Evaluation by Respiration Measurements (OTR, CTR and RQ) of the Biological Activity in Sludge Digestors Operated Under Microaerobic Conditions

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Original scientific paper
Received: July 4, 2006
Accepted: November 14, 2006

Microbial activity measurements (oxygen transfer rate, OTR, carbon dioxide transfer rate CTR and respiratory quotient, RQ) are used to monitor and control biological processes. In this study, the digestion of activated sludge in microaerobic conditions was evaluated. The changes of biological activity during digestion were monitored by respiration activity. The sludge mixtures used were primary plus activated sludge, and baker's yeast plus activated sludge. The biological activity was triggered during the experiments by feeding sugar to the mixtures. The results show that increased activity by adding sugar to digested sludge can be monitored by OTR and CTR, whereas RQ can not be used as an indicator.

Key words:
Oxygen transfer rate, carbon dioxide transfer rate, respiratory quotient, sludge digestion

Introduction

Aerobic digestion is one of the stabilization methods proposed for biological solids. Aerobic digestion may be used to treat waste activated sludge and mixtures of waste activated sludge and primary sludge. Sludge digestion is a process progressing in semi-solid phase. Assuming that non-biodegradable organic matter is present in the liquid phase of a biological sludge, the digestion can be accepted as a process progressing in the endogenous phase of cell growth. The endogenous metabolism is a function of the living or active portion of bacterial mass.

Variations in microbial activity during aerobic digestion can be determined by physicochemical, microbiological and biological activity parameters.[1] Microbial activity parameters are used to determine the efficiency, control the process and determine stability. For example, the value 0.4–1.0 mg O_2 g^{-1} volatile solid h^{-1} is accepted as a good indicator for sludge stability.[2] Microbial activity parameters quantitatively determine the physiological behaviour of the aerobic culture. These parameters can be used in industrial fermentation processes for monitoring and control. Respirometric parameters such as oxygen transfer rate (OTR), oxygen uptake rate (OUR), carbon dioxide transfer rate (CTR), carbon dioxide evolution rate (CER) and respiratory quotient (RQ) within these parameters were used in wide range changed from the characterisation of the organic composition and the estimation of biodegradation kinetics and active biomass[3,4,5,6] to the inhibition test.[7]

Microbial activity of microorganisms in aerobic environments varies depending on consumed substrates, dominating microbial population and physicochemical conditions. As the growth of microorganisms and product synthesis are affected by oxygen availability, OTR and OUR are important parameters for the estimation of the microbial activity. This can also be estimated by CTR and CER.

RQ, which is defined as the ratio of CO_2 production rate to O_2 uptake rate, is an indicator that can determine the metabolism of microorganisms. The real RQ = CER/OUR; in this paper, RQ is calculated as CTR/OTR. Due to pH changes, the difference between CER and CTR values change also and will be reflected by RQ value. If pH, air flow and mixing rate are constant during the process, then CTR can be accepted as equal to CER. This is only at steady-state. CTR = CER holds only when the gas-liquid transfer of CO_2 reaches equilibrium. The stripping process is slow compared to the acid/base buffering equilibriums. Some authors[8,9,10] evaluated the differences between RQ values, calculated using CTR and real CER calculations.

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RQ can be used as a control parameter to supply a process with an optimum concentration of oxygen[11,12] or to control the feed rate.[9] Some estimation techniques for determining the state of biological reactions in an activated sludge process using OUR and the related gas transfer rate coefficient have been reviewed by *Marsili-Libelli* and *Vaggi* (1997).[13]

Wastewater treatment plants (WWTPs) are usually open systems. The plant is exposed to the atmosphere, so RQ can rarely be used. However, optimum RQ values obtained from laboratory and pilot scale units can be considered for scale-up in land-scale application.

In this study, the biological activity of raw sludge and the activity increase after substrate feeding were evaluated in a pilot scale microaerobic sludge digestion unit by using respiratory activity measurements (OTR, CTR and RQ). The best microbial activity indicators reflecting activity increase were determined by means of conclusions from a comparison of these parameters under experimental conditions.

