

PAPER • OPEN ACCESS

Effects of salinity on the respirometric activities of mixed culture bacteria from activated sludge process

To cite this article: Romsan Madmanang and Tongchai Sriwiryarat 2021 *J. Phys.: Conf. Ser.* **1835** 012104

View the [article online](#) for updates and enhancements.



240th ECS Meeting ORLANDO, FL

Orange County Convention Center **Oct 10-14, 2021**

Abstract submission deadline extended: April 23rd

SUBMIT NOW

Effects of salinity on the respirometric activities of mixed culture bacteria from activated sludge process

Romsan Madmanang^{1*} and Tongchai Sriwiryarat²

¹Faculty of Science Technology and Agriculture, Yala Rajabhat University, Yala, 95000, Thailand

²Department of Chemical Engineering, Faculty of Engineering, Burapha University, Chonburi, 20131, Thailand

*Corresponding author's e-mail address: romsan.mad@yru.ac.th

Abstract. Salinity or saltiness is dissolved in water by the relative proportion of salt in a solution. All organisms have to keep their cells alive a water balance in their bodies. The activated sludge process (ASP) containing aerobic microorganisms located nearby the coastal area may be faced the problems of salinity from sea water. In this research, the effects of salinity on the respirometric activities of mixed culture bacteria from activated sludge process were evaluated at different levels of NaCl concentrations ranging between 0-25 g/L in the OxiTop system at the temperature of 20 °C. The oxygen uptake rate (OUR) was used to reveal the effects of salinity. The experiments results indicated that the OURs decreased as the NaCl concentrations increased, resulting in the failures of bacterial osmotic pressure systems and causing the lysis of bacteria. The COD and nitrification were failed because the nitrogen and COD loadings to the systems were increased by the lysis of bacteria.

1. Introduction

Activated sludge process (ASP) is a biological wastewater treatment process for the treatment of sewage and industrial wastewaters [2]. A large variety of ASP design is available; however, all ASP systems in general consist of three main components including an aeration tank, which serves as a bioreactor; a settling tank for the separation of AS solids and treated wastewater and a return activated sludge (RAS), to transfer settled sludge from the clarifier to the aeration basin. Several factors including pH, temperature, dissolved oxygen and others must be properly controlled so the effects of them would not result in the failure of this system [11].

If the ASP is located nearby the coastal areas, it is often encountered the problems of salinity from seawater. Salinity inhibits the microbial activities of microorganisms in the ASP process. Salty solutions can draw water out of bacteria through their cell membranes and this dehydration process reduces or prevents the chemical reactions which they rely on to live and to reproduce because water is a fundamental requirement for life. It was reported that microorganisms required several weeks to acclimatize with salinity and finally to achieve the same initial activities when salt concentrations were less than 5 g NaCl/L. As the salt concentrations increased above 25 g NaCl/L, the acclimatization process was very slow or impossible. Furthermore, salinity is a nutrient that affects different organisms in different ways [6].



In general, the ASP employs aerobic bacteria to decompose organic matters in the wastewater so microbes require oxygen for respiration to sustain life. Bacteria require oxygen for breathing and then releasing carbon dioxide as a product. To evaluate the direct effects of salinity on aerobic bacteria in the ASP system, the respirometric activities of bacteria resulting from the factors could be used to represent the impacts. In this study, the effects of salinity on the respirometric activities of mixed culture bacteria from the AS systems were evaluated at different NaCl concentrations at a temperature of 20 °C.

2. Theoretical Background

2.1. Activated sludge process

Activated sludge process (ASP) is widely used as a biological wastewater treatment system for treating municipal and industrial wastewaters containing organic matters under aerobic conditions and then producing good quality effluents. The basic principle behinds all ASPs is that floc forming microorganisms grow by consuming organic matters and settle to the bottom of the final clarifier, resulting in a clear liquid without organic materials and suspended solids. Recycled sludge containing high extent of biomass from the secondary tank is mixed with influent wastewater is also required. Oxygen is added into the aeration basin to suspend the biomass and to provide oxygen for bacteria. After a period of time, the mixed liquor flows to the final clarifier to settle and separate the effluent. During this settling, a portion of the bacteria is removed to maintain the Solids Retention Time (SRT) or sludge age [10].

2.2. Effects of salinity on activated sludge process

The performances of ASP system located nearby the coastal area could possibly be failed due to the leakage of seawater into the treatment plant. At high seawater level during the rising of tide, seawater could possibly flow into the gutter and then flows into the sewage system. The seawater would affect the performances of the ASP systems, possibly resulting in low suspended solids (SS) concentrations (less than 10 mg/L) and increase of oxygen demand (OD) when salt concentrations increase in the wastewater.

