ISSN:
Print - 2277 - 0593
Online - 2315 - 7461

Journal of Natural Science, Engineering and Technology

EFFECTS OF LOW AND ELEVATED SODIUM ION CONCENTRATIONS ON ACETONE-BUTANOL-ETHANOL (ABE) FERMENTATION AND BIOHYDROGEN PRODUCTION FROM WASTE FIG (*Ficus carica*)

***[1], [2]WASIU AYODELE ABIBU, AND [2], [3]ILGI KARAPINAR**

[1]Department of Microbiology, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria
[2]Department of Biotechnology, Natural and Applied Sciences, Dokuz Eylul University, İzmir, Türkiye
[3]Department of Environmental Engineering, Engineering Faculty, Dokuz Eylul University, İzmir, Türkiye
***Corresponding Author**: abibuwa@funaab.edu.ng **Tel**: +2348062169365

ABSTRACT

The positive role of sodium on Acetone-Butanol-Ethanol (ABE) fermentation, most especially in the improvement of butanol concentration has been documented. The role of sodium in inhibiting biohydrogen production has also been reported. This research therefore focused on the role of low and elevated sodium concentrations on ABE fermentation since ABE fermentation and biohydrogen production share a common biochemical pathway. Waste fig (*Ficus carica*) was pretreated via microwaving hydrolysis. Optimum pretreatment conditions were particle diameter (370 µm), pH (4.96), power (250W) and treatment period (50 mins) with maximum sugar concentration of 82 g/L obtained. Hydrolyzed fig was fermented at low (20 – 100 mg/L) and high (500 – 2000 mg/L) sodium concentrations. A control experiment was conducted. Highest acetone, butanol and ethanol concentrations of 11.63, 6.28 and 2.18 g/L were recorded at 60 mg/L, 60 mg/L and 20 mg/L, respectively. Highest cumulative hydrogen was observed at 40 mg/L (681.84 ml). Results obtained from elevated concentrations were similar to that of the control experiments. This showed that elevated sodium concentration may not be needed when considering fermentation media supplementation with sodium in ABE fermentation studies.

Keywords: Acetone, Butanol, Ethanol, Waste, Fig, Biohydrogen, Sodium concentration.

INTRODUCTION

Butanol obtained from biomass is known as "biobutanol". In the last three decades, biobutanol research has witnessed lots of scholastic contributions from the academia and industry, thus, strengthening the public acceptance on the need for an environmentally friendly fuel alternative when compared with fossil fuel and its accompanying environmental concerns. The possibility for butanol pipeline transportation and engine ignition positioned it as a perfect substitute to gasoline. Initially, biobutanol research was challenged with low productivity, substrate cost, final separation and product recovery. However, biotechnological advancements

have led to discovery of butanol producing Clostridial strains and the use of lignocellulosic biomass has tackled the concerns in biobutanol research. Butanol is widely produced via the ABE (Acetone-Butanol-Ethanol) fermentation process. This method is bi-phasic, consisting of the acid production and solvent production stages. Many scientists have focused on using 2^0 (lignocellulosic biomass) as substrates for ABE fermentation because they are readily available, inexpensive and free from the food versus fuel debate associated with primary biomass category. Butanol is a major well-known biofuel. The World Wars in the 20th century shifted global attention to ABE fermentation. The success recorded by Brazil in ethanol research, reducing its dependence on fossil fuels increased interest in ABE fermentation and butanol production by private and governmental agencies across the globe (Kumar *et al.*,2012). Recent publications have reported the possibility of using ABE products directly as fuel without the need for separation and purification (Veza *et al.*, 2019).

Biohydrogen research stemmed out from the rising interest in renewable energy. It is a fermentation based, microbial mediated value added product and it forms an integral part in the production, promotion and sustainability of green energy technologies. Biohydrogen production technologies can be classified into photo-fermentation, bio-pyrolysis, microbial electrolysis cell, microbial fuel cell and dark fermentation. Enterobacter, Escherichia and Clostridium are important microbial genera with high biohydrogen production potential (Goyal *et al.*, 2013). In biohydrogen production and ABE fermentation, substrates pre-treatment is highly necessary. Pre-treatment makes fermentable sugar readily available for microbial utilization, thereby enhancing fermentation for biohydrogen and for production of ABE solvents. Pre-treatment may be physical, chemical or biological. Lignocellulose materials are well documented in biofuel generation.

