Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 83 (4) 411–423 (2018)
JSCS–5085
UDC 582.23**Clostridium acetobutylicum*:664.4:664.15+544.35.000.57:544.032.72
Original scientific paper

Production of biosolvents and acids by a salinity-adapted strain of *Clostridium acetobutylicum*: Effects of salt and molasses concentrations

REZA ZABIHI[1], DARIUSH MOWLA[2]* and GHOLAMREZA KARIMI[1]

[1]*Department of Chemical Engineering, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran and* [2]*Environmental Research Centre in Petroleum and Petrochemical Industries, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran*

(Received 5 August, revised 22 November, accepted 28 December 2017)

Abstract: In this study, the growth of *Clostridium acetobutylicum* was evaluated in *Clostridium* basal medium (CBM) containing 0.001, 0.5, 1, 2 and 4 % salt concentrations. Although the strain is sensitive to salinity of more than 2 %, the adapted strain was shown to grow even at 6 % salinity. Results indicate adverse effects of salinity on bacterial growth and bioproducts, such as 1--butanol and butyric acid, whereas the produced acetone is increased by salinity in CBM. In addition, the glucose of CBM is substituted by sugar beet molasses, due to its lower price and greater accessibility. Therefore, molasses-based mediums (MBM) at different concentrations of molasses were examined to assess the effect of molasses concentration on the adapted strain at low salinity. The results showed that 4 and 6 % concentrations of molasses are optimum concentrations for bacterial growth and its production of bioproducts at low salinity. Finally, the simultaneous effects of salinity and molasses concentration on the adapted strain were investigated. For this purpose, molasses-based mediums (MBM) containing 2, 3 and 4 % molasses concentration at 4 % salinity were considered. The results demonstrated that the increase in the molasses concentration raises the production of both butyric acid and acetone.

Keywords: *Clostridium acetobutylicum*; adapted strain; salinity; sugar beet molasses; bioproducts.

INTRODUCTION

Clostridium acetobutylicum is a Gram-positive, endospore-forming and anaerobic bacterium from the *Clostridium* species.[1,2] The main products of *C. acetobutylicum* are butanol and butyric acid and its byproducts are acetone, acetic acid, lactic acid and ethanol. However, the amounts of the produced lactic acid

* Corresponding author. E-mail: dmowla@shirazu.ac.ir; dmowla@gmail.com
https://doi.org/10.2298/JSC170822013Z

and ethanol are too small. Since, *C. acetobutylicum* can produce acetone, 1-butanol and ethanol during fermentation, the process is called an ABE fermentation process.[3–5] The ABE process is a biphasic process in which acidogenic (acid-producing) and solventogenic (solvent-producing) phases occur. In the acidogenic phase, the growth of *C. acetobutylicum* is realized and acids, such as lactic acid, acetic acid and butyric acid, are produced. In addition, adenosine triphosphate (ATP) is formed during the production of acids, which is necessary for enzymes activation.[1] In the solventogenic phase, the growth of the bacterium ceases, the produced acids are consumed and solvents such as acetone, ethanol and 1-butanol are generated. Moreover, degradation of carbon sources continues in parallel.[1,2,6,7] Furthermore, it is to be noted that high amounts of gases, such as carbon dioxide and hydrogen, are generated during the ABE process.[3]

Industrial ABE fermentation process was taken into consideration for some decades and was the second largest fermentation process from 1900 to 1950. Since the industrial ABE process could not compete economically with the petrochemical synthesis process, this process ceased after about 1950. However, with the rise in oil prices in the past few decades,[8] biofuels were introduced as a partial substitution of fossil fuels and to mitigate greenhouse gas emissions,[9] the industrial ABE process was restarted in recent years.[2,6,10]

Some researchers have investigated the effect of salinity on *Clostridium* species[11–13] and other bacteria.[14,15] Alshiyab *et al*. investigated the effects of salts, such as sodium chloride and sodium acetate, on hydrogen production using *C. acetobutylicum* NCIMB 13357. They found that increasing the sodium chloride concentration from 0 to 5 g L^{-1} has an adverse effect on hydrogen productivity, biomass concentration and glucose consumption.[12] Rudyk and Søgaard studied the effect of salinity on the *Clostridium tyrobutiricum* strain. They showed that the strain could grow at 50 g L^{-1} salinity, however it was sensitive to greater salinities and hence, adapted the strain to 100 g L^{-1} salinity.[11] Van Niel *et al*. studied the influence of sodium acetate and sodium chloride on hydrogen production by *Caldicellulosiruptor saccharolyticus*. They concluded that cell lysis occurred at sodium acetate or sodium chloride concentrations greater than 200 mM and that the hydrogen production rate was reduced.[14] The effect of sodium chloride on hydrogen production was evaluated by Zheng *et al*. at 0 to 500 mM salinities. They demonstrated that sodium chloride had inhibitory effects on hydrogen production and glucose degradation.[15]