Wong, Y. (1981) studied the effects of high salinity on the performances of ASP and plastic trickling filter as a result of using seawater as flushing water so this flushing water caused an effect on biological wastewater treatment [14]. Different levels of salinity ranging from 5000 mg/L to 25000 mg/L were studied. It was found that the performances of activated sludge decreased as salinity increased in the wastewater. At high salinity dosages, it was required longer recovery time at high salt concentration than low salt concentration. Mesquita et al. (2009) studied the influences of saline wastewater on activated sludge flocs through automated image analysis in a sequencing batch reactor (SBR) system [9]. The sludge settling ability was evaluated by using image analysis at different NaCl concentrations of 0 to 60 g/L. It was found that the sludge volume index (SVI) decreased at 20 g/L and remained constant above this value. Moreover, they found that the salt concentrations between 5 g/L to 20 g/L resulted in a strong deflocculation phenomenon, leading to a heavy loss of aggregated biomass. Anant. (1997) studied the effects of salinity on the nitrogen and phosphorus removals of a 3-stage phoredox activated sludge process at different NaCl concentrations of 5, 10, 20 and 30 g/L [1]. Moreover, they designed to shock the system with a very high NaCl concentration of 70 g/L for four days to study the capability of bacteria to recover the process. From the experiment results, it was found that the increases of NaCl concentrations decreased the COD removal efficiencies from 96.5% to 84.7%, 84.0%, 73.6%, and 60.0% at the NaCl concentrations from 0 to 30 g/L for non-acclimatized bacteria. Total nitrogen removal efficiencies also decreased from 87.7% to 80.4%, 75.8%, 69.5% and 66.9%, respectively. After the process was shocked with high NaCl concentration, it was found that non-acclimatized bacteria needed 10 days of the control system to 8, 6, 5 and 5 days, respectively. So they concluded that NaCl-acclimatized bacteria had a higher ability to recover.

2.3. Effects of salinity on bacteria

It is known that water is necessary for bacteria and the osmosis is one of important processes for transporting the water between bacterial cell membrane and solution. Three types of osmotic conditions including hypotonic, isotonic and hypertonic conditions due to the concentration gradient between outside and inside cells. When the concentration of solution outside the cell membrane is lower than inside called hypotonic condition; therefore, the cell are full of the water. Isotonic condition occurs when there is an equilibrium of solution between inside and outside of the cell. The last type is hypertonic, which is a solution outside the cell is higher than inside the cell as a high salinity in the water, causing the water to move out the cell; thus, bacteria are shrinking and dying. Marquis (1968) studied the effects of salt on *Bacillus megaterium* cells. The results revealed that 26.5% of cell volume decreased when cells were transferred from water to unbuffered non-plasmolyzing NaCl solution [8].

2.4. Respiratory rate measurement

To study on the biological treatment process, Oxygen Uptake Rate (OUR) is one of the important parameters to evaluate the respirometric activities. The OUR is a reliable and recognized measurement of respiratory rate. OUR indicates the relationship between respiration, substrate utilization and growth of bacteria [12]. It appears that the respiration is linked to the substrate conversion and biomass growth. To measure the respiration rate, it is not possible to measure directly from the biomass, but can indirectly be measured with the OUR. In addition, the OUR involves three phases including biomass, liquid phase, and gas-phase; therefore, the OUR can be measured from either liquid or gas phases. There are many ways to measure OUR, but can be grouped into two main types as listed below: Respirometry based on measuring oxygen in the liquid phase focuses on measuring dissolved oxygen (DO) concentration in the liquid phase by using a DO mass balance from the liquid phase. Respirometry is based on measuring oxygen in the gas phase. This method deals with two phases including liquid phase and gas phase. Liquid phase contains the respiring biomass and the oxygen in the gas phase is measured for evaluating the OUR.

Wang et al. (2005) evaluated the effects of salt shock loading on the performances of the ASP at different salinity concentrations of 0.1, 0.5, 2, 5, 10, and 20 g/L [13]. It was found that TOC removal efficiency and OUR was not changed when the salt concentrations were below 0.5 g/L. When the salt concentrations were greater than 5 g/L, the OUR and TOC removal efficiencies were reduced to 35% and 30%, respectively. Furthermore, some studied the effects of salt concentrations ranging from 0, 15, 30, 45, and 60 g/L on the activated sludge with Challenge Model AER204 respirometer [5]. It was found that 20 g/L salt concentration resulted in the maximum effects on growth rate. The OUR decreased with the increase of salt concentrations.