Table 1. Elemental and organic constituents of hydrolyzed Fig

Elements mg/L	Te	0.00078	As	0.000998
	Hg	0.001605	Sr	0.282092
	Li	0.002823	Mo	0.003437
	Be	8.48×10^{-6}	Ag	0.000479
	B	0.803213	Cd	0.001506
	Na	10.85947	Sn	0.005461
	Mg	41.06452	Sb	0.000412
	Al	0.09162	Ba	0.16168
	K	576.6324	Tl	0.000381
	Ca	40.00488	Pb	0.009734
	V	0.000862	Co	0.005106
	Cr	0.000161	Ni	0.115218
	Mn	0.28494	Cu	0.100718
	Fe	1.354785	Zn	0.399009
Nutrients mg/L	$PO^{4-}P$	43.7		
	Total phosphorus	49.4		
	$NH^{3-}N$	60.24		
	Total nitrogen	700		
	Total kjeldahl nitrogen	98		

Fig is widely grown in Turkiye with yearly export of approximately 200,000 tones (Ercisli *et al*., 2012). It is a rich source of sugar and essential minerals (Table 1) - Abibu and Karapinar, (2023). Afflatoxin contaminated fig are disposed by incineration. However, the high sugar content of fig can act as biohydrogen fermentation substrate. Lignocellulosic biomasses are rich in cellulose which are excellent startup material for ABE fermentation.

The effect of cations on ABE fermentation and biohydrogen production has not been widely studied. Works from past authors revealed the positive role of Gadolinium (Gd) in improving butanol yield with a 15.26 ± 0.43 g/L concentration (Liu *et al*., 2023). Metal supplementation in fermentation medium has been proposed to favour H_2 and ABE production. Metal addition mostly involves compounds of cations documented to improve biofuels concentrations (Wang and Yin, 2021). Metals are generally cofactors and activators of enzymes involved in numerous biochemical pathways and promote microbial growth. Thus, their addition to fermentation medium offers a positive role in achieving an increased concentration of bioproducts. The perfect concentration of metals to be added is still a subject of debate amongst scientists. This stem up from differences in substrate and inoculum employed in the fermentation studies. Many authors only use the Plackett Burman experimental design to study the significance of metals on biohydrogen production. Thus, further experiments are needed to determine the optimal concentration of additives needed to achieve increased ABE and H_2 production. Zinc is the only documented cation favouring butanol production. According to Mukherjee *et al.* (2019), Zn^{2+} addition (0.001 g $ZnSO4.7H_2O$) into the fermentation gave 15.79 g/L butanol. Zinc acts as cofactor for butyrylaldehyde dehydrogenase & butanol dehydrogenase activities, thus, favouring butanol production. From our recent study involving the effects of metals on H_2 and ABE production using the Plackett burman design, Na^+ was found to support ABE fermentation while reducing hydrogen production alongside Zinc, which is known to favour ABE fermentation. There is therefore the need to properly study the effects of sodium on ABE fermentation and H_2 production. This research was conducted to determine the effects of low and elevated sodium concentrations on ABE fermentation and H_2 production with Fig and *C. pasteuranium* DSM 525 as substrate and microbial source, respectively.

MATERIALS AND METHODS

Substrate preparation and Inoculum preparation

Waste figs (*Ficus carica*) obtained from Izmir, Turkiye were pre-treated at microwaving conditions; substrate concentration 100 g L^{-1}, particle diameter 370.72 μm, pH 4.96, microwave power 253.67 W and treatment period of 50 mins, with total sugar concentration of 82 g L^{-1} obtained in line with previous report (Abibu and Karapinar, 2023). Butanol producing *C. pasteuranium* DSM 525 supplied by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) was used as microbial source. 10% v/v inoculum was achieved in growth media of 40 g/L glucose, 20 g/L $CaCO_3$, 10 g/L yeast extract and 0.1 g/L L-cysteine with incubation at 37°C, pH 7.0 and 48 hrs.

