
Conditioning	Head-space free amber bottle	Amber bottle

4.2. Application of the adapted formation potential test to model precursors.

In order to validate the formation potential test described in Table 5, tests were conducted with two model precursors, i.e., DMA and DOX, and compared to the results obtained from the nitrosamine formation potential with chloramine. These compounds were chosen as surrogates for secondary and tertiary amine respectively and because their reactivity is already reported in the literature [23, 43, 44].
The preliminary tests showed that pH 5 was preferred pH for the formation potential test in terms of FNA stability. To validate the test, the impact of pH on NDMA formation potential was determined. The experiments were conducted with high initial concentration of the model precursors (20 to 250 mg/L) in order to be able to quantify NDMA using HPLC-DAD.

4.2.1. NDMA formation potential of a secondary amine

The impact of pH on NDMA formation was investigated using a 100 mg/L DMA solution. A 30 mg-N/L nitrite solution was adjusted to pH between 3 and 6. The NDMA concentrations generated after 7 days are given in Figure 4.

Figure 4 and 5 indicate that NDMA formation depends on pH (i.e. FNA concentration). The highest NDMA concentration was generated at pH 4-4.5 regardless of the DMA/NO_2 ratio with an NDMA formation yield of 0.24% (Figure 5). Then NDMA formation potential decreases with increasing pH. No NDMA was detected above the limit of detection for pH 7.2. Although the initial reaction rate for nitrosation of secondary amines is maximal at pH 3 and 3.4 (pKa of HNO_2) [21, 22], the yield for prolonged reaction periods is larger at pH 4 to 5 [21]. This can be explained by the faster decomposition of FNA at low pHs. After 7 days, the tests conducted at pH 3 had a nitrite residual of 4 mg/L NO_2-N (87% decomposition), while no FNA decomposition was observed for pH 5 and 6 (nitrite residual of 30 mg/L NO_2-N). At low pH, FNA is instable and total nitrite concentration decreases together with nitrate formation. Nitrates do not form nitrosamines directly.

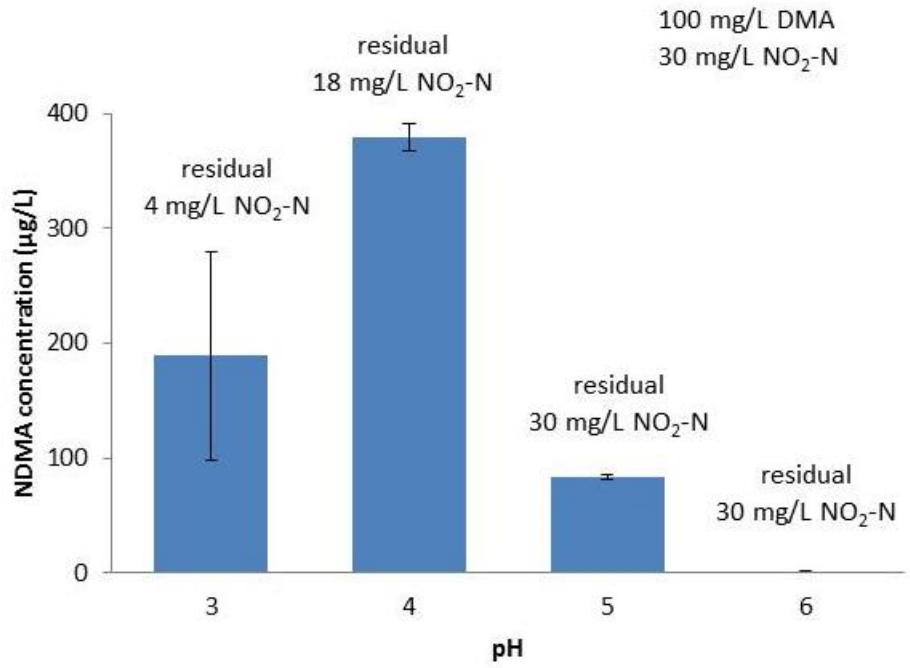


Figure 4. NDMA formation with DMA (100 mg/L) and nitrite (30 mg/L as N) as a function of pH.

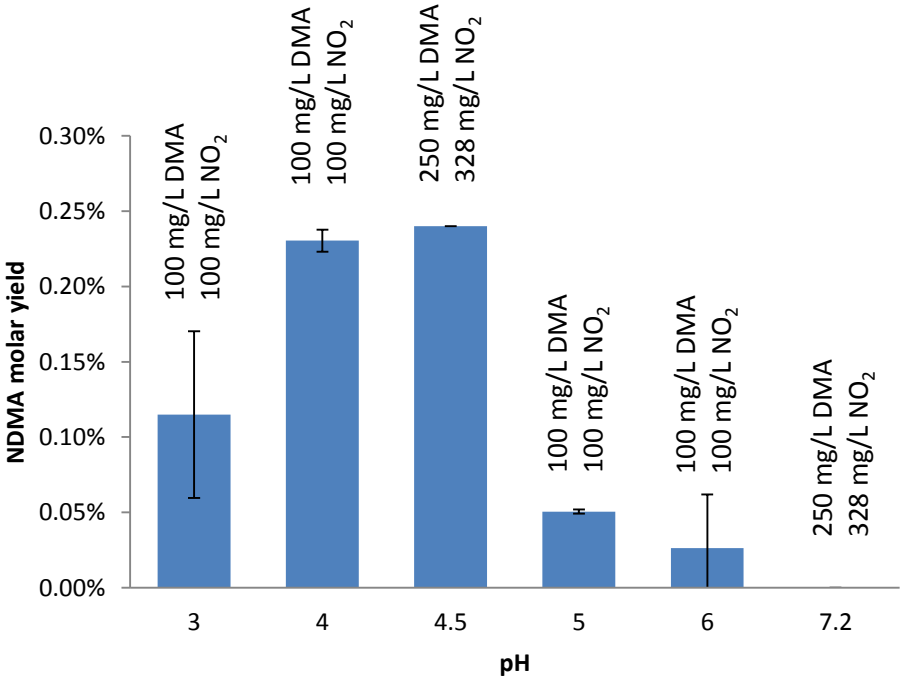


Figure 5. NDMA formation with DMA (100 or 250 mg/L) and nitrite (30 or 100 mg/L as N) as a function of pH.