2.5. OxiTop respirometer

OxiTop system (WTW, Weiheim, Germany) was accepted as a reliable method to measure the OUR [3]. The OxiTop measures the OUR by using a pressure sensor to determine a pressure drop in the gaseous phase of a closed system [7]. The OxiTop system can be used to measure the BOD₅ by determining pressure drops and calculated with the Equation (1).

$$\text{BOD} = \frac{M(\text{O}_2)}{RT_m} \left(\frac{v_t - v_i}{v_i} + \alpha \frac{T_m}{T_0} \right) \Delta p(\text{O}_2) \quad (1)$$

where $M(\text{O}_2)$ is the molecular weight of oxygen (32,000 mg/mol); R is the constant of gas (83,144 L hPa/mol/K), T_m is the temperature of 273.15 °K; T_0 is the temperature of 293.15 °K; v_t is the volume of sample bottle (mL), v_i is the volume of sample (mL), α is the bunsen absorption coefficient (0.03103); and $\Delta p(\text{O}_2)$ is the difference of oxygen pressure (hPa).

Then, the cumulative oxygen uptake rate (COU) can be calculated as listed in Equation (2). The data is then graphically plotted between COU and time. The specific oxygen uptake rate (SOUR) can be calculated from Equation (3).

$$\text{COU} = \left(\frac{\Delta p}{RT_m} \right) \left(\frac{V_{\text{gas}}}{VS} \right) \quad (2)$$

where COU is the cumulative oxygen uptake rate (mmol O₂/kg VS); R is the constant of gas (83,144 L hPa/mol/K); T_m is the temperature of 293.15 °K; V_{gas} is the difference between sample bottle volume and sample volume (mL); Δp is the difference of oxygen pressure (hPa); and VS is the weight of volatile suspended solids of sample (kg).

$$\text{SOUR} = \frac{\text{COU}}{t} \times M(\text{O}_2) = \left(\frac{\Delta p}{RT_m} \right) \left(\frac{V_{\text{gas}}}{VS} \right) \left(\frac{M(\text{O}_2)}{\Delta t} \right) \quad (3)$$

where SOUR is the specific oxygen uptake rate (mg O₂/kg VS-hr); COU is the cumulative oxygen uptake rate (mmol O₂ /kg VS); M(O₂) is the weight of oxygen (32 g/mol); and Δt is time (hr).

In addition, Equation (3) can be rearranged to Equation (4) to calculate the SOUR.

$$\text{SOUR} \left(\frac{\text{mg O}_2}{\text{g VS} \cdot \text{day}} \right) = \frac{(\Delta p \text{ hPa}) \left(\frac{100 \text{ Pa}}{\text{hPa}} \right) \left(\frac{1 \text{ N}}{\text{m}^2} \right) \left(\frac{32 \text{ g}}{\text{mol}} \right) \left(\frac{1000 \text{ mg}}{\text{g}} \right)}{\left(\frac{8.314 \text{ J}}{\text{mol} \cdot \text{°K}} \right) \left(\frac{1 \text{ N} \cdot \text{m}}{\text{J}} \right) \cdot T(\text{°K}) \cdot W(\text{g}) \cdot \frac{VS}{100}} \quad (4)$$

where SOUR is the specific Oxygen uptake rate (mg O₂/g VS·day); Δp is the difference between the initial pressure and final pressure (hPa); V is the volume of the vessel (m³); T is incubation temperature (K); W is the weight of the sample (g), and VS is volatile solid (%).

To calculate the respiration rate in the form of OUR in the wastewater, Equation (5) can be applied.

$$\text{OUR} \left(\frac{\text{mg O}_2}{\text{L} \cdot \text{h}} \right) = \frac{\left(\frac{\Delta P \text{ hPa}}{t \text{ h}} \right) \cdot \left(\frac{100 \text{ Pa}}{\text{hPa}} \right) \cdot \left(\frac{1 \text{ N}}{\text{m}^2} \right) \cdot \left(\frac{32 \text{ g}}{\text{mol}} \right) \cdot \left(\frac{1000 \text{ mg}}{\text{g}} \right)}{\left(\frac{8.314 \text{ J}}{\text{mol} \cdot \text{°K}} \right) \cdot \left(\frac{1 \text{ N} \cdot \text{m}}{\text{J}} \right) \cdot T(\text{°K}) \cdot \left(\frac{1000 \text{ L}}{\text{m}^3} \right)} \quad (5)$$

The researchers described the changes of a typical pressure drop in the OxiTop system as illustrated by Figure 1 that the variations in the pressure during the first 2-3 hours are resulted from the temperature differences between sample and incubator (phase A) [7]. Later, a lag period is required during phase B when microorganisms adjust themselves to new conditions. After that, the pressure drops down linearly to reach a quasi-steady state. The pressure drop during this period is related to the oxygen uptake rate of microorganisms (phase C). Microorganisms use oxygen to degrade the organic matters, resulting in carbon dioxide production. Carbon dioxide is subsequently trapped by absorption process on NaOH pellets in the OxiTop heads. The pressure drop is recorded with a data logger and is observed until oxygen is depleted (phase D).