Experimental design and set up

The effects of sodium at low and elevated sodium ion concentrations were employed. Low (Na^+) concentrations (20 -100 mg/L)

and elevated concentrations (500 – 2000 mg/L) were tested. A control experiment with microwave pre-treated fig hydrolysate was also setup. Fermentation media composition with $CaCO_3$ (5.35 g/L), $FeSO4.7H_2O$ (0.019 g/L), $MgSO_4.7H_2O$ (0.2 g/), L-cysteine (0.1 g/L), Yeast extract (0.6 g/L) and $(NH_4)_2SO_4$ (2.0 g/L) was employed. Three hundred and ten milliliter (310 ml) capacity serum bottles with fermentation total working volume of 200 ml were used. The mixture was adjusted to an initial pH 6.5 followed by autoclave sterilization at 121°C for 15 min. This was followed with 10% v/v inoculum transfer into the sterilized fermentation composition. Anaerobiosis was ensured with passing the experimental serum bottles under nitrogen gas from bottles' head space before incubation at 37°C and 100 rpm rotating speed. The study lasted for 120 hrs. Ten (10) ml culture aliquots were collected daily for solvent analysis.

Analytical Methods

Daily hydrogen production from the experimental set-ups were carefully monitored via taking gas samples through the head space of the bottles and also determining the total gas volume by water displacement method (Tosuner *et al.*, 2019). Agilent Gas Chromatograph (1100 series) with TCD connected to ChemStation (a computer software) was used for percentage hydrogen determination (Eker and Erkul, 2018). The H_2 production rate in this study represents the overall cumulative H_2 production per reactor volume in 1 h (mL H_2 Lreactor volume_1 h-1).

pH range 6.5-7 was ensured throughout the study. Daily collection of samples was done for seven (7) days. The Dubois method (Dubois *et al.*, 1956) was used for the total sugar analysis while the high performance liquid chromatography HPLC (Agilent 1200 series, USA) was used for organic acid analysis.

The Plackett Burman experimental design was used for the effects of metals on ABE fermentation and biohydrogen production (Table 2) - Abibu and Karapinar, (2024).

Table 2: Plackett-Burman actual experimental points for 11 metals

Run order	Co^{2+}	Cu^{2+}	Mn^{+}	Fe^{2+}	Na^{+}	Mg^{2+}	Zn^{2+}	Ca^{2+}	K^{+}	Mo^{3+}	B^{3+}
						mg L^{-1}					
1	0.9	0.1	300	10	16	41	0.4	40	1000	0.003	16
2	0.9	5	0.3	1.4	11	400	0.4	100	1000	0.003	16
3	0.9	5	0.3	10	16	400	0.4	40	577	0.5	0.8
4	0.005	0.1	0.3	1.4	11	41	0.4	40	577	0.003	0.8
5	0.005	0.1	0.3	10	11	400	50	40	1000	0.5	16
6	0.9	0.1	0.3	1.4	16	41	50	100	577	0.5	16
7	0.9	5	300	1.4	11	41	50	40	1000	0.5	0.8
8	0.9	0.1	300	10	11	400	50	100	577	0.003	0.8
9	0.005	5	0.3	10	16	41	50	100	1000	0.003	0.8
10	0.005	5	300	1.4	16	400	50	40	577	0.003	16
11	0.005	0.1	300	1.4	16	400	0.4	100	1000	0.5	0.8
12	0.005	5	300	10	11	41	0.4	100	577	0.5	16

RESULTS AND DISCUSSION

The ABE fermentation and biohydrogen production are two microbial mediated processes with similar production pattern. Varieties of lignocellulosic substrates have been employed in studying these bioproducts. However, this is the second known study that studied ABE and biohydrogen production using fig (*Ficus carica*) as fermentation's carbon source after the study conducted by Abibu and Karapinar (2024) on the effects of metals on ABE fermentation and biohydrogen production. Butanol (produced from ABE fermentation) and biohydrogen are important biofuels established to have beneficial physical and chemical characteristics countering the concerns associated with fossil fuels.