Glucose is the main carbon source for *C. acetobutylicum* to grow and generate bioproducts. Since glucose is an expensive product and is not economically viable, some other carbohydrate sources, such as cane molasses and sugar beet molasses, are utilized in ABE industrial processes.[5,10,16] Sugar beet molasses, one of the byproducts of sugar companies, contains sucrose, fructose and glu-

cose, as well as minerals and vitamins.[17] Sugar beet molasses is inexpensive and accessible, therefore it could be an alternative to glucose.[3,10,18]

Previous studies focused only on the growth and the rate of hydrogen production by *C. acetobutylicum* and, to the best of our knowledge, no researches are to be found in which the effect of salinity on the rate of produced biosolvents and acids was considered. Therefore, in this research, first the effects of salinity on the sensitive strain *C. acetobutylicum* and on a strain adapted to high salinity were investigated. In the next stage, glucose was substituted by sugar beet molasses at low salt concentration and the influence of its concentration was assessed on the adapted strain growth and bioproducts. Finally, fresh water was replaced by synthetic brine similar to seawater. Then, the effect of molasses concentration was evaluated on the adapted strain at high salinity.

EXPERIMENTAL

Chemicals

Sugar beet molasses was obtained from the Marvdasht Sugar Company in the Fars province, Iran. Resazurin sodium salt was purchased from Sigma–Aldrich. Sodium hydroxide solution and lactic acid (AnalaR grade) were supplied by the BDH Company. Extra pure sodium chloride (more than 99.5 %) was provided by Dr. Mojallali Chemical Complex Co., Iran. Other chemicals, such as glucose, acetone, 1-butanol, *etc*., of analytical grade, were purchased from the Merck Company.

Microorganism and culture medium

In this study, *Clostridium acetobutylicum* was obtained from the Persian Type Culture Collection (PTCC) as a freeze-dried strain, which is introduced as PTCC 1492. This center states that another collection number for the bacterium is NRRL B-591.[19] The strain was prepared from beef liver medium according to the ATCC protocol.[7,20] After 24 h, the culture containing the microorganism was stored at 4 °C as a bacterial stock. Since the preparation of beef liver medium is expensive and time consuming, clostridium basal medium (CBM) described by Monot *et al*.[21] was used for the other experiments. The composition of CBM consisted of (per L): glucose, 20 g; KH_2PO_4, 0.5 g; $K_2HPO_4{\cdot}3H_2O$, 0.5 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; $MnSO_4{\cdot}H_2O$, 0.01 g; $FeSO4{\cdot}7H_2O$, 0.01 g; NaCl, 0.01 g; ammonium acetate, 2.2 g; *p*-aminobenzoic acid (PABA), 1 mg; thiamin·HCl, 1 mg; biotin, 1 µg; resazurin sodium salt, 1 mg. Then, the pH of the CBM was adjusted to 8 using 1 M sodium hydroxide and was autoclaved for 20 min at 121 °C.

Inoculation of microorganism and bioreactor experiments

In the first stage, the prepared CBM was poured into a 50 mL sterile tube and 1 vol.% of bacterial stock was inoculated. Then, sterile liquid paraffin was added to apply anaerobic conditions and incubated for 48 h at 37 °C. Thus, inoculums were prepared for bioreactor tests.

For all bioreactor tests, 500 mL of CBM was prepared in each bioreactor, resazurin solution and vitamins were added and 1 vol.% of an inoculum containing the bacterium was inoculated. The optical density of the inoculum was in the range of 0.7 to 0.8. Then, pure nitrogen was introduced for 5 min to produce anaerobic conditions in the bioreactor. Finally, the bioreactor was placed into a shaker incubator model SI-100R (HYSC Ltd., Korea) at 100 rpm and 42 °C.

Bioreactor tests were divided into four groups. In the first group, CBMs containing 0.001, 0.5, 1, 2 and 4 % salinities were prepared and the effects of salinity on bacterial growth, pH variation, and the production of bioacids and biosolvents by the sensitive strain were studied during fermentation.

Since the bacterium could not tolerate high salinity, adaptation of the bacterium to high salinity was performed in the second group. For this purpose, the highest salinity in which the bacterium could grow in the first group was considered and samples were taken from it. Then, the adapted strain was inoculated for other bioreactor tests. In this group, the salinity effect was investigated in CBM containing 2.5, 3, 4 and 6 % salinities.

In the third group, glucose was substituted by sugar beet molasses as the carbohydrate source because molasses is much cheaper than glucose and it is byproduct of sugar factories.[3,10,16] The elemental concentration of the sugar beet molasses used in the experiments is given in Table I. The carbon and nitrogen concentrations were determined using a FlashEA 1112 elemental analyzer and the concentrations of the trace elements were measured using a PerkinElmer Optima 8000 ICP-OES. The concentration of Cl was determined using the chlorine standard method (4500-Cl) for the examination of water and wastewater. Therefore, MBM containing 2, 4, 6, 8, 10, 12 % molasses were prepared at 0.001 % (low) salinity. Afterward, the effect of molasses concentration was assayed on the adapted strain.

As Iranian seawaters (especially Persian Gulf seawater) have a salinity of about 4 %, MBMs containing 2, 3 and 4 % molasses were prepared with 4 % salinity in the fourth group. Subsequently, the effects of molasses and salinity were simultaneously examined on the adapted strain.