4.2.2. NDMA formation potential of a secondary and a tertiary amine with FNA and monochloramine

Tertiary amine are also able to form nitrosamine [11]. Experiments were also conducted to compare the reactivity of tertiary amines toward nitrosation and chloramination. Figure 6 shows the NDMA concentration generated with 20 mg/L of DMA and DOX using the formation potential test with FNA and monochloramine. As expected, the tertiary amine

(DOX) showed lower NDMA formation potential than DMA. After chloramination, DOX formed NDMA with a molar yield of 4.2%, which is in a similar range than previous work [43]. No NDMA was detectable after formation potential test with FNA, confirming that tertiary amine are less reactive towards nitrosating agents [5, 11].

The results show that chloramination is much more critical regarding NDMA formation than FNA. A NDMA molar yield of 4.6% (1522±21 µg/L) was obtained with monochloramine versus 0.03% with FNA (11±0 µg/L) for DMA. With DOX, a NDMA molar yield of 4.2% (228±6 µg/L) was obtained with NH_2Cl, while no NDMA was detected with FNA. The formation of NDMA of tertiary amine via nitrosation is slow, due to the necessity of C-N bonds breakage.

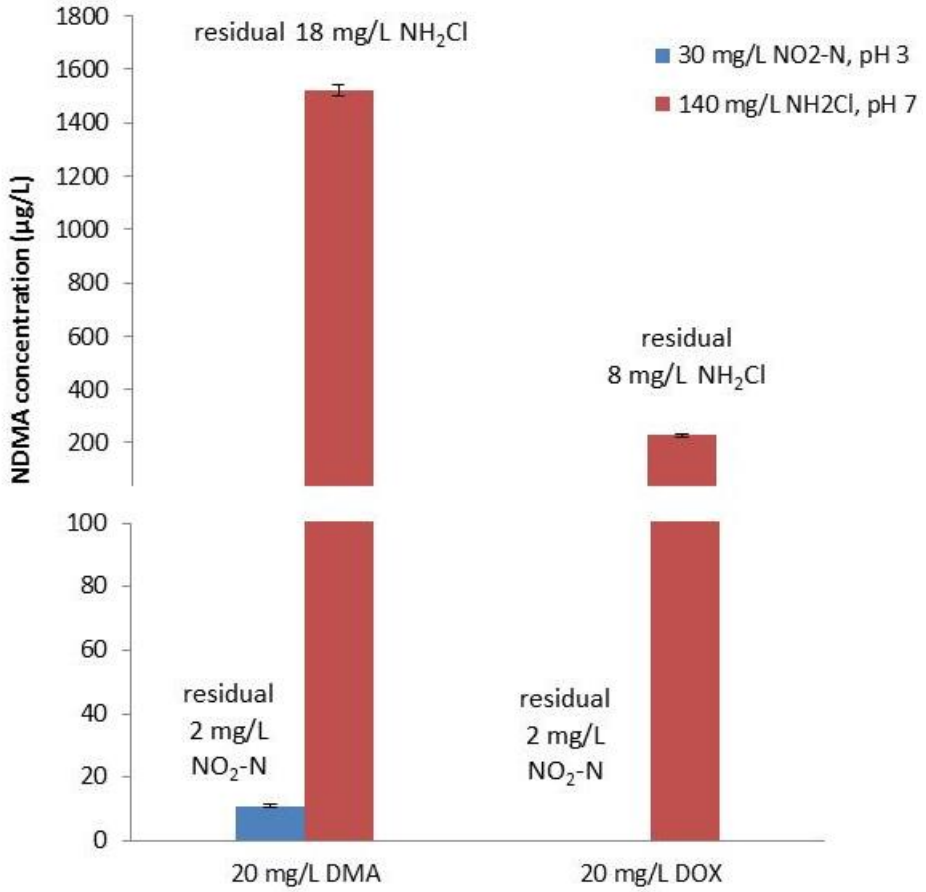


Figure 6. Comparison of the NDMA formation potential of a secondary amine (DMA, 20 mg/L) and a tertiary amine (DOX, 20 mg/L) with either nitrite (30 mg/L as N) or monochloramine (140 mg/L).

4.3. Application of the nitrosamine formation potential test to sewage

The nitrosamine formation potential was measured in sewage with both FNA and chloramines following the previously described tests. Sewage is a complex matrix and contains a larger variety of precursors. It can be seen from the data in Figure 7 that NDMA was the main nitrosamine formed from FNA reaction and the only species formed during chlormaination. The higher NDMA formation potential was observed at pH 5 (1230 and 1620

ng/L for sewage batch #1 and #2 respectively). This result validates the choice of pH for the formation potential test with FNA. At higher pH, NDMA formation dramatically decreases. At pH 4 and 3, the NDMA formation potential decreases, which is consistent with a decrease of nitrite residual (18 and 2 mg/L as N respectively).

As observed previously for model precursors, the NDMA formation potential (5611 ng/L) for chloramination is much higher than FNA (all applied pH) (<1250 ng/L). However, while with NH_2Cl, other nitrosamines were not formed in detectable amounts, when FNA was applied, other nitrosamines such as NDEA, NMOR, NPip and NDnBA were detected. As seen before, the formation of other nitrosamines is also maximized at pH 5.