Fig (*Ficus carica*) is an edible meditarenean fruit rich in C6 sugar. It is lignocellulosic in nature and serves as an excellent substrate for fermentation. Previous work on the fermentable sugar production from fig via autoclaving and microwaving pre-treatment revealed that a total sugar concentration of ≥ 80 g/L is obtainable from microwave hydrolysis of fig wastes (Abibu and Karapinar, 2023). Also, fig contains numerous macro and micro nutrients responsible for its health benefits. Of all elements present in fig, only calcium, zinc and magnesium have scholarly work been conducted on them for their roles in ABE fermentation. Zinc has been the only element reported to favour butanol production in the ABE fermentation process. Thus, the need for a Plackett burman design was necessary to test other elements present in fig to understand their effects on ABE fermentation and biohydrogen production. Alongside zinc, sodium and molybdenum were found to have a strong positive significance in achieving a higher butanol production. This was the only known study that reported this novelty (Abibu and Karapinar, 2024). The Placket Burman only gives an idea of the significance based on the range of independent factors subjected to the experimental analysis. A further experiment study is always necessary to determine the optimal concentrations of these factors to achieve best results. Sodium (Na+) was selected as the target element of this study because it has a positive significance for ABE fermentation and an insignificant effect on hydrogen production rate from our previous work (Abibu and Karapinar, 2024). Biobutanol and biohydrogen share a common biochemical pathway. The dehydrogenase and hydrogenase enzymes are respectively, responsible for biobutanol and biohydrogen production. By implication, the two bioproducts exhibit an inverse relationship in their interaction despite having a common biochemical production pathway. Thus, it is worth embarking on a study that assures present and future researchers whether the Na+ naturally present in hydrolyzed fig or the maximum Na+ concentration (16 mg/L) tested in previous studies (Abibu and Karapinar, 2024) are enough to increase butanol production concentration, while reducing hydrogen production or the need for an external addition of sodium is necessary. The determination of optimum Na+ concentration for best results is also necessary. Thus, the need to study the effect of Na+ at low and elevated concentrations.

The role of sodium in the ABE fermentation and biohydrogen production can be likened to the function of electron mediators like Methyl viologen and Neutral red in ABE fermentation. Methyl viologen accelerates acetyl CoA conversion into butyryl CoA, thus, increasing butyric acid and butanol production. Methyl viologen as an electron me-

diator improves butanol yield by increasing NADH levels and diverting biochemical pathway to favour butanol rather than hydrogen, which reduces NADH to favour its production (Bao *et al*., 2021).

Cumulative hydrogen production (ml) upon 120 hrs of anaerobic fermentation for the low concentrations (20, 40, 60, 80 and 100 mg/L) of Na^+ tested were 566.37, 681.84, 440.25, 421.62 and 545.05ml, respectively. Highest and lowest cumulative hydrogen were observed in 40 mg/L and 80 mg/L, respectively. Cumulative hydrogen production increases up till 40 mg/L (Figure 1). Above this concentration, 35-40% reduction in cumulative hydrogen production was recorded. Similarly, hydrogen production rates of 46.65, 56.63, 28.74, 26.79 and 50.98 ml/L/hr were recorded for 20, 40, 60, 80 and 100 mg/L respectively. In the same vein, highest and lowest hydrogen production rates were found in 40 mg/L and 80 mg/L, respectively. This shows that 40 mg/L is the highest Na^+ concentration needed to maximize hydrogen production rate and cumulative hydrogen formation. For the high concentrations (500, 1000, 1500 and 2000 mg/L) of Na^+ tested (Figure 2) cumulative hydrogen production obtained were at 35 – 57% reduction when compared with the highest cumulative hydrogen production obtained at 40 mg/L. Thus, low Na^+ (up till 40 mg/L) concentrations favour cumulative hydrogen production and hydrogen production rates. The role of sodium in hydrogen production from food waste was studied by Cao and Zhao (2009). Na (5.03–28.7 g/l) was the range tested in the study. It was discovered that hydrogen production increased within 5.03–14.41 g/L Na concentration. However, at concentrations > 20 g/L, hydrogen production declined possibly due to high osmotic pressure, weakening the activity of microorganisms. Similarly, Taheri *et al*. (2018) studied the role of NaCl on hydrogen production at concentration (0.5 - 30 g/L). The highest hydrogen production results were obtained at 0.5 and least at 30 g/L NaCl. Thus, it is easy to affirm the negative role of Na on hydrogen production at higher concentrations. Cao and Zhao, (2009) also concluded that increasing sodium concentrations also had a direct relationship with microbial lag phase of growth. Microbial tolerance to salinity varies; most halophiles are not commonly employed as inoculums in biofuel studies. Usually, microorganism exposed to low sodium ion concentrations easily progress to the logarithmic phase of growth (easily overpowering the lag phase) with timely production of organic acid and progression to the solventogenic stage of the ABE fermentation than those exposed to high saline concentrations. This indicates that ABE solvent production may be favoured at high saline concentrations (which reduce hydrogen production). This therefore reinforces the possibility of achieving the main objective of this study. Furthermore, low concentrations of acetic and butyric acids which are precursors for acetone and butanol production in the solventogenic stage of ABE fermentation implies prompt conversion of these organic acids into acetone and butanol respectively.