TABLE I. Chemical composition of the employed sugar beet molasses

Element	Concentration, mg L^{-1}	Element	Concentration, mg L^{-1}
C	310070	N	11877
K	15100	Na	12600
Ca	3000	Mg	354
P	143	Fe	64
Mn	15.3	Zn	14.3
Cu	2.9	Cl	26625

Optical density and pH measurements

In order to determine bacterial growth, samples were removed from the bioreactor and divided into two parts. The first part was centrifuged at 4000 rpm for 30 min to achieve a supernatant that was then used as blank. The optical density (*OD*) of the second part was measured at 600 nm[1,9,21] using a model DR 2800 HACH spectrophotometer (USA). The *OD* presents the total number of cells (live and dead), whereas the colony-forming units (CFU) presents only the number of live cells. Therefore, the correlation between *OD* and CFU mL^{-1} was determined. The correlation was $y = 6\times10^9x - 5\times10^7$; where y represents CFU mL^{-1}; x represents the *OD* value; R^2 is 0.9958. Moreover, the pH of the culture was measured using a Milwaukee model MW102 pH meter (Romania) at room temperature.

Bioproducts analysis

Various concentrations of acetic acid, butyric acid, lactic acid, acetone, 1-butanol and ethanol in ethyl acetate were prepared and standard curve was achieved for each component (Fig. S-1 of the Supplementary material to this paper). Then for analysis of the bioproducts,

0.5 mL of the prepared supernatant was added to 0.5 mL of ethyl acetate in a vial and vortexed for 1 min to extract the bioproducts from the aqueous phase to ethyl acetate.[6,22] Then, 100 µL of extracted phase was injected into a Varian model 3700 gas chromatography (GC) instrument equipped with a flame ionization detector (FID) and an OV-101 column. Helium was used as the carrier gas. The flow rates of helium, air and hydrogen were set at 30, 300 and 30 mL min^{-1}, respectively. The detector and injector temperatures were adjusted respectively to 270 and 240 °C. Initial column temperature was adjusted to 35 °C for 2 min, then the column temperature was increased to 100 °C at 10 °C min^{-1} and finally, the column was maintained at 100 °C for 3 min. A typical GC chromatogram is presented in Fig. S-2. Each test was repeated at least two times.

RESULTS AND DISCUSSION

Effect of salinity on the sensitive strain of C. acetobutylicum

The effects of salinity on the growth of the sensitive strain and on the concentrations of bioproducts, such as butyric acid, lactic acid, acetone and 1-butanol, after 10 days are illustrated in Fig. 1. As could be seen from this figure, the *OD* was reduced from 2.019 to 1.002 as the salinity increased from 0.001 to 2 %, while there was no bacterium growth at 4 % salinity. The same results were achieved by Qureshi *et al*. for *Clostridium beijerinckii* P260 in a batch process and they stated that NaCl concentrations above 2 % had an inhibitory effect on cell growth and ABE fermentation.[13] Moreover, investigation of salinity effect on *Clostridium acetobutylicum* P262 was performed by Maddox *et al*. and their results showed that bacterial growth was inhibited at 3 % and higher concentrations of sodium chloride.[23]

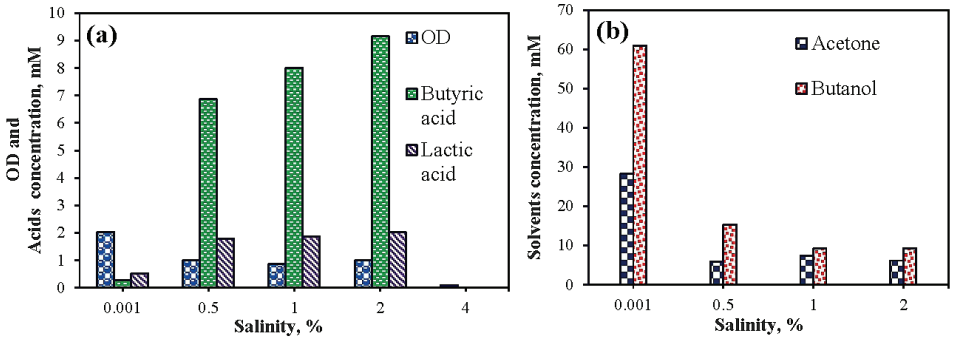

Fig. 1. Effect of salinity on: a) the sensitive strain growth and acids concentration and b) solvents concentration after 10 days.