Materials and methods

Experiments were conducted in a steel reactor of $V = 100$ L volume. The reactor was operated under pressure and low mixing rate to keep the dissolved oxygen level (DO) > 0.5 mg L^{-1} during the entire process. During digestion, because OUR level vs. time was reduced, the desired DO level was kept by adjusting the mixing rate.

The effluent gas was led through a silica gel column to purify the water content and then analysed in a gas analyser (SERVOMEX); O_2 and CO_2 concentrations were determined. The biological activity of activated sludge was evaluated by respiratory activity parameters (OTR, CTR and RQ). These parameters were calculated according to eq. 1, 2, and 3, measuring O_2 and CO_2 concentrations of influent (fresh air) and effluent (exhaust) gas.

$$\mathrm{OTR} = \frac{Q_{\mathrm{air}} M_{\mathrm{O_2}}}{V_{\mathrm{L}} V_{\mathrm{N}}} \cdot \left[X_{\mathrm{O_2,in}} - X_{\mathrm{O_2,out}} \left(\frac{1 - X_{\mathrm{CO_2,in}} - X_{\mathrm{O_2,in}}}{1 - X_{\mathrm{CO_2,out}} - X_{\mathrm{O_2,out}}} \right) \right] \tag{1}$$

$$\mathrm{CTR} = \frac{Q_{\mathrm{air}} M_{\mathrm{CO_2}}}{V_{\mathrm{L}} V_{\mathrm{N}}} \cdot \left[X_{\mathrm{CO_2,out}} - X_{\mathrm{CO_2,in}} \left(\frac{1 - X_{\mathrm{CO_2,in}} - X_{\mathrm{O_2,in}}}{1 - X_{\mathrm{CO_2,out}} - X_{\mathrm{O_2,out}}} \right) \right] \tag{2}$$

$$\mathrm{RQ} = \frac{\mathrm{CTR}}{\mathrm{OTR}} \frac{32}{44} \tag{3}$$

where

Q_{air} = air flow rate, $m^3\ h^{-1}$

V_{L} = liquid volume, m^3

$M_{\mathrm{O_2}} = 32$ kg $kmol^{-1}$, molar mass of O_2

$M_{\mathrm{CO_2}} = 44$ kg $kmol^{-1}$, molar mass of CO_2

V_{N} = volume of 1 mol gas under normal conditions (22.4116 $m^3\ kmol^{-1}$)

$X_{\mathrm{O_2,in}}$, $X_{\mathrm{O_2,out}}$ = O_2 mole fractions of fresh and exhaust air

$X_{\mathrm{CO_2,in}}$, $X_{\mathrm{CO_2,out}}$ = CO_2 mole fractions of fresh and exhaust air

$X_{\mathrm{O_2,in}} = 0.21$ for fresh air

$X_{\mathrm{CO_2,in}} = 0.00035$ for fresh air

In the determination of CTR and CER, two special features of CO_2 gas should be taken into consideration.[14] First, CO_2 is highly soluble in water. Henry's law constants given the solubility of a gas in water are 1.44 Pa mol^{-1} fraction and 40.63 Pa mol^{-1} fraction at 20 °C for CO_2 and O_2, respectively. It depends on the medium pH. It is more soluble than O_2 in fermentation broths. Second, CO_2 dissolved in liquid forms carbonic acid which dissociates to give bicarbonate and carbonate ions. Aqueous CO_2 is formed when atmospheric CO_2 dissolves in water; we can find its concentration in fresh water using Henry's law. Aqueous CO_2 then forms carbonic acid (H_2CO_3) which, in turn, ionizes to form hydrogen ions (H^+) and bicarbonate (HCO_3^-)

$$\mathrm{CO_{2(aq)} + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-} \tag{4}$$

HCO_3^- ionizes to form more hydrogen ion and carbonate (CO_3^{2-})

$$\mathrm{HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}} \tag{5}$$

If sufficient time is allowed for the system to reach equilibrium, then the equilibrium constant for reactions (4–5) can be used to analyze the system. Reaction 4 results in