Hao *et al*., (2006) studied the effects of Na+ (0 - 16,000 mg/L) on hydrogen production from sucrose. The study had the range 1000 – 2000 mg/L as the best concentration range for maximum cumulative hydrogen production, hydrogen yield and hydrogen production rates. Butyric acid production, which is the precursor organic acid for the production of butanol (in the ABE fermentation process) was found to decrease with increasing Na^+ concentration. Thus, the work of

Hao *et al.* (2006) showed that microorganisms with hydrogen production potential rapidly utilize sucrose for biohydrogen production at lower Na^+ concentrations and experiences difficulty in adapting to high Na^+ concentrations. This is because most hydrogen producing bacteria are not halophiles.

The role of Na (0.01-40 g/L) on *Clostridium acetobutylicum* ATCC 824 activity for ABE fermentation was studied by Zabihi *et al.*, (2019). It was reported that increasing NaCl concentration adversely affects bacterial performance. Hence, low ABE products result. However, Zhao *et al.* (2016) affirmed the negative role of Na on *Clostridium acetobutylicum* ATCC 824 for ABE fermentation at high concentrations. It was the opinion that the adverse effect only has to do with the acidogenesis stage of ABE fermentation. According to Zhao *et al.* (2016), increasing sodium ion concentrations result in ATP deposition in membranes and NADH accumulation. These have a resultant effect on the ABE fermentation biochemical pathway, and hence, possibility of a reduced ABE fermentation products. Furthermore, Zhao *et al.* (2016) conducted a metabolomics study of high sodium response in *Clostridium acetobutylicum* ATCC 824 during ABE fermentation. The study revealed that elevated sodium concentrations, though impede cellular performance, however, possess a comparative metabolic activity advantage via cell robustness and potential of increased ABE solvents production.