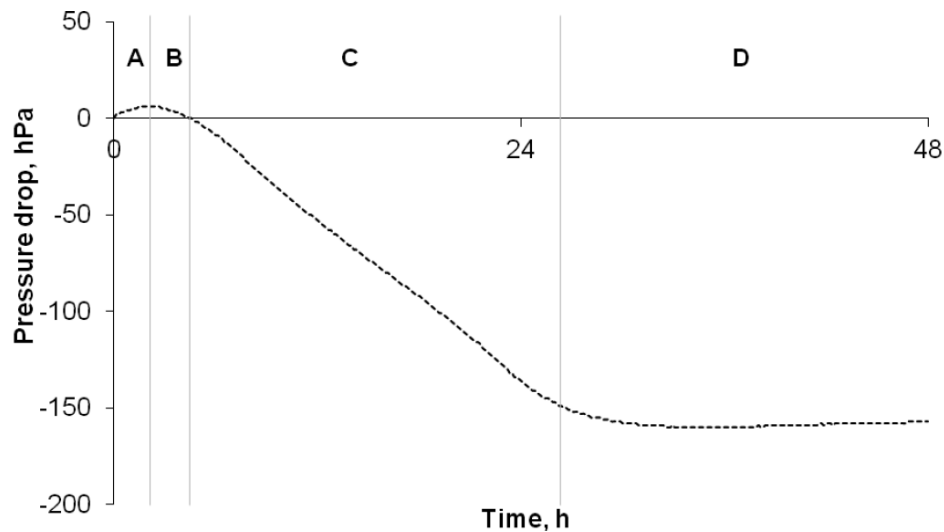


Figure 1. A typical pressure drop during a 2-day respiration test [7]

For all researches that we found, it appears that salinity affected to microorganisms in the ASP systems. The OUR would decrease with increasing salinity in the wastewater. In this study, the objective of this research was to evaluate the effects of salinity on the OUR by using OxiTop system to deeply understand more details of bacteria respiration. The range of NaCl concentrations not more than 35 g/L were employed in this study because seawater has a salinity of 35 g/L and possibly can be leaked to the ASP at a lesser extent because it may be diluted by the wastewater. Furthermore, all previous studies indicated that the maximum NaCl concentration of 30 g/L affected on the growth rate of bacteria [6]. Therefore, the effects of NaCl concentrations ranging between 0-25 g/L on the respirometric activities of mixed culture bacteria from the ASP system were evaluated in the OxiTop system.

3. Methods

3.1. Mixed culture bacteria

Mixed culture bacteria were taken from the aeration tank of a pilot-scale wastewater treatment system operating in the Environmental Engineering Laboratory of the Faculty of Engineering, Burapha University. Sludge was settled and washed out with distilled water. The procedures were repeated twice to ensure that the sludge was free from existing carbon sources and nutrients. The MLVSS concentration of 1050 mg/L was added into each OxiTop bottle at the volume of 500 mL.

3.2 Preparation of synthetic wastewater and sodium chloride

Synthetic wastewater was prepared by using chemicals including NH_4Cl 0.1 g, NaHCO_3 1.3 g, K_2HPO_4 0.4 g, MgSO_4 0.4 g, CaCl_2 0.02 g and MnSO_4 0.01 g at the volume of 3.5 L. Additional minor nutrients were added into the synthetic wastewater in the volume of 50 μL . Chemicals for minor nutrients were prepared from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 50 g, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 10 g, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 10 g, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 10 g, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 10 g in 1.0 L of distilled water.

After mixing to achieve homogeneous wastewater, the wastewater was divided into 6 aliquots, each one had the volume of 500 mL, and transferred to each OxiTop bottle. After adding biomass taken from the activated sludge system running in the Environmental Engineering Laboratory of Burapha University, additional chemicals were added into each OxiTop bottle at the volume of 2 mL. This synthetic wastewater (i.e., 7.7 g of CH_3COONa g, 7.9 g of milk powder and 1.4 g of extract yeast in 1.0 L of distilled water) had the COD concentration of about 40 mg COD/L and ammonia nitrogen of about 35-40 mg N/L.

Sodium chloride with the masses of 0, 2.5, 5, 7.5, 10, and 12.5 g were dissolved in the OxiTop bottles No. 1, 2, 3, 4, 5, and 6 to obtain the NaCl concentrations of 0, 5, 10, 15, 20, and 25 g/L, respectively.