Fig. 1 also shows that increasing salinity resulted in the production of more butyric and lactic acids by the sensitive strain. As was observed, when the salinity was increased from 0.001 to 2 %, the concentrations of butyric acid and lactic acid were increased from 0.28 to 9.16 mM, and from 0.53 to 2.03 mM, respectively. It is necessary to note that since the acetic acid concentration was

lower than 40 mM, interference of peaks produced by the GC occurred and it was impossible to determine its concentration. Fig. 1 also shows that salinity had a negative effect on the production of solvents; the 1-butanol and acetone concentrations were decreased from 60.93 to 9.24 mM and from 28.24 to 6.17 mM, respectively, as the salinity was increased from 0.001 to 2 %. Maddox *et al*. and Zhao *et al*. declared that *C. acetobutylicum* produced more acetic and butyric acids than acetone and 1-butanol on increasing the salinity up to 2–3 %. In the other words, the bacterium cannot convert acids to solvents at high salinity. Some researchers suggested that the adverse effect of salinity is related to the presence of sodium ions.[13,22]. Hartmanis *et al*. announced that sodium ions inhibit acetoacetyl-CoA:acetate (butyrate) CoA-transferase, which solely converts acetate and butyrate into acetyl-CoA and butyryl-CoA.[24] It is necessary to note that although low concentrations of sodium are necessary for the oxidation of NADH and the formation of ATP in anaerobic bacteria,[25] high sodium concentrations not only increase bacterial growth, but also destructively influence microorganism activity.[25,26]

Adapted strain of C. acetobutylicum *and effect of salinity*

Effect of salinity on the growth of the adapted strain and pH variations. The effect of salinity on the growth rate of the adapted *C. acetobutylicum* strain and pH variations of the medium are illustrated in Fig. S-3 of the Supplementary material. The lag phase for the normal strain of the bacterium in the medium containing 2 % salinity was prolonged to 80 h, however, the lag phase for adapted strain in the mediums containing 2.5 and 3 % salinities was prolonged by only 6.5 h, *i.e.*, the necessary time period for adaptation of the bacterium was reduced for the adapted strain compared to the sensitive strain. Furthermore, the adapted strain could grow in a medium containing more salinity, such as 4 and 6 %, and the lag phase was prolonged to 28 h. Moreover, the specific growth rate and maximum *OD* decreased with salinity for the adapted strain. The adverse effects of salt on bacteria growth was reported by some other researchers.[11,12,14,15,27] These researchers declared that the ionic strength increased with increasing salt concentration and this caused higher osmotic pressures. In fact, when bacteria are exposed to higher osmolarity environments, the water exits from the cytoplasm and dehydration of cytoplasm or plasmolysis occurs.[11,12,14,15,25,26] Moreover, each bacterium needs a certain level of ions to activate enzymes and co-enzymes. As the amount of the ions is much greater than that required, chemical binding of the ions to enzymes arises. As a result, the activities and structures of enzymes are disrupted. Therefore, high salinity results in reduction of bacterial growth, changes bacterial metabolic pathways and even can be inhibitor or toxic,[12,15,25–27] In addition, McCarty and McKinney stated that cations, such as sodium, potas-

sium, calcium and magnesium, play key roles in salt toxicity, since these cations affect anaerobic digestion.[26]

The effects of pH variation for various salinities are shown in Fig. S-3b of the Supplementary material. As could be seen, for 2.5 and 3 % salinities, the pH of the medium initially decreased, remained constant, decreased again and reached about 5, then finally increased. However, for 4 and 6 % NaCl concentrations, the culture pH initially decreased and finally was uniform during fermentation. Additionally, it could be seen that the maximum acid production occurred at 4 % salinity, which resulted in an intensive pH drop. At 2.5 and 3 % salinity, the initial pH reduction is indicative of the production of acids, whereas the pH increase indicates the consumption of acids and the production of solvents.[7]

Salinity effect on butyric and lactic acid production by the adapted strain. The butyric and lactic acids production by the adapted strain at various salt concentrations is shown in Fig. 1, from which it could be seen that the concentrations of butyric and lactic acids increased during the log phase at 2.5 and 3 % salinities, and then decreased during the stationary and dead phases that is in good agreement with the trend in the pH variation. However, no butyric acid was produced by the adapted strain at 4 and 6 % salinities and significantly, less lactic acid was produced. Since pH reduction occurred intensively at 4 and 6 % salinities, Fig. S-3b, significantly more acetic acid must have been produced at these salinities.

Comparison of Figs. 2 and S-3 illustrates that a large pH reduction and a high rate of acid production occurred at a high bacterial growth rate. At a high bacterial growth rate, *Clostridium* bacteria require high energy and more ATP. During acetic acid and butyric acid production, 4 and 3 mol of ATP are produced, respectively. Since acetic acid can produce more ATP than butyric acid, acetic acid production occurs initially and then butyric acid is produced.[1,28]

Effect of salinity on acetone and 1-butanol production by the adapted strain. The amounts of acetone and 1-butanol produced by the adapted strain at various salt concentrations are also exhibited in Fig. 2. Based on this figure, acetone production occurred during pH reduction and when the pH was constant at salinities of 2.5 and 3 % during the log and stationary phases, respectively. However, the adapted strain produced 1-butanol at the end of stationary phase. On the other hand, no 1-butanol was produced at 4 and 6 % salinities, as was also observed for butyric acid (Fig. 2). For production of 1-butanol and butyric acid, activities of enzymes from acetyl-CoA to butyryl-CoA are necessary in the pathway.[1,29,30] Since 1-butanol and butyric acid were not produced at 4 and 6 % salinities, high salinity influences the enzymes activities from acetyl-CoA to butyryl-CoA and inactivates them. On the other hand, large amounts of acetone were produced during the stationary and dead phases at 4 and 6 % salinities (Fig. 2). Thus, the

adapted strain produced higher acetone in CBM with higher salinity and salinity had a beneficial effect on acetone production.