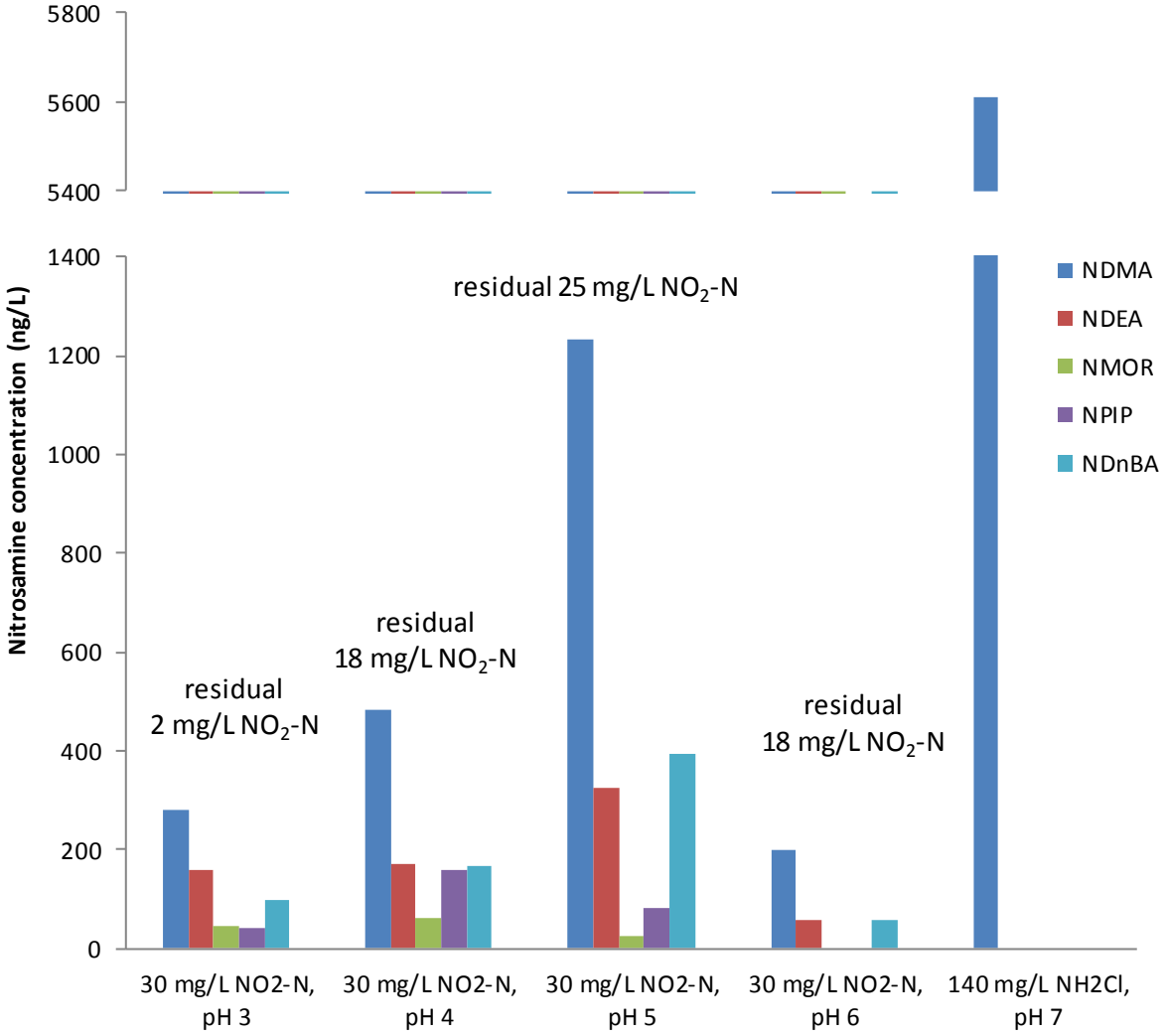


Figure 7. NDMA formation with sewage and nitrite (30 mg/L as N) as a function of pH. Comparison of the NDMA formation potential during FNA application and chloramination (140 mg/L) (n=2).

Figure 8 presents the NDMA formation potential with different sewage batches and nitrite concentrations (10 or 30 mg/L as N) as a function of pH. The results confirmed the previously observed results, i.e., higher NDMA formation potential was observed at pH 5.

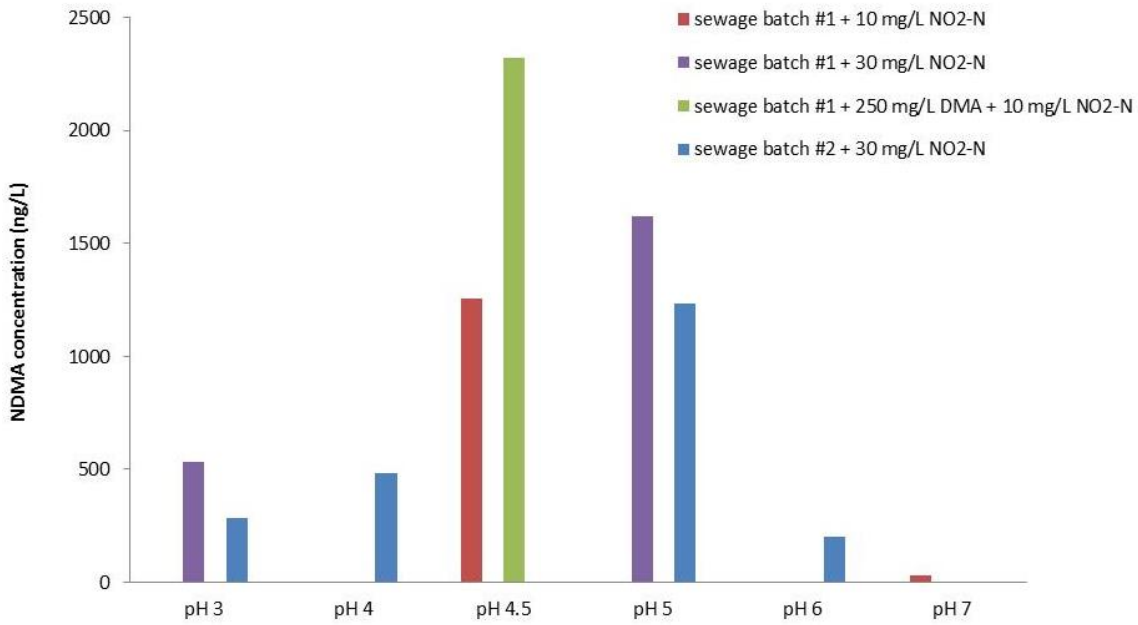


Figure 8. NDMA formation with different sewage batches and nitrite concentration (10 or 30 mg/L as N) as a function of pH.