3.3. Respiration measurement

The measurements were conducted in two modes including pressure and BOD₅ modes in the OxiTop systems. A 3-L of synthetic wastewater was prepared with the addition of ATU inhibitor to inhibit nitrification reaction during the measurements in BOD₅ mode. The pH was adjusted to obtain a neutral pH with sodium hydroxide and sulfuric acid. The volumes of concentrated sludge and synthetic wastewater were calculated so that the MLVSS concentration of 1000 mg/L in each OxiTop bottle with the same COD concentrations were obtained. The net volume of solution in each OxiTop bottle was 500 mL. A magnetic bar was added into each bottle and then the OxiTop heads were used to close the bottles. All OxiTop bottles were incubated in the BOD incubator controlled the temperature of 20 °C. After 5 days, the data from the data logger were retrieved to the computer for further analyses.

4. Results and Discussion

4.1. Effects of salinity on pressure drops and oxygen uptake rates in pressure mode

The experiments measured the pressure drops in the OxiTop system containing only synthetic wastewater without any NaCl addition as a control data so that effects of salinity on pressure drops and OURs in other OxiTop systems could be evaluated. The pressure drop data are shown in Figure 2.

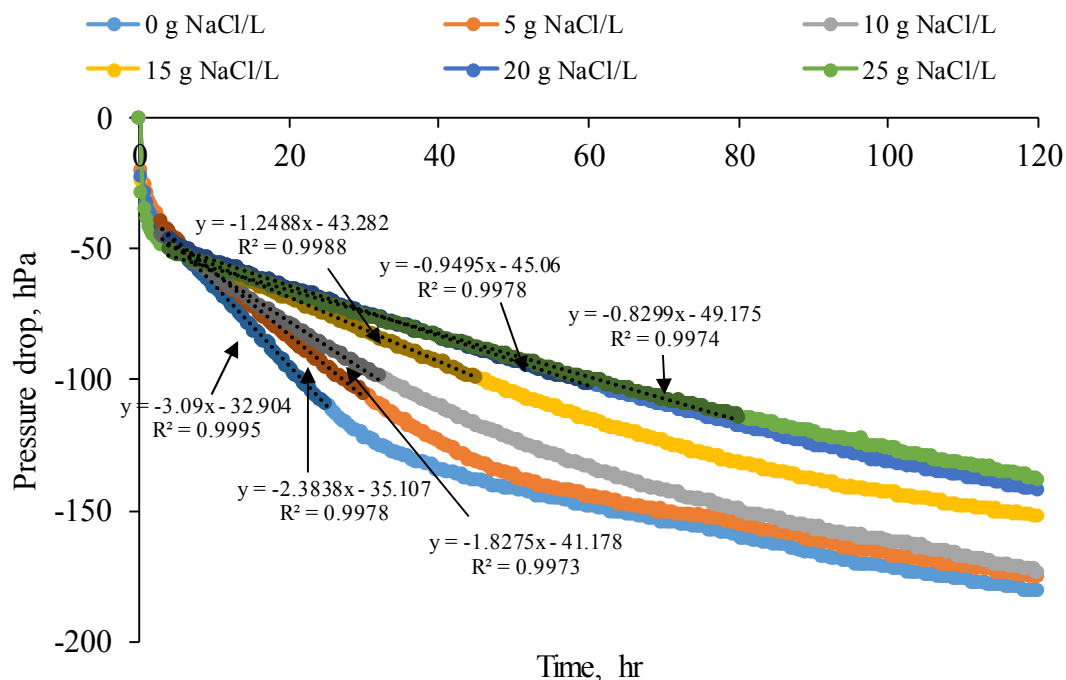


Figure 2. Pressure drops with time in the OxiTop systems containing 40 mg COD/L synthetic wastewater at the NaCl concentrations of 0, 5, 10, 15, 20, and 25 g NaCl /L, respectively.

It was found that pressure in gaseous phase dropped immediately from the beginning, indicating that there was no phase A (Figure 1) because there was no difference in temperature between the sample and incubator. Subsequently, the pressure decreased non-linearly for about 3 hours, indicating that microbes consumed oxygen during this period, possibly the oxygen consumption due to the organic matters. Later

on, the pressures decreased linearly, indicating that steady microbial oxygen consumption was found. It is suggested that nitrification occurred during this period because the COD was exhausted. Finally, the pressure decreased slowly as a result of limited oxygen in the OxiTop bottle. As indicated by the slope of the linear line, which was $\Delta P/t$, in light blue line, the pressure drop rate is 3.09 hPa/hr ($R^2 = 0.9995$). According to Equation (5), OUR could be determined. The OUR for mixed culture bacteria feeding with synthetic wastewater without NaCl was 4.05 mg O₂/L-hr. When the OUR was divided by the MLVSS concentration of 1050 mg VSS/L for SOUR determination, the SOUR was 3.86 mg O₂/g VSS-hr. The results including OUR and SOUR from this system were used to compare with other OxiTop systems containing different NaCl concentrations.