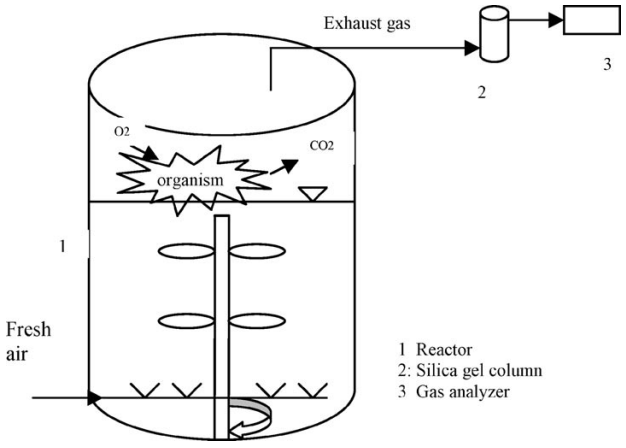
$$\frac{[\mathrm{H^+}] \cdot [\mathrm{HCO_3^-}]}{[\mathrm{CO_{2(aq)}}]} = K_1 = 4.47 \cdot 10^{-7}\ \mathrm{mol\ L^{-1}} \quad (\text{for } 25\ ^\circ\mathrm{C}) \tag{6}$$

and 5 yields

$$\frac{[\mathrm{H^+}] \cdot [\mathrm{CO_3^{2-}}]}{[\mathrm{HCO_3^-}]} = K_2 = 4.68 \cdot 10^{-11}\ \mathrm{mol\ L^{-1}} \quad (\text{for } 25\ ^\circ\mathrm{C}) \tag{7}$$

Due to these features, one part of the generated CO_2 dissolves in water, depending on process conditions, and the other part escapes as gas. Transferred CO_2 is determined by analysing the influent and effluent gas composition. CTR can be analysed using a gas balance, whereas CER can not be directly measured. Due to the low solubility of O_2, the oxygen transfer rate (OTR) and oxygen uptake rate (OUR) can be assumed to be equal. OTR = OUR holds only when the gas-liquid transfer of O_2 reaches equilibrium. The latter requires that OUR is approximately constant and the gas transfer rate coefficient (K_{La}) is constant, and the data should taken after an initial dynamic phase of the mass transfer.

TOC (total organic carbon) and IC (inorganic carbon) analysis were performed in raw sludge and the supernatant obtained by centrifuging the sludge at $n = 3000$ rpm for 5 min. Ionics 1 555B carbon analyzer was used for TOC and IC analysis. WTW Oxi 323B oxygen meter for the dissolved oxygen (DO) was used. Solid matter was made by the described method in APHA (1995).[15] The experimental set-up is shown in Fig. 1. Three experiments with different sludge were conducted:



F i g . 1 – *Experimental set-up*

1 – Aerobic digestion of a mixture consisting of primary and activated (PS+AS) sludge. The mixture of primary and activated sludge (60 L at ratio $PS/AS_{OS/AS} = 1:1$) optained from the WWTP Izmit-Turkey, where industrial and domestic wastewaters are treated simultaneously. The reactor was operated under pressure of $25 \cdot 10^3$ Pa. The sludge mixture was characterised by IC 310 mg L^{-1}, TOC 4940 mg L^{-1}, dry matter 17.93 g L^{-1} and pH 7.32. 1 L of molasses (sucrose content 45 % w/v) was added to digested sludge of 60 L at the 4094th min of digestion. During digestion, the mixture was monitored by respiratory activity parameters to evaluate the consumption of the organic matter in primary sludge by the activated sludge microorganisms and the activity increase after molasses feeding to the digested sludge mixture.

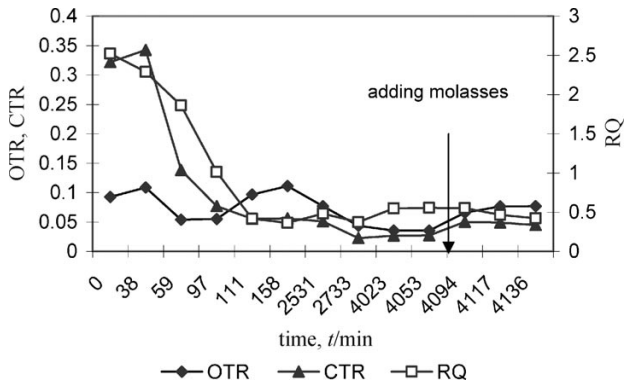
2 – Aerobic digestion of activated (AS) sludge. The activated sludge (60 L) obtained from the WWTP of a yeast factory in Izmit-Turkey. The reactor was operated under pressure of $25 \cdot 10^3$ Pa. The sludge was characterised by IC 600 mg L^{-1}, TOC 5600 mg L^{-1}, dry matter 18.48 g L^{-1} and pH 6.52. The digestion of the activated sludge was monitored by respiratory activity parameters to evaluate the microbial activity of the activated sludge of endogenous phase