4.4. Application of the nitrosamine formation potential test to cleaning solutions

After validation of the nitrosamine formation potential test with model precursors and sewage, formation potential experiments were conducted with simulated RO membrane cleaning solutions. Biofouling was collected by scraping a known surface area of a RO module and foulant was mixed with 1 litre of FNA cleaning solutions during 7 days. The impact of pH, nitrite concentration, temperature and the nature of foulant were investigated. The results of these experiments are presented in Figures 9.a-h. The following main conclusions can be drawn:

- ✓ Nitrosamine formation potential (Figure 9-14)

All the nitrosamine formation potential tests showed similar NDMA concentrations ranging between 5 and 20 ng/L, regardless of the experimental conditions applied. This indicates that NDMA precursors were in limited amounts in membrane foulant. Unlike formation potential tests conducted with sewage, NDEA was the main nitrosamine formed in all cleaning conditions investigated. NDnBA was also detected in a higher level than NDMA. Low level of NMOR were also detected in specific conditions such as high temperature, low pH or high nitrite concentration, while NPip was not found.

The highest formation of nitrosamines was also observed between pH 4 and 5, except for NDMA, which generated similar concentration regardless the pH applied.

- ✓ Impact of pH

While NDMA was generated at a relatively constant concentration (8±3 ng/L) regardless of the pH applied (from 3 to 6), the other nitrosamines (NDEA, NDnBA and NMOR) followed the same trend previously observed, i.e. highest formation potential at pH 4-5 and lower formation potential at pH 3 potentially due to FNA degradation (Figure 9).

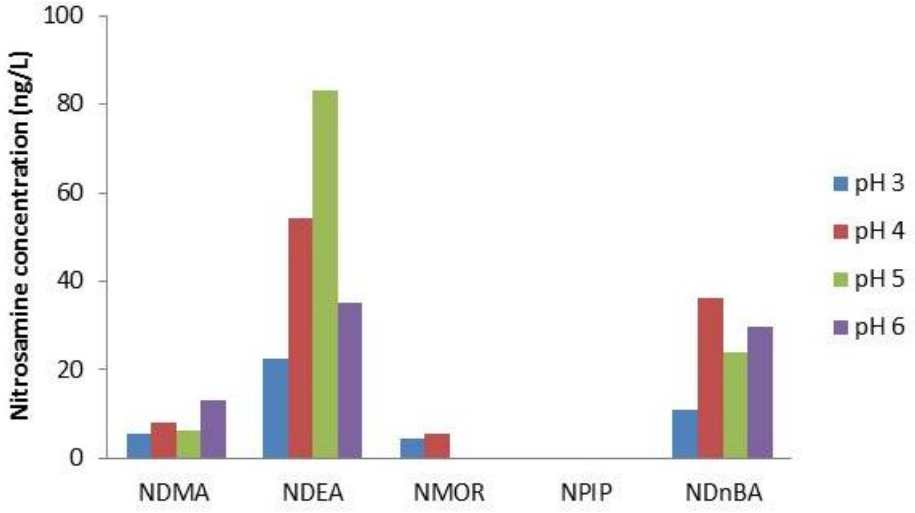


Figure 9. NDMA formation with RO cleaning solution and nitrite, impact of pH values.

- ✓ Impact of Nitrite

At pH 4 and 5, NDMA formation potential is similar for the different nitrite concentrations, while for the three other measured nitrosamines (NDEA, NMOR and NDnBA), the formation potential increased with nitrite concentration (Figure 10.a-b).

Figure 10.c presents the nitrosamine formation potential for similar FNA concentrations (1.73, 3.63 and 4.07 mg/L HNO_2-N for pH 3, 4 and 5 respectively). Although similar reagent concentrations were applied, the nitrosamine formation potential increases with pH.