At a NaCl concentration of 5 g/L indicated by the orange line, there was no difference in temperature between samples and incubator. The pressure decreased immediately but non-linearly, indicating that the 40 mg/L of COD was degraded during this period. Subsequently, the pressure dropped linearly at the slope of 2.38 hPa/hr ($R^2 = 0.9978$) due to the nitrification. The OUR and SOUR were 3.14 mg O₂/L-hr and 2.99 mg O₂/g VSS-hr, respectively. It is evident that additional NaCl at 5 g/L reduced the respirometric activities of bacteria. In the OxiTop bottle containing 10 g NaCl/L, the pressure data as shown in the grey line indicated that the pressure constantly decreased as a result of microbial oxygen consumption without temperature adjustment between sample and incubator. The linear slope of hPa/hr was 1.83 hPa/hr ($R^2 = 0.9973$); therefore, the OUR and SOUR were 2.33 mg O₂/L-hr and 2.22 mg O₂/g VSS-hr, respectively. When the NaCl increased in the wastewater to 15 g/L, microorganisms in the OxiTop bottle consumed the oxygen immediately; however, initial pressure drop data indicate that bacteria required time to acclimatize with wastewater. Subsequently, the steady microbial oxygen consumption indicates that the pressure constantly decreased linearly with the slope of 1.25 hPa/hr ($R^2 = 0.9988$); therefore, the OUR and SOUR were 1.61 mg O₂/L-hr and 1.53 mg O₂/g VSS-hr, respectively. For the fifth OxiTop bottle, the NaCl was increased to 20 g/L in synthetic wastewater, under the same incubation time, it was found from the red line that the pressure decreased at a constant rate with the slope of 0.95 hPa/hr ($R^2 = 0.9978$). It appears that pressure dropped linearly but at a slope less than previous OxiTop bottles containing 0, 5, 10, and 15 g/L, respectively. The OUR and SOUR were 1.22 mg O₂/L-hr and 1.16 mg O₂/g VSS-hr, respectively. Lastly, when the NaCl was increased to 25 g/L, the pressure drop slope was 0.83 hPa/hr ($R^2 = 0.9974$), resulting in OUR and SOUR of 1.18 mg O₂/L-hr and 1.13 mg O₂/g VSS-hr, respectively.

To evaluate the effects of NaCl concentrations on the respirometric activities of mixed culture bacteria from the activated sludge system at different NaCl concentrations of 0, 5, 10, 15, 20 and 25 mg NaCl/L. The OURs were plotted as shown in Figure 3. The OUR decreased linearly as the salt concentrations increased from 0 to 25 g/L. It is confirmed that NaCl created more effects on the mixed culture bacteria cells, suggesting that their osmotic pressure systems are likely affected, causing the shrinking and dying of bacteria [8].

4.2. Effects of NaCl on BOD₅

The experiments were also conducted by using the OxiTop in BOD modes. The OxiTop systems containing mixed culture bacteria with different NaCl concentrations of 0, 5, 10, 15, 20, and 25 g/L, respectively. Figure 4 illustrates the BOD₅ profiles in the OxiTop bottles containing mixed culture bacteria treating synthetic wastewater with COD concentration of 40 mg COD/L and different NaCl concentrations.

At first, mixed culture bacteria consumed oxygen rapidly when synthetic wastewater contained no NaCl. However, when the NaCl increased in the wastewater from 5 g/L to 25 g/L, the oxygen uptake rate decreased correspondingly. It can be concluded that adding different levels of NaCl clearly resulted in different BOD₅ concentrations in the OxiTop bottles. The BOD₅ results followed the same results from the OxiTop systems in the pressure mode.

