

**PERFORMANCE ENHANCEMENT OF ANAEROBIC AMMONIUM
OXIDATION REACTOR START-UP AND OPERATION WITH THE
EXTERNAL HYDRAZINE ADDITION**

by

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LIST OF ABBREVIATIONS

$(\text{N}_2\text{H}_5)\text{HSO}_4$	Hydrazinium sulphate
AMO	Ammonium oxygenase
Anammox	Anaerobic ammonium oxidation
AOB	Ammonium oxidizing bacteria
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
$\text{C}_6\text{H}_{12}\text{O}_6$	Glucose
CANON	Completely autotrophic nitrogen removal over nitrite
CAS	Conventional activated sludge system
CO_2	Carbon dioxide
CoCl_2	Cobalt chloride
COD	Chemical oxygen demand
CuSO_4	Copper sulphate
DI	Deionised water
DO	Dissolved oxygen
DOE	Department of Environment
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular polymeric substances
EQA	Environmental Quality Act
EQR	Environmental quality report
FA	Free ammonia
FBR	Fixed/Fluidized bed reactor
FeSO_4	Iron sulphate
FNA	Free nitrous acid

H ₂ O	Water
H ₃ BO ₃	Boric acid
HAO	Hydroxylamine oxidoreductase
HDH	Hydrazine dehydrogenase
HRT	Hydraulic retention time
HZS	Hydrazine synthase
IC	Inhibition percentage
IP	Inhibitory percentage
K ₂ HPO ₄	Di-potassium phosphate
KCl	Potassium chloride
KH ₂ PO ₄	Potassium di-hydrogen phosphate
KHCO ₃	Potassium hydrogen carbonate
MBR	Membrane bioreactor
MF	Membrane filter
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MnCl ₂	Manganese Chloride
N ₂	Nitrogen
N ₂ H ₄	Hydrazine
N ₂ O	Nitrous oxide
Na ₂ MoO ₄	Sodium molybdate
Na ₂ SeO ₄	Sodium selenate
Na ₂ SO ₄	Sodium sulphate
NaCl	Sodium chloride
NaOH	Sodium hydroxide

NaNO ₂	Sodium nitrite
NH ₂ OH	Hydroxylamine
NH ₃	Ammonia
NH ₄ Cl	Ammonium chloride
NH ₄ -N	Ammonium nitrogen
NiCl ₂	Nickel chloride
NIR	Nitrite oxidoreductase
NLR	Nitrogen loading rate
NO	Nitric oxide
NO ₂ -N	Nitrite nitrogen
NO ₃ -N	Nitrate nitrogen
NOB	Nitrite oxidizing bacteria
NOR	Nitric oxic reductase
NOS	Nitrous oxide synthase
NRA	Nitrate reductase
NRE	Nitrogen removal efficiency
NRR	Nitrogen removal rate
NXR	Nitrate oxidoreductase
O ₂	Oxygen
OLR	Organic loading rate
PBS	Phosphate buffer solution
PN	Protein
PS	Polysaccharide
RBC	Rotating biological contactor
RPM	Rotation per minute

SBR	Sequencing batch reactor
SHARON	Single reactor system for high ammonium removal rate over nitrite
SRT	Sludge retention time
Tem	Temperature
TN	Total nitrogen
UASB	Upflow anaerobic sludge blanket reactor
UBF	Upflow biofilter
UV-VIS	Ultraviolet-visible spectrophotometry
VSS	Volatile suspended solids
WTP	Water/wastewater treatment plant
WWTP	Wastewater treatment plant
ZnSO ₄	Zinc sulphate

LIST OF SYMBOLS

$\%$	Percentage
Cu^{2+}	Copper ion
H^+	Hydrogen ion
NH_4^+	Ammonium ion
NO_2^-	Nitrite ion
NO_3^-	Nitrate ion
r_1	Anammox activity and time t_1
r_2	Anammox activity and time t_2
$^{\circ}C$	Degree Celsius
€	Euro
μL	micro litre
μ_{max}	The maximum specific growth rate
μmol	micro molar
μ_{obs}	Anammox activity over time
A	Absorbance value
Ar	Argon
B	Weight of empty filter paper
b_{AN}	Decay rate
c	Concentration of the solution
C	Weight of filter paper from MLSS test
d	day
D	Weight of filter paper after furnace ignition
e^-	Electron
E	Weight of filter paper plus sample