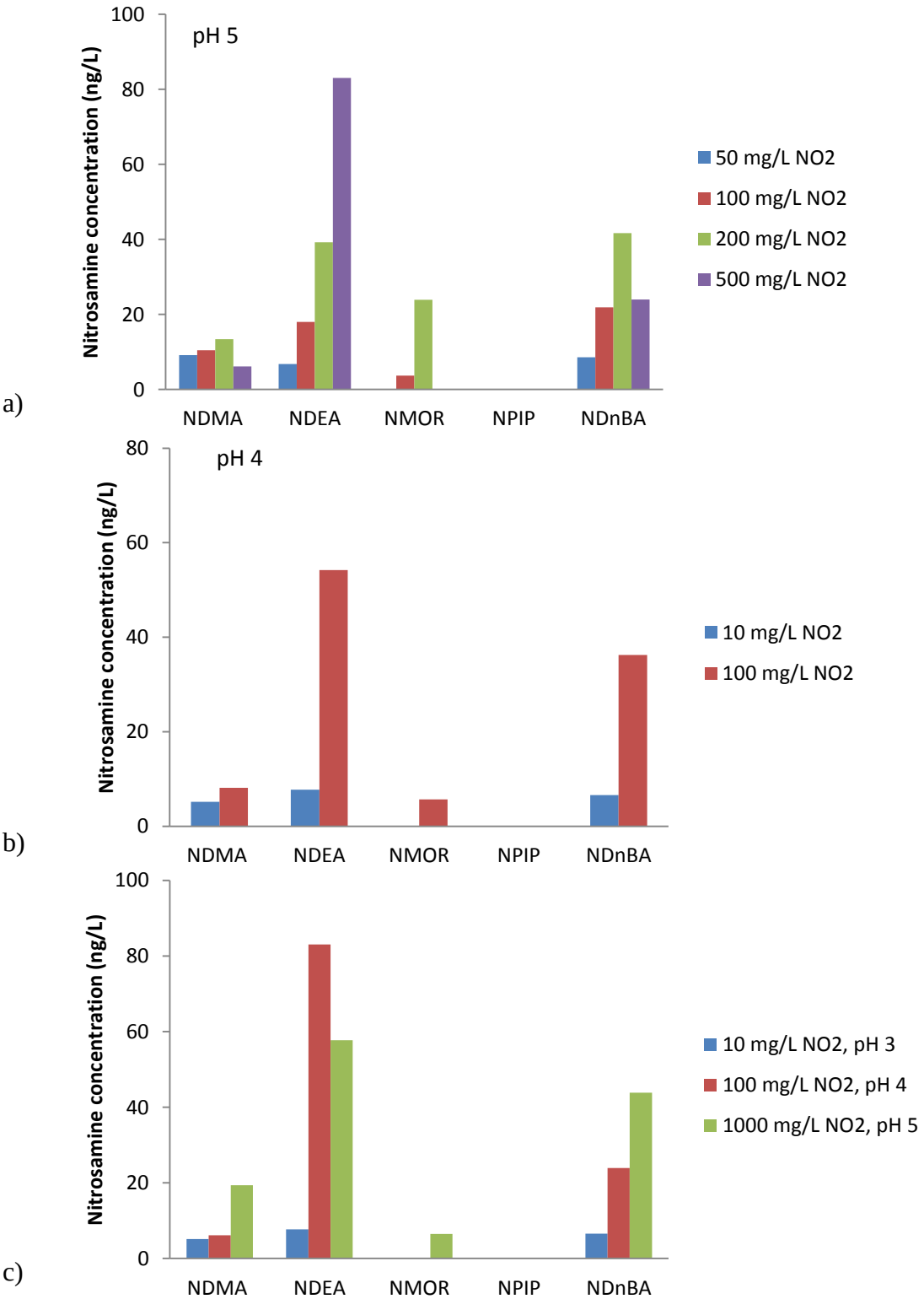


Figure 10. NDMA formation with RO cleaning solution and nitrite, (a) impact of nitrite concentration at pH 5 (b) impact of nitrite concentration at pH 4 and (c) impact of nitrite concentration and pH.

✓ Impact of the temperature

Figure 11 shows the nitrosamine formation potential with FNA for three different temperatures. The maximum formation potential was observed for the highest temperature (37°C) for the four nitrosamines detected. Although this result is consistent with the literature (Nitrosation tend to proceed faster when the temperature is raised), this was not confirmed by the experiments conducted at 22 and 30°C. The results are inconclusive. One hypothesis is that two factors are competing, such as kinetics and FNA decomposition. However, the nitrite residual after 7 days (28-30 mg/L as NO_2-N) did not demonstrate FNA degradation.

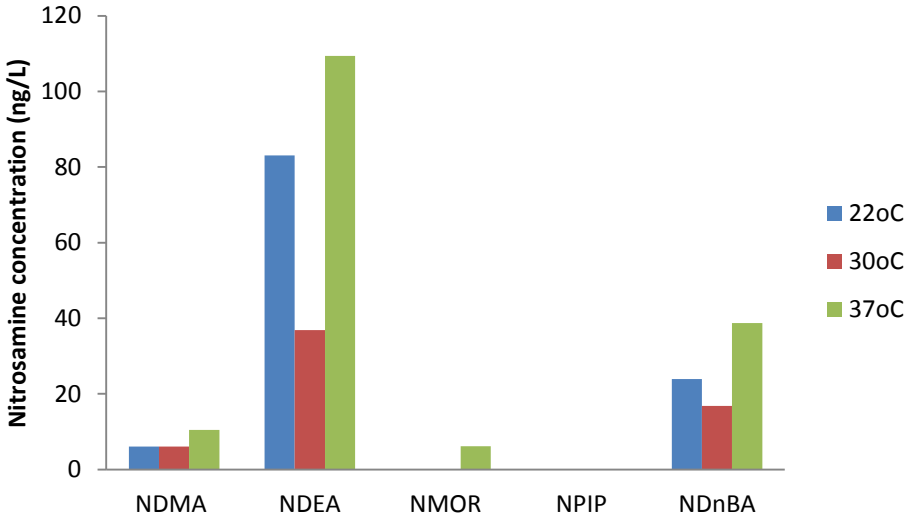


Figure 11. NDMA formation with RO cleaning solution and nitrite, impact of the temperature.

✓ Impact of nature of the foulant (formation potential with cleaning solution ROFNA-2)

In order to investigate the effect of foulant nature, nitrosamine formation potential tests were conducted using biofouled RO modules from two different origins (30 mg/L NO_2-N, pH 5): ROFNA-1 from the Beenyup ground water replenishment plant (Water Corporation, Perth) and ROFNA-2 from an industrial water recycling plant. Both membranes generated the same NDMA concentration, i.e., 8 ng/L (Figure 12). This value is below the regulation limit at 10 ng/L used at Public Health Regulations in Queensland in recycled water to augment a supply of drinking water. Similarly NDnBA was generated at 35 and 25 ng/L for ROFNA-1 and ROFNA-2 respectively. The fouling layer of ROFNA-1 presents a higher NDEA formation potential than the one from ROFNA-2 (43 versus 13 ng/L).

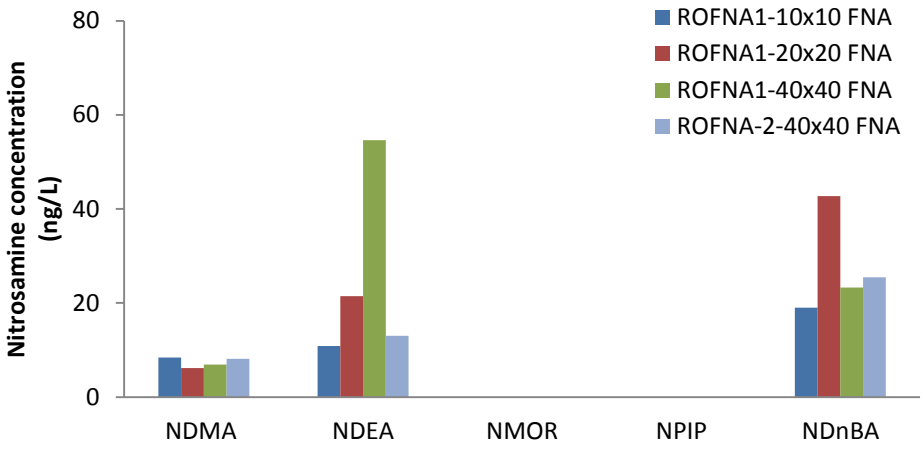


Figure 12. NDMA formation with RO cleaning solution and nitrite, impact of membrane foulant and quantity.