3 – Aerobic digestion of a mixture consisting of activated sludge (AS) and baker's yeast. 2.4 kg dried baker's yeast was added to $V = 60$ L of activated sludge obtained from the WWTP of a yeast factory in Izmit-Turkey. Baker's yeast consists of carbohydrates 45–35 %, crude protein 40–50 %, lipids 4–7 %, and ash 5–8 %. The mixture was digested under microaerobic conditions. The reactor was operated under pressure of $p = 15 \cdot 10^4$ Pa. After a digestion of 7 d, 1.5 kg dextrose was fed to the sludge and the response was monitored by respiratory activity parameters.

Results and discussion

Aerobic digestion of a mixture consisting of primary and activated sludge

Raw organic matter in the primary sludge was the substrate for the activated sludge microorganisms during the digestion. Fig. 2 gives the respiratory parameters OTR, CTR and RQ vs time, during the digestion of the mixture of the primary and activated sludge (0 to 4094 min) and after molasses feeding (4094 to 4136 min). At the beginning of the experiment, the DO concentration was 0.553 mg L^{-1}. In the period from 0 to 38th min, OTR in-



F i g . 2 – *Respiratory activity parameters (OTR (kg O_2 m^{-3} h^{-1}), CTR (kg CO_2 m^{-3} h^{-1}), RQ) during the digestion of the mixture of primary and activated sludge*

creased from 0.09 to 0.11 kg O_2 m^{-3} h^{-1} and CTR from 0.32 to 0.34 kg CO_2 m^{-3} h^{-1}, due to digestion of the raw organic matter in the primary sludge. In this period, RQ decreased from 2.5 to 2.3. After the 38th min, OTR, CTR and RQ decreased dramatically, most likely because the organic matter was consumed. DO value increased from the beginning 0.553 mg L^{-1}, to 4.531 mg L^{-1}, due to the reduced biological activity. With sudden increase of molasses, OTR increased from 0.035 to 0.066 kg O_2 m^{-3} h^{-1} and CTR from 0.027 to 0.05 kg CO_2 m^{-3} h^{-1}; DO value decreased to 0.6 mg L^{-1}. RQ value of 0.55 did not change.

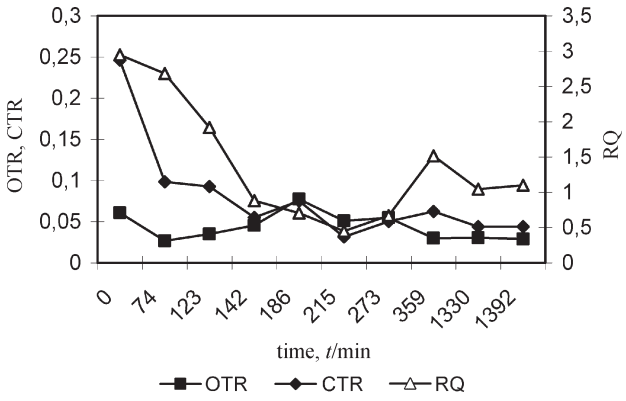
Because molasses were added to biomass without acclimation at the level of created substrate inhibition, these activity values determined by respiratory activity parameters did not represent the real activity of biomass. The aim of this experiment was to determine the best activity indicator shown the activity increase created by adding molasses.