<i>exp</i>	Exponential
ε	Milimolar extinction coefficient
<i>F</i>	MLVSS valued in the SBR
<i>g</i>	grams
<i>G</i>	MLSS value in the effluent
<i>HRT</i>	Hydraulic retention time
<i>kg</i>	kilograms
<i>KHz</i>	Kilohertz
K_I	Non-substrate inhibition constant
K_{ie}	Edwards inhibition constant
K_S	Half saturation constant
<i>l</i>	Length of solution cell
<i>L</i>	Litre
<i>m</i>	metre
<i>M</i>	Molarity
m^3	metre cube
<i>mg</i>	milligrams
<i>mmol</i>	mili molar
<i>nm</i>	nano metre
<i>NRR</i>	Nitrogen removal rate
NRR_{max}	Maximum nitrogen removal rate
<i>R11</i>	Reactor inhibited with 1100 mg N/L substrate
<i>R11_H</i>	Recovering reactor inhibited with 1100 mg N/L substrate with hydrazine addition
<i>R13</i>	Reactor inhibited with 1300 mg N/L substrate

$R13_H$	Recovering reactor inhibited with 1300 mg N/L substrate with hydrazine addition
$R9$	Reactor inhibited with 900 mg N/L substrate
$R9_H$	Recovering reactor inhibited with 900 mg N/L substrate with hydrazine addition
R_a	Starvation reactor with the presence of ammonium
R_h	Starvation reactor with the presence of hydrazine
R_n	Starvation reactor with the presence of nitrite
S	Substrate concentration
t	Time of inhibition/starvation
TAN	Total ammonia nitrogen
t_{HL}	Half life time
TN_{eff}	Total nitrogen concentration in effluent
TN_{in}	Total nitrogen concentration in influent
TNN	Total nitrite nitrogen
$t_{reaction}$	Reaction time
t_{total}	Total batch time
VSS_t	VSS value after inhibition/starvation
Y_I	NRR after inhibition
$Y_{control}$	NRR before inhibition

**KESAN PENAMBAHAN HIDRAZIN TERHADAP PENGAKTIFAN DAN
OPERASI REAKTOR YANG MENJALANKAN PROSES
PENGOKSIDAAN AMMONIUM SECARA ANAEROBIK (ANAMMOX)**

ABSTRAK

Pengoksidaan ammonium secara anaerobik (Anammox) adalah salah satu rawatan biologi yang digunakan secara meluas. Bagaimanapun, pengaktifan sistem Anammox mengambil masa yang panjang. Tambahan pula, bakteria Anammox juga sensitif terhadap turun naiknya nilai substrat dimana ia mudah mengalami proses perencatan dan kebuluran. Objektif pertama kajian ini adalah bagi memperkayakan bakteria Anammox dalam reaktor kelompok penjujukan (SBR) dengan isipadu 8L. Benih enapcemar bagi objektif ini diperolehi daripada reaktor yang menjalankan proses separa nitrifikasi dan sebuah reaktor anaerobik. Bagi 75 minggu pertama, substrat yang diberikan, dinaikkan secara berperingkat iaitu dari 100 - 900 mg N/L. Substrat yang disediakan (amonium + nitrit) berada dalam keadaan nisbah seimbang (1: 1). SBR utama mencapai lebih daripada 90% penyingkiran nitrogen dalam tempoh 14 minggu. Objektif kedua kajian ini adalah untuk mengkaji kesan tambahan hidrazin dalam membantu pengaktifan reaktor Anammox. Kesan 5 kepekatan hidrazin berbeza dalam mengaktifkan reaktor Anammox (0, 5, 10, 15, 20 mg / L) dikaji. SBR dengan tambahan 10 mg/L hidrazin didapati mengambil masa 7 minggu untuk mencapai (kecekapan penyingkiran nitrogen) NRE sebanyak 86%. SBR tanpa penambahan hidrazin mengambil masa 11 minggu dimana NRE sebanyak 83.5% sahaja dicapai. Objektif ketiga kajian ini dilakukan untuk menilai kesan perencatan substrat terhadap bakteria Anammox dan keupayaan hidrazin untuk membantu