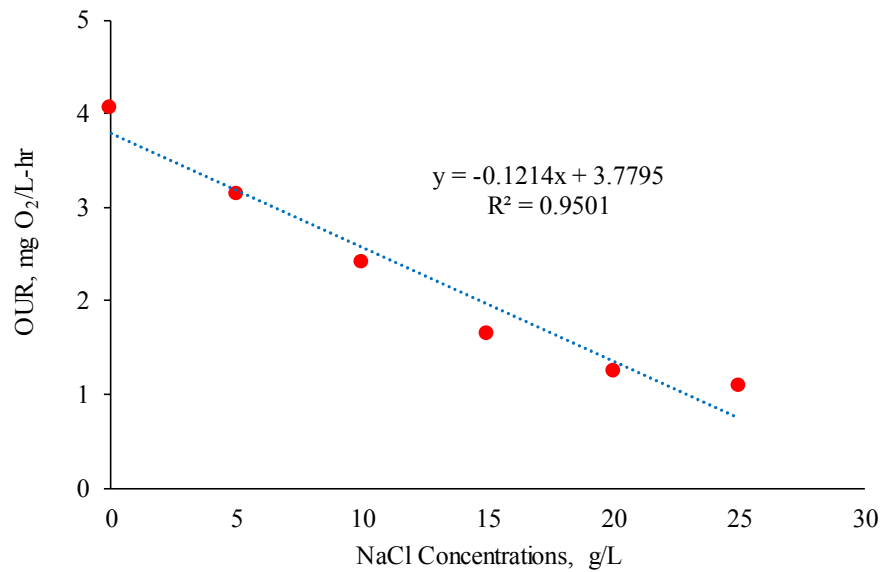


Figure 3. Variation of OUR at different NaCl concentration

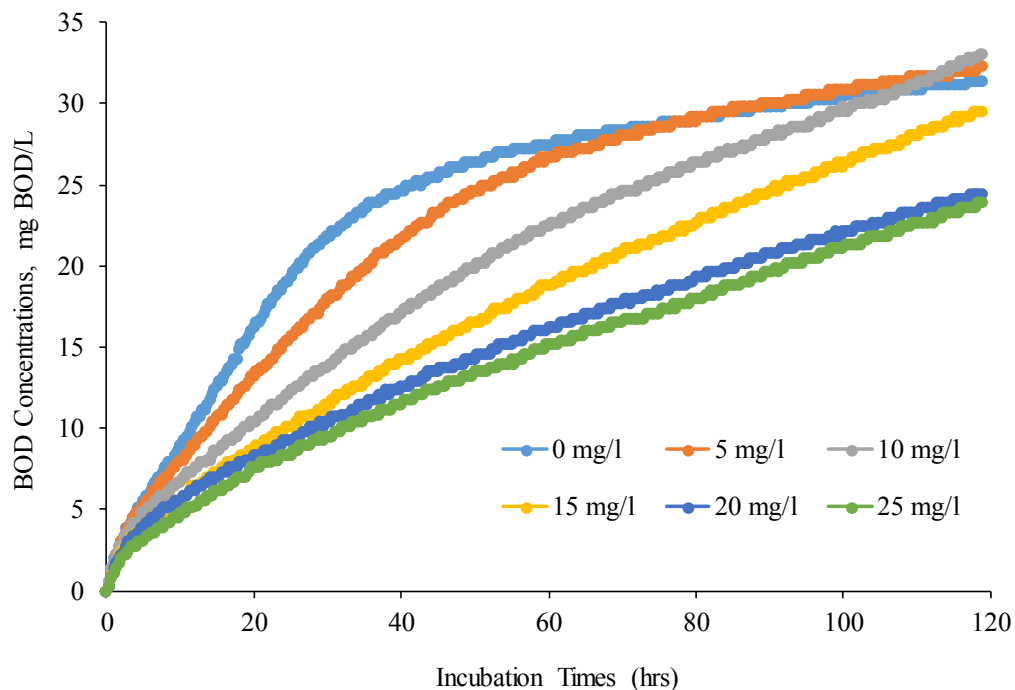


Figure 4. BOD5 profiles in the OxiTop systems containing mixed culture bacteria with different NaCl concentrations and 40 mg COD/L

4.3. Effects of NaCl on COD removal and nitrification

The quality of effluents from OxiTop bottles was measured for biomass, nitrogen, and organic matters. The results are listed in Table 1. Carbon removal indicated as COD was completed because a minimal amount of COD was added throughout the experiments and organics were biodegradable. However, the COD in effluent contained 40 mg COD/L are likely non-biodegradable COD which was probably produced from microbes. It appears that nitrification was completed in the OxiTop bottles containing synthetic wastewater without NaCl because nitrite nitrogen was not accumulated and nitrate nitrogen was produced, resulting in the ammonia removal efficiency of 97.9%.

Table 1 COD and nitrification from OxiTop bottles operating in pressure mode

NaCl (g/L)	Effluent Concentrations				Suspended Solids		NH ₄ ⁺ Removal Efficiency
	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	COD	MLVSS	MLSS	
0	0.73	0	31.3	40	1070	1070	97.9%
5	1.34	0	35.8	40	1170	1420	96.2%
10	3.7	18.7	20.2	40	1100	1560	89.4%
15	7.33	19.4	24.1	200	1170	1830	79.1%
20	12.18	21.1	14.8	200	1180	2070	65.2%
25	30.77	13.9	3.93	360	1080	2350	12.1%

Note: Initial MLSS equal to 1060 mg SS / L; Initial MLVSS equal to 1050 mg VSS / L; Influent COD equal to 40 mg COD / L; Ammonium nitrogen equal to 35 mg N / L; Nitrite and nitrogen nitrate were not found in synthetic wastewater.