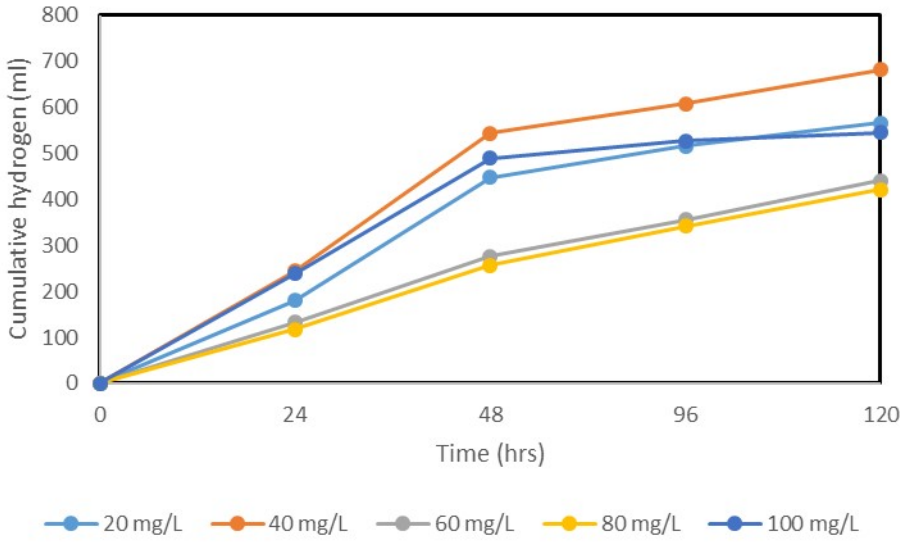

Figure 1: Variation of cumulative hydrogen volume with time at low Na^+ concentrations

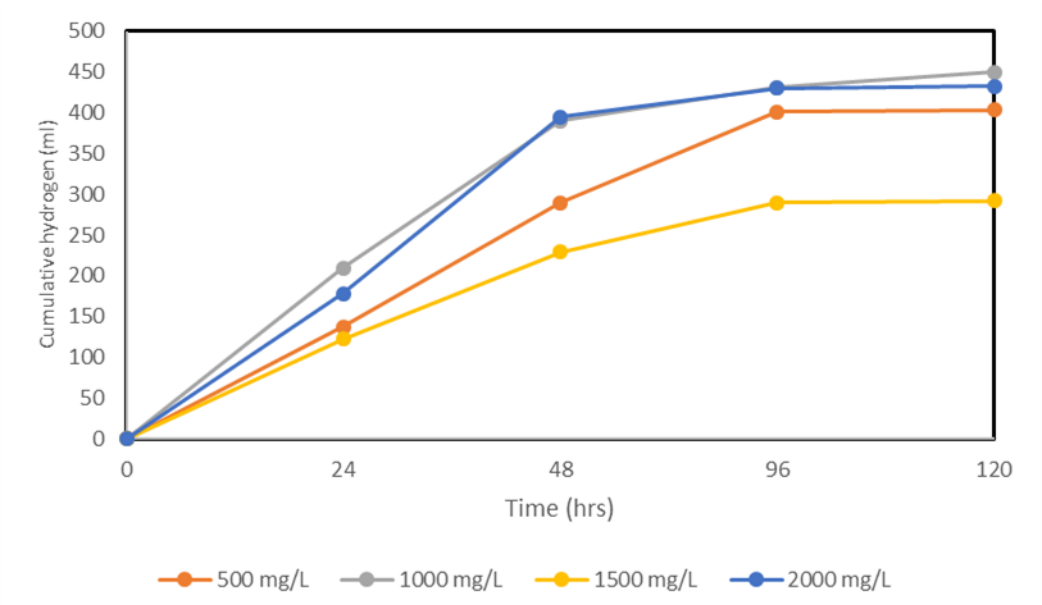

Figure 2: Variation of cumulative hydrogen volume with time at high Na^+ concentrations