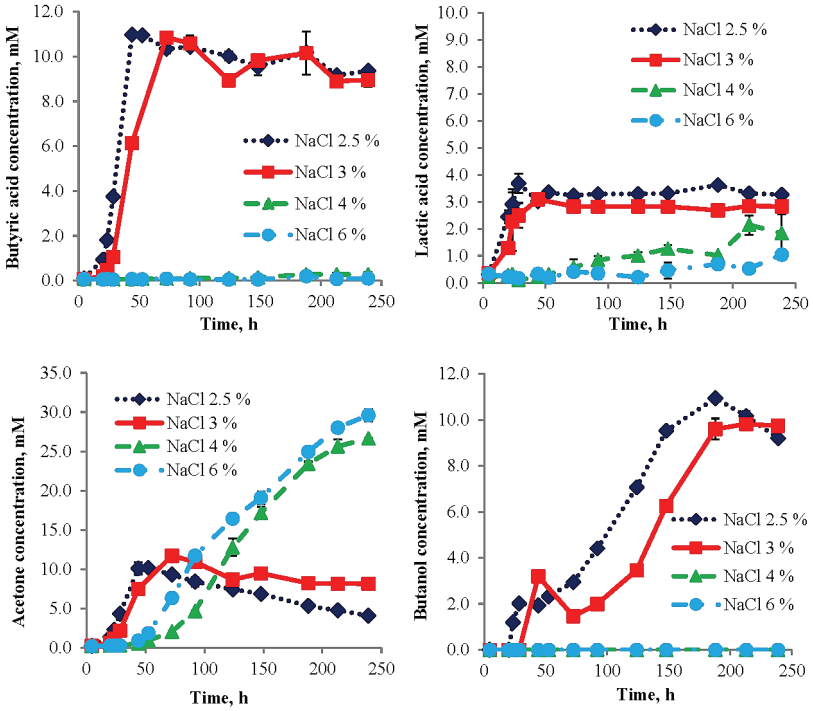

Fig. 2. Produced acids and solvents by adapted strain at various salinities.

Molasses effect on the adapted strain in the medium with low salinity

Effect of molasses on bacterial growth and pH variation. The growth of the adapted strain and pH variation in medium with various concentrations of molasses from 2 to 12 % and the lowest salinity are presented in Fig. S-4. The *OD* increased with concentration of molasses (Fig. S-4a). When the molasses concentration increases, MBM approaches saturation and affects *OD*. It is necessary to mention that the inoculum volume was the same for the various MBMs. In addition, the MBM containing a greater concentration of molasses had a greater specific growth rate; thus, the specific growth rates were 0.137 h^{-1} for 2 %, 0.155 h^{-1} for 3 %, 0.109 h^{-1} for 4 %, 0.16 h^{-1} for 6 %, 0.229 h^{-1} for 8 % and 0.221 h^{-1} for 10 % concentration of molasses. Molasses contains sugars, such as glucose and fructose, and glucose is the main carbohydrate source for the bacterium fermentation.[3,10,16] Thus, increasing of molasses in the medium can raise the specific growth rate. OD_{max} of the adapted strain increases with increasing molasses concentration from 2 to 3%, nevertheless more increasing in molasses concentration from 3 to 12 % results in lower OD_{max}. The molasses contains not only

sugars, but also mono- and divalent-cations which increase the ionic strength in MBM containing more molasses [16]. Higher ionic strength causes more osmotic stress and the bacterium shrinks as was mentioned before.[11,12,15]

In addition, greater pH reduction occurs at higher molasses concentration in the culture (Fig. S-4b). In the other words, increasing the molasses concentration resulted in increased production of acids by the adapted strain. Since a higher specific growth rate was evidenced at higher concentrations of molasses, the bacterium required more ATP to grow and therefore, more acids were produced and the pH reduction was greater.[1,28]

Effect of molasses on butyric and lactic acids. Produced butyric and lactic acids by the adapted strain at low salt concentration are presented in Fig. 3, which shows that the production of butyric acid was reduced considerably at concentrations of molasses above 6 %. Moreover, the production of lactic acid was low and remained nearly constant at molasses concentrations above 3 %. Therefore, it seems that the pH reduction was due to increased acetic acid production at higher concentrations of molasses, especially at 10 and 12 % salinity (Fig. S-4a).