When the OxiTop bottles were added with different concentrations of NaCl, it was found that COD removal could be accomplished when NaCl concentrations were less than 10 g/L. The COD in effluents increased when NaCl increased. It is explained that when NaCl increased in the wastewater, the osmotic pressure systems were deteriorated due to NaCl causing the shrinking and dying of bacteria [8]. The lysis of cell released particulates as shown by MLSS in Table 1, resulting in soluble COD and ammonia nitrogen. According to Chen et al. (2017) studied the effects of salinity on removal performances and activated sludge characteristics in sequencing batch reactors [4]. The results revealed that the ammonia, total phosphorus and COD removal efficiencies decreased from 95.34%, 93.58% and 94.88% at the salinity of 0 g/L to 62.98%, 55.64% and 55.78% at the salinity of 20 g/L, respectively. The studies concluded that increasing salinity decreased the performances of activated sludge system.

As shown in Figure 3 and 4 the oxygen uptake rates and BOD₅ decreased with the increase of salt concentrations because microbes were dead and nitrogen loadings were increased from the cell lysis. The accumulation of nitrite nitrogen at the NaCl concentrations of 10, 15, 20, 25 g/L was found due to the limitation of oxygen as COD loadings increased from the bacterial lysis.

5. Conclusion

The experiments evaluated the effects of salinity on the respirometric activities of mixed culture bacteria taken from the activated sludge process. Six different NaCl concentrations of 0, 5, 10, 15, 20, and 25 g/L were added to the synthetic wastewater. Respiration rates were evaluated by the OxiTop in pressure and BOD modes at a temperature of 20 °C for five days. From the experimental results, the salt concentrations decreased the oxygen uptake rates, causing the failures of bacterial osmotic pressure systems causing the lysis of bacteria. The COD and nitrification were completely accomplished when the system contained no NaCl; however, NaCl decreased the nitrification and COD removal by increasing the nitrogen and COD loadings to the systems by the loss of bacteria.

Acknowledgments

The authors are grateful for all the supports from the Environmental Engineering Laboratory of Chemical Engineering department, Burapha University, Thailand. Special thanks are extended to Viranphat Singrakphon and Kansiri Sinrat, former engineering undergraduate students of the department of Chemical Engineering, Burapha University, for their data in this research.

References

- [1] Anan C 1997 Effects of salinity on the nitrogen and phosphorus removal of a 3-stage phoredox activated sludge process *Master's thesis Chulalongkorn University* (Bangkok, Thailand)
- [2] Beychok M R 1967 Aqueous wastes from petroleum and petrochemical plants 1st ed (UK: John Wiley & Sons Ltd.)
- [3] Binner E, Böhm K and Lechner P 2012 Large scale study on measurement of respiration activity (AT(4)) by Sapromat and OxiTop *Waste Manage. Res.* **32**(10), pp 1752-1759
- [4] Chen Y, He H, Liu H, Li H, Zeng G, Xia X and Yang C 2018 Effect of salinity on removal performance and activated sludge characteristics in sequencing batch reactors *Bioresour. Technol.* **249** pp 890-899
- [5] Frigon M D and Liu D 2016 Effect of high salinity on yeast activated sludge reactor operation *Water Sci. Technol.* **74**(9) pp 2124-2134
- [6] Linarić M, Markić M and Sipos L 2013 High salinity wastewater treatment. *Water Sci. Technol.* **68**(6) pp 1400-1405
- [7] Malińska, K. Application of a modified OxiTop respirometer for a laboratory composting studies *Arch. Environ. Prot.*, **42**(1) pp 56-62
- [8] Marquis R E 1968 Salt-induced contraction of bacterial cell walls *J. Bacteriol.* **95**(3) pp 775-781
- [9] Mesquita D P, Amaral A L, Ferreira E C and Coelho M A Z 2009 Study of saline wastewater influence on activated sludge flocs through automated image analysis *J. Chem. Technol. Biotechnol.* **84**(4) pp 554-560
- [10] Muralikrishna I V and Manickam V 2017 Wastewater treatment technologies, eds I V Muralikrishna and V Manickam Environmental Management (Oxford:Elsevier) pp. 249-293
- [11] Rajasulochana P and Preethy V 2016 Comparison on efficiency of various techniques in treatment of waste and sewage water—A comprehensive review. *Resour.-Effic. Technol.* **2**(4) pp 175-184
- [12] Vanrolleghem P A 2002 Principles of respirometry in activated sludge wastewater treatment *Proc. Int. Work. on Recent Development in Respirometry for Wastewater Treatment Plant Monitoring and Control.* (Taiwan: Taipei) p 2
- [13] Wang J L, Zhan X M, Feng Y C and Qian Y 2005 Effect of salinity variations on the performance of activated sludge system. *Biomed. Environ. Sci* **18** pp 5-8
- [14] Wong Y 1981 The effect of high salinity on the performances of activated sludge process and plastic trickling filter. *Master's thesis, The University of Hong Kong* (Pokfulam, Hong Kong)