- ✓ Impact of H_2O_2

The combined biocidal effects of FNA and hydrogen peroxide (H_2O_2) on anaerobic wastewater biofilm has been investigated and showed that H_2O_2 greatly enhances the inactivation of microorganisms by FNA [45]. Thus, the effect of H_2O_2 in combination with FNA was evaluated in terms of nitrosamine formation potential with an initial H_2O_2 concentration of 150 mg/L. It has been shown that addition of H_2O_2 can inhibit the formation of NDMA from dimethylamine [18] due to the loss of available nitrite by quantitative conversion into nitrate [46]. However, in our experiments no significant effect of H_2O_2 addition was noticed for NDMA and NDnBA formation potential (Figure 13). NDMA and NDnBA were generated at 8 and 35 ng/L with FNA and 11 and 42 ng/L with FNA/H_2O_2, respectively. However, NDEA concentration dramatically decreases with the addition of H_2O_2 (43 ng/L with FNA versus 20 ng/L with FNA/H_2O_2).

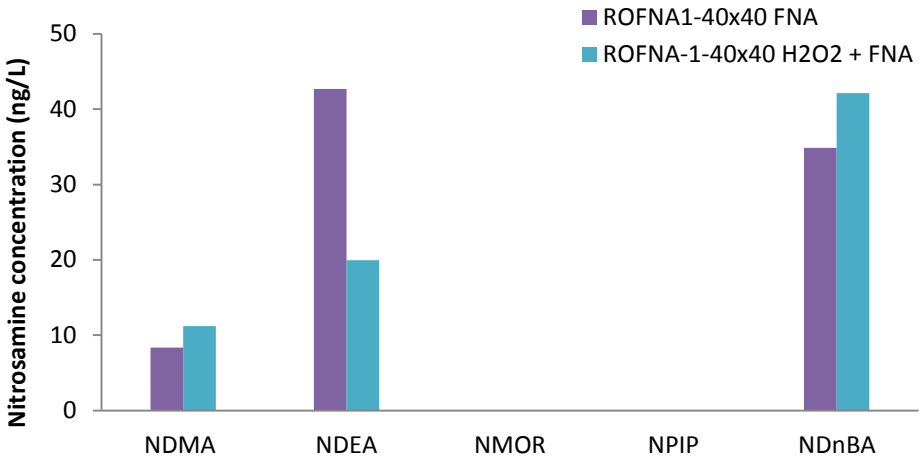


Figure 13. NDMA formation with RO cleaning solution and nitrite, impact of hydrogen peroxide addition .

- ✓ Comparison with monochloramine

The formation potential tests conducted with FNA and NH_2Cl again confirmed the results previously observed from sewage: while with NH_2Cl, only NDMA was formed above the limit of detection, other nitrosamines such as as NDEA and NDnBA were detected during FNA application (Figure 14). Interestingly, in that case both formation potential tests generated the same NDMA concentration (7 and 8 ng/L for NH_2Cl and FNA respectively), although a significantly higher NDMA concentration was expected with chloramination. These results confirmed the limit amount of NDMA precursors in RO fouling layer.

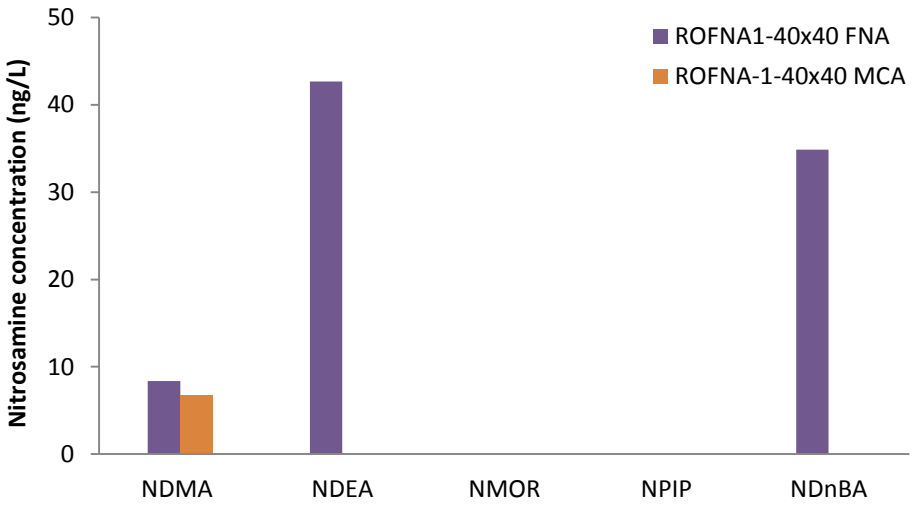


Figure 14. NDMA formation with RO cleaning solution and nitrite, comparison of the NDMA formation potential during nitrosation and chloramination (140 mg/L).

5. Conclusion

The formation potential of five nitrosamines, including NDMA, was investigated during the application of FNA for RO membrane cleaning and biofilm removal.

First, a nitrosamine formation potential test was developed at the laboratory scale for FNA application in order to quantify and compare under controlled conditions the concentration of precursors in different matrices (i.e. ideally different RO membrane cleaning solution). Based on the results, pH 5 was selected as the recommended pH values that can be used for the formation potential test without significant FNA degradation after 7 days of contact time. Absence of head-space in the bottles employed for the test and buffer did not show any impact on nitrosamine formation tests.

Preliminary experiments were performed using dimethylamine and doxylamine as surrogates for secondary and tertiary amine, respectively. Their nitrosamine formation potential when using FNA was compared with the one observed when using monochloramine and already well reported in the literature. Results indicate that:

- ✓ The formation of NDMA via nitrosation was lower than via chloramination.
- ✓ NDMA formation from FNA depends on pH having a maximum formation at pH 4-5, regardless of the DMA/NO_2 ratio. Although the initial reaction rate for nitrosation of secondary amines has its maximum at pH 3-3.4 (pKa of HNO_2), the yield for prolonged periods is largest at pH 4-5 due to a faster decomposition of nitrous acid at lower pH values.