During the first part of the experiment, as a result of the initial anoxic conditions of the sludge, the specific oxygen uptake rate (approximately 0.006 g g^{-1} MLVSS h^{-1}) presented relatively low values compared to those typically recorded in active sludge units. In an activated sludge system, typical values of the rate of oxygen used by the microorganisms vary from 2 to 7 g g^{-1} MLVSS[16]. As the experiment progressed and as oxygenation and feeding conditions stabilized, the microorganisms evolved and the consumption rate increased, reaching its maximum value. From this point onwards, this quantitiy registered an exponential decrease, indicating reduced respiratory activity. Similar results were obtained by *Oviedo* et al. (2005) and *Sanchez* et al. (2006) who evaluated biosolids stability in aerobic digestion systems.[17,1]

Aerobic digestion of activated sludge

Activated sludge is more stable than primary sludge. Therefore, it is expected that the biological activity of activated sludge will be lower than that of the activated and primary sludge mixture. Indeed, OTR and CTR of digested activated sludge decreased from 0.06 to 0.0266 kg O_2 m^{-3} h^{-1} and from 0.246 to 0.098 kg CO_2 m^{-3} h^{-1}, respectively in the first 60 min. In this period, the RQ value decreased from 2.94 to 2.68 (Fig. 3). The parameters for the activated and primary sludge mixture decreased in the same period (0–60th min.): OTR from 0.09 to 0.05 kg O_2 m^{-3} h^{-1}, CTR from 0.32 to 0.138 kg CO_2 m^{-3} h^{-1} and RQ from 2.5 to 1.86 (Fig. 2).

At the beginning of the experiments, the RQ values are high. This fact can be explained with an



F i g . 3 – *Respiratory activity parameters (OTR (kg O_2 m^{-3} h^{-1}), CTR (kg CO_2 m^{-3} h^{-1}), RQ) during the digestion of the activated sludge*

abiotic process. The experiments begin in an anaerobic environment in the reactor; the oxygen is totally consumed. The pH of the sludge is determined by the CO_2–HCO_3^- balance. Other elements that effect buffering capacity are ammonium and ammonia.

The pH was 6.5 at beginning and increased up to 7.6 during the experiment. By increasing pH, the dissolved CO_2 escapes as gas according to equation 8 and 9 so that CTR value increases. The increase of CTR at the beginning of the experiment is also due to transfer of CO_2 from liquid to gas phase by air stripping. The abiotic reactions causing dissolved CO_2 leaving the system as gas are the beginning aeration and the following pH increase. These factors cause a high CTR.

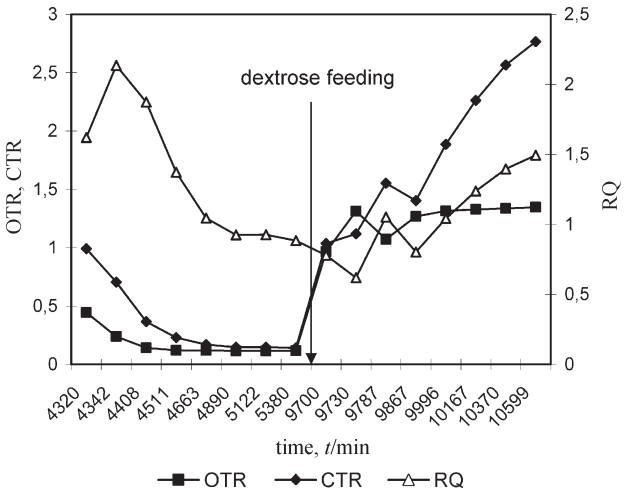
$$\mathrm{pH} = \mathrm{p}K_a + \log\left[\frac{\text{base}}{\text{acid}}\right] \qquad (8)$$

Dissociation constant and pK for bicarbonate ion at 25 °C are $6.31 \cdot 10^{-11}$ and 10.2, respectively. Eq. 8 yields equation 9.[18]

$$\mathrm{pH} = 10.2 + \log\left[\frac{HCO_3^-}{CO_2}\right] \qquad (9)$$

Aerobic digestion of a mixture consisting of activated sludge (AS) and baker's yeast

Since sludge digestion conditions are unsuitable for yeast growth, yeast cells were lysed and became substrate for the activated sludge microorganisms. This fact was verified by the increase of the TOC from 2740 to 6480 mg L^{-1} in the supernatant in the first 3 days of the experiment. In this period, 18 % of the TOC was consumed. High OTR (0.45 kg O_2 m^{-3} h^{-1}), CTR (0.99 kg CO_2 m^{-3} h^{-1}) and RQ (1.62) values at the end of 4320 min of the digestion period show that the biological activ-