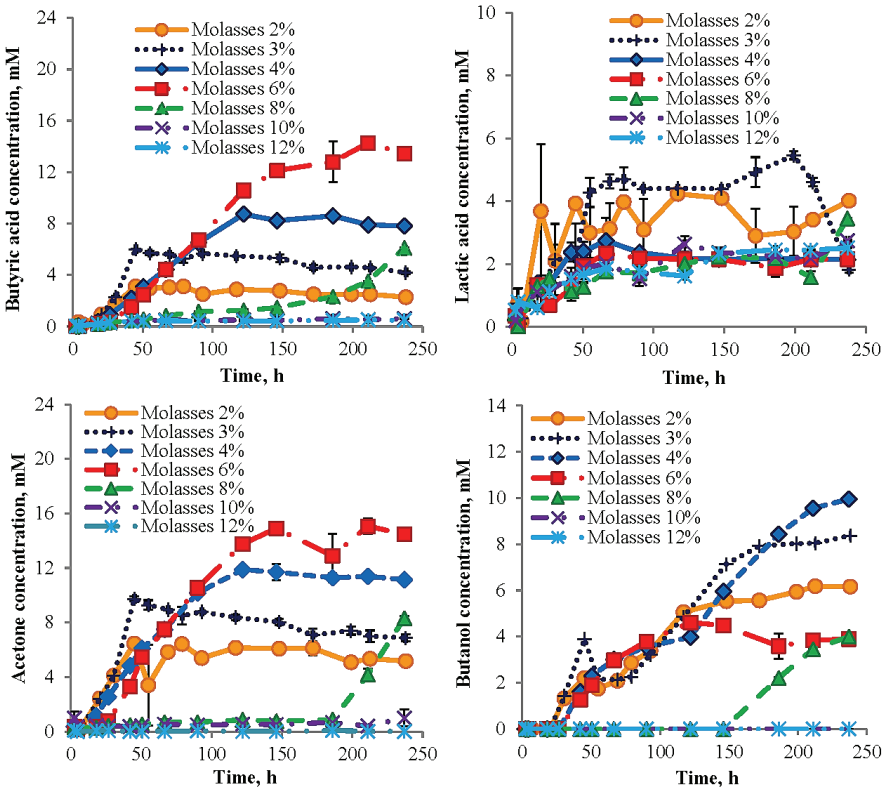

Fig. 3. Effect of molasses on the production of acids and solvents by the adapted strain at low salt concentration.

Effect of molasses on acetone and 1-butanol production. The amounts of produced solvents in mediums with different concentrations of molasses at low salinity are illustrated in Fig. 3. Although the production of acetone increased with increasing concentration of molasses from 2 to 6 %, it intensively decreased at higher concentrations of molasses. In addition, the amount of produced 1-butanol increased with molasses concentration between 2 to 4 %, but then decreased considerably at higher concentrations of molasses.

Effect of molasses on the adapted strain in high salinity medium

Effect of molasses on bacterial growth and pH variation. As could be seen in Fig. S-5a of the Supplementary material, bacterial growth initially increased and then decreased with increasing of concentration of molasses at higher salinity (40 g L^{-1} salt concentration). Moreover, according to Fig. S-5b, the pH increased at concentrations of molasses from 2 and 3 % during the death phase, which indicates consumption of acids, while the pH remained constant at a molasses concentration of 4 % and 40 g L^{-1} salinity.

Comparison of Figs. S-4a and S-5a illustrates that salinity not only reduces the bacterial growth, but also increases it at constant concentrations of molasses. Since the bacterium was adapted to salinity at the initial stages, it seems that the salinity-adapted strain can grow in a high salinity medium more effectively than in a low salinity medium. Therefore, the bacterium grows better in the presence of salt at a constant concentration of molasses.

Based on Fig. S-5b, the pH variation was more considerable at 3 % molasses concentration than at 2 and 4% at 40 g L^{-1} salinity. In addition, comparison of Figs. S-4b and S-5b illustrates that the existence of salt in MBM resulted in a smaller pH reduction during the log phase and pH increase during the stationary or dead phase at the same concentration of molasses.

Effect of molasses on the production of butyric and lactic acids. Fig. 4 indicates that a higher concentration of molasses caused more butyric acid to be produced at 40 g L^{-1} salinity. On the other hand, comparison of Figs. 3 and 4 revealed that a higher salt concentration resulted in more butyric acid being produced at constant molasses concentrations. However, the concentration of lactic acid showed no variation at different salinities.

Effect of molasses on the amount of produced acetone and 1-butanol. The obtained results showed that increasing the concentration of molasses had a positive effect on the production of acetone at higher salinity so that the produced acetone was about 12 mM at 4 % molasses concentration (Fig. 4). Indeed, salinity had no adverse effect on the production of acetone at a constant concentration of molasses; however, salinity considerably reduced 1-butanol production, as seen in Figs. 3 and 4. Thus, less butyric acid converts to 1-butanol at higher salinity and constant molasses concentration.