The nitrosamine formation potential of sewage was measured in two independent samples as a second test matrix to evaluate the testing protocol. As observed previously, the NDMA formation potential during chloramination was much higher than during FNA application for all tested pHs with the highest formation potential observed at pH 5. Interestingly, NDMA was the only species measured during chloramination while NDEA, NMOR, NPIP and NDnBA were also detected during nitrosamine formation potential tests with FNA.

Finally, formation potential tests with both chloramine and FNA were conducted in simulated RO membrane cleaning solutions. The concentration of nitrosamine found were much lower than for the two seawage samples. The impact of cleaning conditions was investigated and the results indicate that:

- ✓ NDMA formation was measured at concentrations below 20 ng/L in all the cases. Although the NDMA formation potential with FNA was much lower than during chloramination, the formation of other nitrosamines, such as NDEA and NDnBA, was observed. In particular, NDEA was measured up to 110 ng/L during formation potential test at 37^{o}C.
- ✓ High temperature (37^{o}C), high nitrite concentrations (500 mg/L as NO_2) and pH values of 4-5 enhanced nitrosamine formation, in particular NDEA and NDnBA. Low concentrations of NMOR were detected under these specific conditions, while NPIP was not measured above the limit of detection.

- ✓ The highest nitrosamine formation potential is observed between pH 4 and 5 and lower formation potential at pH 3 due to FNA degradation.
- ✓ NDEA and NDnBA formation potential increased with nitrite concentration.
- ✓ No significant effect of H_2O_2 addition was noticed for NDMA and NDnBA formation potential, while the addition of H_2O_2 inhibited the formation of NDEA.

In summary, the results show that small amounts of nitrosamines may be formed during the membrane cleaning process with FNA. The formation potential tests conducted with FNA cleaning solutions generated NDMA (max 20 ng/L), NDEA (max 110 ng/L), NDnBA (max 45 ng/L) and NMOR (max 25 ng/L). Guideline values of 10, 10 and 1 ng/L for NDMA, NDEA and NMOR respectivey have been established for indirect potable water reuse in Queensland (Australia) [9].

During the cleaning process, FNA cleaning solution would not be in direct contact with the RO feed water. However, nitrosamines precursors (e.g. proteins, secondary amines) can be accumulated on the membrane surface or trapped in the biofilm. In this case, nitrosamines formation potential has to be considered in case they may pass through the membrane.

Commonly, RO membrane cleaning is conducted at low pressure to compensate for the pressure drop from feed to concentrate. Consequently, little permeate can be produced [47].

NDMA rejection by RO membranes was reported between 10 and 70% in the literature [13, 15] and may pass through the membrane. Nevertheless, the concentration from the cleaning solution formation potential test with FNA was low (max 20 ng/L). The other nitrosamines (i.e.NDEA, NMOR, NDnBA) were detected at much higher concentrations (especially NDEA). However, they have shown 90% or greater rejection by four RO membranes [13].

At the end of the cleaning procedure, RO permeate or deionized water is used for flushing out the cleaning solution and membrane foulants using high flow rate. Therefore, the nitrosamines potentially formed during RO membrane cleaning process using FNA will be flushed out similarily and are likely to be discharged with the cleaning solution to a sewer with a high dilution effect.

To summarize, the higher risk of nitrosamine formation is related to the low rejection of NDMA by RO membranes. However, NDMA formation is low and other nitrosamines will be rejected even if they are formed at high concentration. Based on these results, the application of FNA alone or in combination with hydrogen peroxide shows a low risk level for nitrosamine formation, including NDMA, NDEA, NMOR, NPIP and NDnBA.

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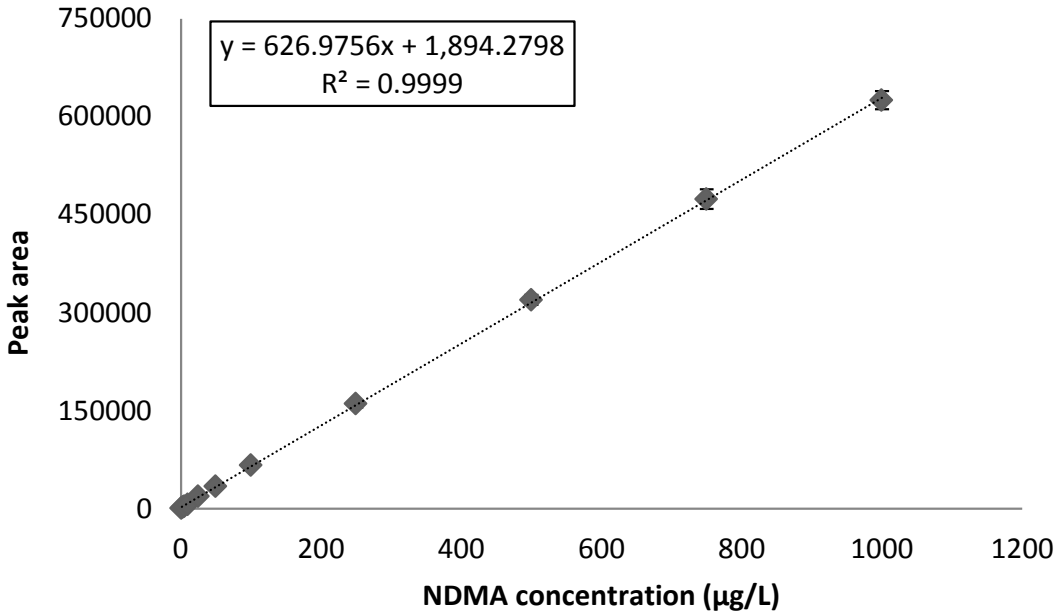
Appendix

A. Calibration curve of NDMA from HPLC-DAD data (n=4)



B. Results of the stability tests

Time (min)	pH value				
	pH 2	pH 3	pH 4	pH 5	pH 6
0	2.0	2.9	4.0	5.0	6.0
5	1.9	2.8	4.1	5.2	6.3
24	2.0	2.8	3.8	5.0	6.3
48	2.0	2.8	3.7	4.8	6.3
72	2.0	2.8	3.8	5.0	6.2
164	2.0	2.8	3.9	5.2	6.1