F i g . 4 – *Respiratory activity parameters (OTR (kg O_2 m^{-3} h^{-1}), CTR (kg CO_2 m^{-3} h^{-1}), RQ) during the digestion of the mixture of activated sludge and baker's yeast*

ity of the sludge was still high. Fig. 4 shows the course of the respiratory parameters after this period (4320th min). At 5380th min, OTR, CTR and RQ were 0.12 kg CO_2 m^{-3} h^{-1}, 0.14 kg m^{-3} h^{-1} CO_2 and 0.88, respectively. In order to increase the activity with an impulse at 9700th min of the digestion, 1.5 kg dextrose was fed to 60 L sludge at 9700th min. The system's response was an increase of respiratory parameters: OTR 0.97 kg O_2 m^{-3} h^{-1}, CTR 1.04 kg CO_2 m^{-3} h^{-1}, whereas RQ decreased to 0.78 (Fig. 4). DO decreased 2.9 mg L^{-1} to 0.77 mg L^{-1} after the dextrose impulse.

In the first experiment, adding of 0.45 kg sucrose within 1 L molasses triggered an OTR and CTR increase of 1.89 and 1.85 times respectively, RQ value did not change (according to value at 4053th min and 4094th min). In the third experiment, the increase of OTR and CTR were 8.08 times and 7.43 times respectively after adding of 1.5 kg dextrose. RQ value decreased for 0.1 units (according to value at 5380th min and 9700th min). The increase was overproportional regarding the substrate quantity. This difference may be due to the kind of substrates (sucrose and dextrose) and the number of active microorganisms. The activated sludge fed with baker's yeast should have had more active microorganisms than the mixture of activated and primary sludge.

Lysed yeast cells served as subsrate for the activated sludge. Because the endogenous phase began after the diminishing of this substrate supply, the active microorganism level was high at prolonged time. Also, because baker's yeast consists of carbohydrates 45–35 %, the activated sludge microorganism adapted to carbohydrate easily decomposed dextrose.

Conclusions

Raw sludge was stabilized by reduction of biological activity during the digestion process. In this study, the biological activity of the sludge was monitored using respiratory activity parameters OTR, CTR and RQ. In the aerobic digestion of a mixture consisting of primary and activated sludge, OTR and CTR increased due to digestion of the raw organic matter in the primary sludge within 38 min, whereas RQ decreased. During the digestion after 38 min, the respiratory activity parameters were reduced. With sudden increase of molasses OTR and CTR increased, the RQ did not change.

Results show that the biological activity of the activated sludge was lower than a mixture of primary and activated sludge. Primary sludge serves as substrate for activated sludge. For example, in the first 60 min of digestion OTR decreased from 0.06 to 0.027 kg O_2 m^{-3} h^{-1} and from 0.09 to 0.32 kg CO_2 m^{-3} h^{-1}, for the activated sludge and the activated sludge and primary sludge mixture, respectively.

The aerobic digestion of a mixture consisting of activated sludge and baker's yeast show that the activated sludge fed with baker's yeast should have had more active microorganisms than the mixture of activated and primary sludge. Because baker's yeast cells lyse and their mainly content of carbonhydrates are used as substrate for the activated sludge fed with yeast at the long time.

Therefore, the endogenous phase began after the diminishing of this substrate supply and the active microorganism level was high at prolonged time. Also, because baker's yeast consists of carbohydrates 45–35%, the activated sludge adapted to carbohydrate easily decomposed dextrose.

RQ was found unsuitable as an indicator. The activity increase after substrate feeding in the first and third experiments was determined by increase of OTR and CTR, whereas RQ value did not change.

The activity of stabilized sludge can be enhanced by adding substrates like molasses containing sucrose or dextrose. The activity increase can be expressed by OTR and CTR. The digestion results of different sludge show that activity increase was proportional to the quantity and quality of the microbial population.

List of symbols

K_1 and K_2 – equilibrium constant, mol L^{-1}
M – molar mass, kg $kmol^{-1}$
n – rotation speed of centrifuge, rpm
p – pressure, Pa
K – dissociation constant, $pK = -\log K$

Q – volume flow rate, $m^3\ h^{-1}$

t – time, min, h

T – temperature, °C

V – volume, L, m^{-3}

V_N – molar volume, $m^3\ kmol^{-1}$

X – mole fraction, 1

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