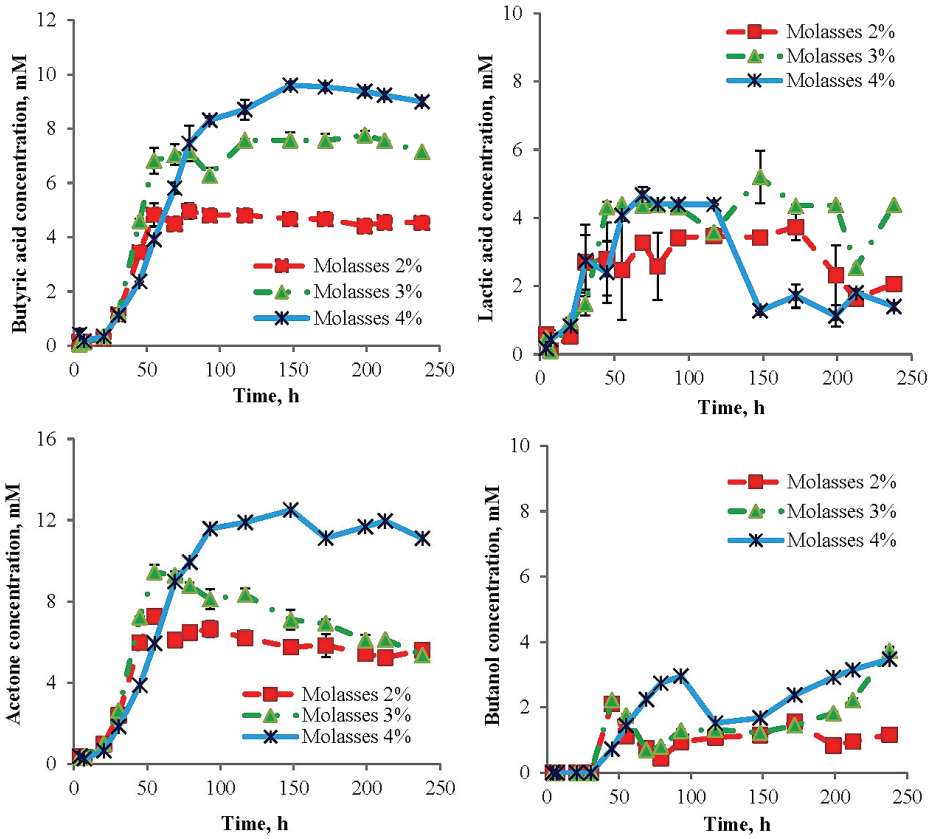

Fig. 4. Effect of molasses on the production of acids and solvents by the adapted strain at high salt concentration.

CONCLUSIONS

According to the data obtained in different experiments and their evaluations, the following conclusions could be drawn:

– *Clostridium acetobutylicum* NRRL B-591 strain was sensitive to 40 g L^{-1} salinity, while the adapted strain could grow at higher salinities, as much as 60 g L^{-1}.

– By increasing the salinity in CBM, bacterial growth was intensively reduced. Butyric acid and 1-butanol production ceased at salinities greater than 30 g L^{-1}, however, acetic acid and acetone were considerably produced. The produced acetone was increased from 8.1 mM at 30 g L^{-1} salinity to 29.6 mM at 60 g L^{-1}.

–The specific growth rate of the adapted strain was increased with molasses concentration form 2 to 12 % at 0.01 g L^{-1} salinity. In addition, the maximum *OD* occurred at 3 % molasses concentration.

– More butyric acid and acetone were produced by increasing the concentration of molasses from 2 to 6 % in MBM at 0.01 g L^{-1} salinity. However, higher concentrations of molasses had an adverse effect on the production of butyric acid and acetone. Although no 1-butanol was produced at 10 and 12 % molasses concentration, acetic acid production was increased extremely. Additionally, the maximum 1-butanol production occurred at 4 % molasses concentration.

– Butyric acid and acetone were considerably produced by increasing the molasses concentration from 2 to 4 % at 40 g L^{-1} salinity. In addition, the results demonstrated that the simultaneous presence of salt and molasses in MBM caused their antagonistic effect and thus, a salinity increase from 0.01 to 40 g L^{-1} resulted in an extreme decrease in 1-butanol production, and increased the amount of butyric acid significantly.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of the journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

ПРОИЗВОДЊА БИОРАСТВАРАЧА И КИСЕЛИНА ПРИМЕНОМ СОЈА *Clostridium acetobutylicum* АДАПТИРАНОГ НА СО: УТИЦАЈ КОНЦЕНТРАЦИЈЕ СОЛИ И МЕЛАСЕ

REZA ZABIHI[1], DARIUSH MOWLA[2] и GHOLAMREZA KARIMI[1]

[1]*Department of Chemical Engineering, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran и* [2]*Environmental Research Centre in Petroleum and Petrochemical Industries, School of Chemical and Petroleum Engineering, Shiraz University, Shiraz, Iran*

У овом раду испитан је раст бактерије *Clostridium acetobutylicum* у основном медијуму (CBM) који садржи 0,001; 0,5; 1; 2 или 4 % соли. Иако је бактерија осетљива на салинитет већи од 2 %, адаптирани сој може расти и у средини са 6 % соли. Резултати су показали да салинитет има негативан ефекат на раст бактерија и биопроизводњу 1-бутанола и бутерне киселине, док је производња ацетона повећана у присуству соли. Даље је испитан утицај замене глукозе у CBM меласом, јер је меласа јефтинија и лакше доступна. Анализиран је ефекат различитих концентрација меласе у медијуму (MBM) на адаптирани сој при малом салинитету. Резултати су показали да су 4 и 6 % концентрације меласе оптималне за раст бактерија и њихову биопроизводњу. На крају, испитан је комбиновани ефекат већег салинитета и меласе на адптирани сој. Коришћен је медијум MBM са 2, 3 или 4 % меласе и 4 % соли. Пораст концентрације меласе је повећао производњу и бутерне киселине и ацетона.