proses pemulihan. 3 kepekatan substrat yang dikaji adalah 900, 1100 dan 1300 mg N/L. Peratusan perencatan (IP%) yang diperolehi untuk reaktor dengan kepekatan substrat 900, 1100 dan 1300 mg N/L selepas 28 hari perencatan adalah 27, 38 dan 75%. Model perencatan substrat (Edwards Model) didapati dapat memberikan penjelasan lebi baik berkaitan impak perencatan substrat. Pemalar ketepuan separuh (K_s) dan Pemalar perencatan (K_{IE}) yang diperolehi ialah 361.62 mg/L dan 731.3 mg/L. Dalam proses pemulihan, reaktor yang dengan tambahan hidrazin menunjukkan keupayaan untuk pulih secara mampan jika dibandingkan dengan reaktor tanpa tambahan hidrazin. Keputusan terbaik diperolehi dari reaktor yang direncat dengan 900 mg N/L dan dipulih dengan tambahan hyrazin (R_{9H}) dimana NRE sebanyak 94% dicapai manakala keputusan terendah dicapai oleh R13(tanpa hidrazin) dengan NRE sebanyak 80%. Objektif terakhir kajian ini menilai kesan keadaan kebuluran berbeza terhadap pemulihan bakteria Anammox. Tiga keadaan kebuluran yang dikaji adalah keadaan kebuluran dengan kehadiran ammonium (R_a), nitrit (R_n), dan hidrazin (R_h). Kadar kematian yang setelah 15 hari untuk R_a , R_n , dan R_h adalah 0.032/hari, 0.042/hari dan 0.019/hari. Akhir sekali, keupayaan pemulihan yang terbaik dan terburuk selepas proses kebuluran ditunjukkan oleh reaktor R_h dan R_n di mana kadar pertumbuhan yang diperolehi adalah 0.092/hari dan 0.011/hari.

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EXTERNAL HYDRAZINE ADDITION**

ABSTRACT

Anaerobic ammonium oxidation (Anammox) is one of the widely used biological treatments to remove nitrogenous compounds from wastewater. Despite this, Anammox system has long start-up period and the Anammox bacteria can easily undergo inhibition or starvation due to fluctuation of feed in wastewater treatment plants. In the first part of the study, Anammox bacteria were enriched in a sequencing batch reactor (SBR) with a working volume of 8L. In 75 weeks the substrates were increased step wise from 100 – 900 mg-N/L. The substrates provided (ammonium + nitrite) were in equimolar balance of 1:1. The parent SBR achieved more than 90% of nitrogen removal efficiency (NRE) in 14 weeks. The second part of the study was to investigate the effect of external hydrazine addition on aiding the start-up of Anammox reactor. Effects of 5 different externally added hydrazine concentration on reactor start-up were studied (0, 5, 10, 15, 20 mg/L). According to the results obtained, SBR with 10 mg/L hydrazine addition only took 7 weeks with an NRE of 86%. However, the SBR with no hydrazine addition took 11 weeks to stabilize with a NRE of 83.5%. The third part of the study was done to evaluate the effect of substrate inhibition on Anammox bacteria and ability of external hydrazine addition to aid the recovery of Anammox bacteria. 3 substrate concentrations were studied which were 900, 1100 and 1300 mg-N/L. The outcomes show that the inhibition percentage (IP%) obtained for reactors with substrate concentration of 900, 1100

and 1300 mg-N/L after 28 days of inhibition were 27, 38 and 75%, respectively. It was found that the substrate inhibition model (Edwards model) is the best model to represent the inhibition towards Anammox bacteria. Half saturation constant (K_s) and inhibition constant (K_{IE}) obtained were 361.62 mg/L and 731.3 mg/L, respectively. During the recovery studies, reactors that had hydrazine addition showed better recovering capabilities compared to the one without hydrazine addition. The best result in terms of NRE was obtained from the reactor inhibited with 900 mg-N/L and recovered in the presence of hydrazine (R_{9H}) which was 94%. Evidently, the best growth rate was also obtained from reactor R_{9H} at 0.22/day and the lowest growth rate was obtained for R13 which was 0.07/day. The final part of this study was to evaluate the effect of different starvation condition on Anammox bacteria and its recovery. Three different starvation were studied which were starvation with the presence of ammonium (R_a), nitrite (R_n), and hydrazine (R_h). The decay rates calculated after 15 days for R_a , R_n , and R_h were 0.032/day, 0.042/day and 0.019/day, respectively. Finally, the best and the worst recovering capabilities after starvation were shown by reactor R_h and R_n where the growth rate value tabulated were 0.092/day and 0.011/day, respectively.