Time (min)	Nitrite concentration (mg NO_2-N/L)				
	pH 2	pH 3	pH 4	pH 5	pH 6
0	15.5	30.4	30.4	30.4	30.4
5	9.2	21.0	27.5	30.5	31.7
24	4.5	21.2	25.5	30.8	31.6
48	2.6	19.9	24.8	30.9	31.4
72	1.3	19.7	24.0	30.5	31.5
164	0.7	15.3	21.2	31.1	31.9

Time (min)	Nitrate concentration (mg NO_3-N/L)				
	pH 2	pH 3	pH 4	pH 5	pH 6
0	-	0.0	0.0	0.0	0.0
5	-	8.7	1.3	0.9	0.9
24	-	9.2	3.7	0.6	0.6
48	-	10.3	6.0	0.7	0.6
72	-	10.6	6.6	0.6	0.7
164	-	15.7	10.7	2.0	1.9

Time (min)	FNA (mg FNA-N/L) @ T=25^{o}C (Thermodynamic simulation)				
	pH 2	pH 3	pH 4	pH 5	pH 6
0	14.4	17.6	3.7	0.41	0.042
5	8.5	12.1	3.3	0.41	0.043
24	4.2	12.3	3.1	0.42	0.043
48	2.4	11.5	3.0	0.42	0.043
72	1.2	11.4	2.9	0.41	0.043
164	0.7	8.8	2.6	0.42	0.044

Time (min)	pH value, pH 5					
	No buffer	Phosphate buffer	Acetate buffer	No buffer	Phosphate buffer	Acetate buffer
	Amber bottle			Head space free amber bottle		
0	4.9	5.2	5.0	4.9	5.2	5.0
5	5.7	5.3	5.0	5.7	5.3	5.0
24	5.7	5.3	5.0	5.3	5.2	4.9
48	5.7	5.3	4.9	5.4	5.2	4.9
72	6.2	5.4	5.1	5.9	5.3	5.0
164	6.0	5.3	5.0	5.6	5.3	5.0

Time (min)	Nitrite concentration (mg NO2-N/L)					
	No buffer	Phosphate buffer	Acetate buffer	No buffer	Phosphate buffer	Acetate buffer
	Amber bottle			Head space free amber bottle		
0	30.4	30.4	30.4	30.4	30.4	30.4
5	31.1	31.0	32.1	30.1	32.5	31.4
24	31.4	30.9	32.3	31.4	30.6	32.4
48	31.8	30.8	32.5	31.2	30.7	32.2
72	31.0	30.7	31.9	34.4	34.1	32.0
164	31.7	30.8	32.5	31.6	30.9	32.3

Time (min)	Nitrate concentration (mg NO3-N/L)					
	No buffer	Phosphate buffer	Acetate buffer	No buffer	Phosphate buffer	Acetate buffer
	Amber bottle			Head space free amber bottle		
0	0.0	0.0	0.0	0.0	0.0	0.0
5	3.1	3.5	2.6	2.2	3.6	2.9
24	1.8	1.8	1.9	1.9	1.9	2.0
48	2.0	1.9	2.1	1.9	1.8	2.1
72	1.8	1.9	2.1	2.3	2.4	2.3
164	2.2	1.8	1.7	2.0	1.7	2.4

Time (min)	FNA (mg FNA-N/L) @ T=25oC (Thermodynamic simulation)					
	No buffer	Phosphate buffer	Acetate buffer	No buffer	Phosphate buffer	Acetate buffer
	Amber bottle			Head space free amber bottle		
0	0.41	0.41	0.41	0.41	0.41	0.41
5	0.42	0.42	0.44	0.41	0.44	0.43
24	0.43	0.42	0.44	0.43	0.42	0.44
48	0.43	0.42	0.44	0.42	0.42	0.44
72	0.42	0.42	0.43	0.47	0.46	0.43
164	0.43	0.42	0.44	0.43	0.42	0.44

C. Results of the formation potential tests with cleaning solutions

Membrane	Membrane area (cm^2)	$[NO_2]$ (mg/L)	$[H_2O_2]$ (mg/L)	pH	Temperature (^oC)	Nitrosamine concentration (ng/L)				
						NDMA	NDEA	NMOR	NPip	NDnBA
ROFNA-1 batch #1	40x40	0		5	22	0.0	0.0	0.0	0.0	0.0
	10x10	100		5	22	8.4	10.9	0.0	0.0	19.0
	20x20	100		5	22	6.1	21.5	0.0	0.0	42.7
	40x40	100		5	22	6.9	54.6	0.0	0.0	23.3
ROFNA-1 batch #2	40x40	100		5	22	8.4	42.7	0.0	0.0	34.9
	40x40	100	150	5	22	11.2	20.0	0.0	0.0	42.1
	40x40	$[NH_2Cl]$ 140 mg/L		5	22	6.7	0.0	0.0	0.0	0.0
ROFNA-1 batch #3	40x40	100		3	22	5.4	22.4	4.5	0.0	10.9
	40x40	100		4	22	8.1	54.2	5.7	0.0	36.2
	40x40	100		5	22	6.1	83.0	0.0	0.0	24.0
	40x40	100		6	22	13.0	35.2	0.0	0.0	29.9
	40x40	100		5	30	6.1	36.9	0.0	0.0	16.8
	40x40	100		5	37	10.4	109.4	6.2	0.0	38.7
	40x40	50		5	22	9.2	6.8	0.0	0.0	8.6
	40x40	200		5	22	10.5	18.0	3.7	0.0	21.9
	40x40	500		5	22	13.4	39.3	23.9	0.0	41.7
	40x40	10		4	22	5.1	7.7	0.0	0.0	6.6
	40x40	1000		6	22	19.4	57.7	6.5	0.0	43.8
ROFNA-2 batch #1	40x40	100		5	22	8.2	13.0	0.0	0.0	25.4