(Примљено 5. августа, ревидирано 22. новембра, прихваћено 28. децембра 2017)

REFERENCES

1. J. Zigová, E. Šturdík, *J. Ind. Microbiol. Biotechnol.* **24** (2000) 153
2. T. Lütke-Eversloh, H. Bahl, *Curr. Opin. Biotechnol.* **22** (2011) 634
3. M. M. K. Bagy, M. H. Abd-Alla, F. M. Morsy, E. A. Hassan, *Int. J. Hydrogen Energy* **39** (2014) 3185
4. N. Kaid, N. Al-shorgani, M. Hafez, M. Isa, W. Mohtar, W. Yusoff, M. Sahaid, A. Abdul, *Renewable Energy* **86** (2016) 459
5. E. A. Hassan, M. H. Abd-Alla, M. Mohamed, K. Bagy, *Anaerobe* **34** (2015) 125

6. C. M. Cooksley, Y. Zhang, H. Wang, S. Redl, K. Winzer, N. P. Minton, *Metab. Eng.* **14** (2012) 630
7. D. T. Jones, A. Van der Westhuizen, S. Long, E. R. Allcock, S. J. Reid, D. R. Woods, *Appl. Environ. Microbiol.* **43** (1982) 1434
8. F. Heidari, M. A. Asadollahi, A. Jeihanipour, M. Kheyrandish, H. Rismani-Yazdi, K. Karimi, *RSC Adv.* **6** (2016) 9254
9. S. Sreekumar, Z. C. Baer, A. Pazhamalai, G. Gunbas, A. Grippo, H. W. Blanch, D. S. Clark, F. D. Toste, *Nat. Protoc.* **10** (2015) 528
10. Y. Ni, Z. Sun, *Appl. Microbiol. Biotechnol.* **83** (2009) 415
11. S. Rudyk, E. Søgaard, in *Applied Microbiology and Molecular Biology in Oil Field Systems*, C. Whitby, T. Lund Skovhus, Eds., Springer, Heidelberg, 2010, pp. 179
12. H. Alshiyab, M. S. Kalil, A. A. Hamid, Y. W. M. Wan, *Pak. J. Biol. Sci.* **11** (2008) 2193
13. N. Qureshi, B. C. Saha, R. E. Hector, M. A. Cotta, *Biomass Bioenergy* **32** (2008) 1353
14. E. W. J. van Niel, P. A. M. Claassen, A. J. M. Stams, *Biotechnol. Bioeng.* **81** (2003) 255
15. X. J. Zheng, Y. M. Zheng, H. Q. Yu, *Environ. Technol.* **26** (2005) 1073
16. J. Cheng, *Biomass to renewable energy processes*, CRC Press, Boca Raton, FL, 2009
17. M. R. Nićetin, L. L. Pezo, B. L. J. Lončar, V. S. Filipović, D. Z. Šuput, V. M. Knežević, J. S. Filipović, *J. Serb. Chem. Soc.* **82** (2017) 253
18. M. Wagner, D. Lungerhausen, H. Murtada, G. Rosenthal, *Development and application of a new biotechnology of the molasses in-situ method; detailed evaluation for selected wells in the Romashkino carbonate reservoir*, BDM Oklahoma, Inc., Bartlesville, OK, 1995
19. *Persian Type Culture Collection*, http://ptcc.irost.org/DBank-details.asp?id= =103&code=0 (2017)
20. *ATCC*, https://www.atcc.org (2017)
21. F. Monot, J.-R. Martin, H. Petitdemange, R. Gay, *Appl. Environ. Microbiol.* **44** (1982) 1318
22. X. Zhao, S. Condruz, J. Chen, M. Jolicoeur, *Sci. Rep.* **6** (2016) 28307
23. I. S. Maddox, N. Qureshi, K. Roberts-Thomson, *Process Biochem.* (*Oxford, U.K.*) **30** (1995) 209
24. M. G. N. Hartmanis, T. Klason, S. Gatenbeck, *Appl. Microbiol. Biotechnol.* **20** (1984) 66
25. Y. Chen, J. J. Cheng, K. S. Creamer, *Bioresour. Technol.* **99** (2008) 4044
26. P. L. McCarty, R. E. McKinney, *J. Water Pollut. Control Fed.* **33** (1961) 399
27. X. Hao, M. Zhou, H. Yu, Q. Shen, L. Lei, *Chinese J. Chem. Eng.* **14** (2006) 511
28. D. Michel-Savin, R. Marchal, J. P. Vandecasteele, *Appl. Microbiol. Biotechnol.* **34** (1990) 172
29. G. N. Bennett, F. B. Rudolph, *FEMS Microbiol. Rev.* **17** (1995) 241
30. L. Girbal, C. Croux, I. Vasconcelos, P. Soucaille, *FEMS Microbiol. Rev.* **17** (1995) 287.