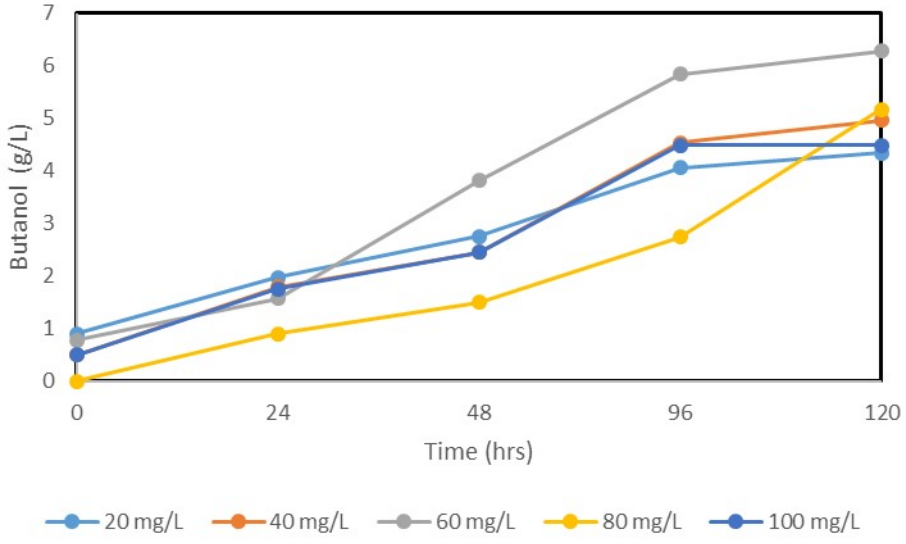

Figure 3: Variation of butanol production with time at low Na^+ concentrations

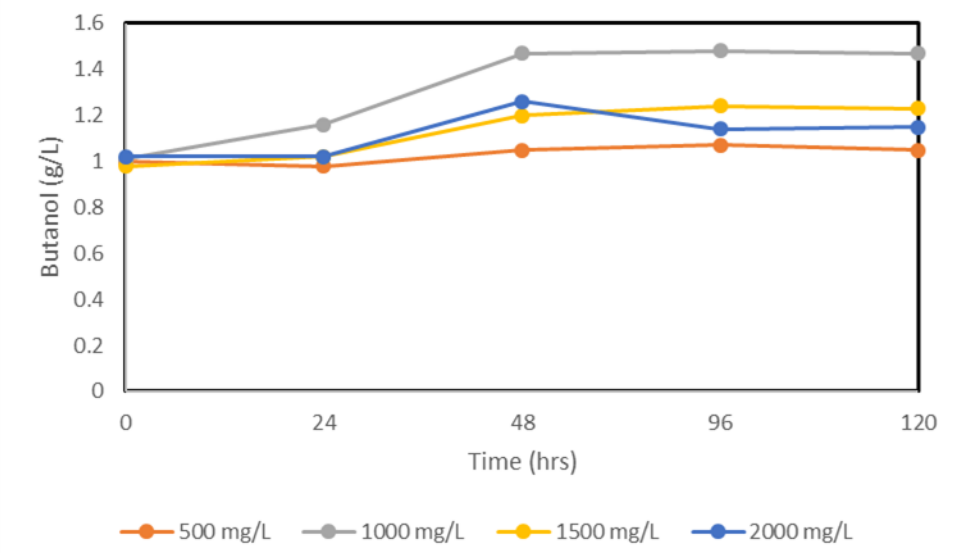

Figure 4: Variation of butanol production with time at high Na^+ concentrations

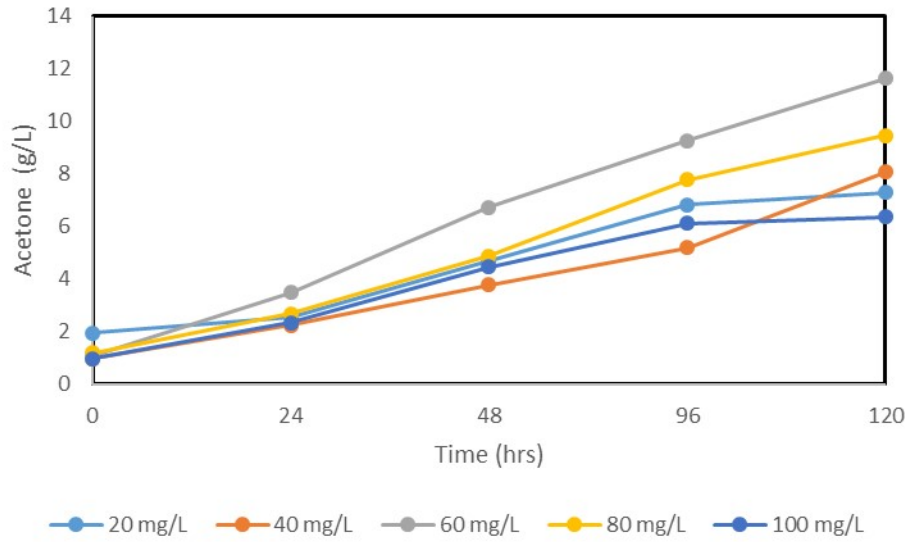

Figure 5: Variation of acetone production with time at low Na^+ concentrations

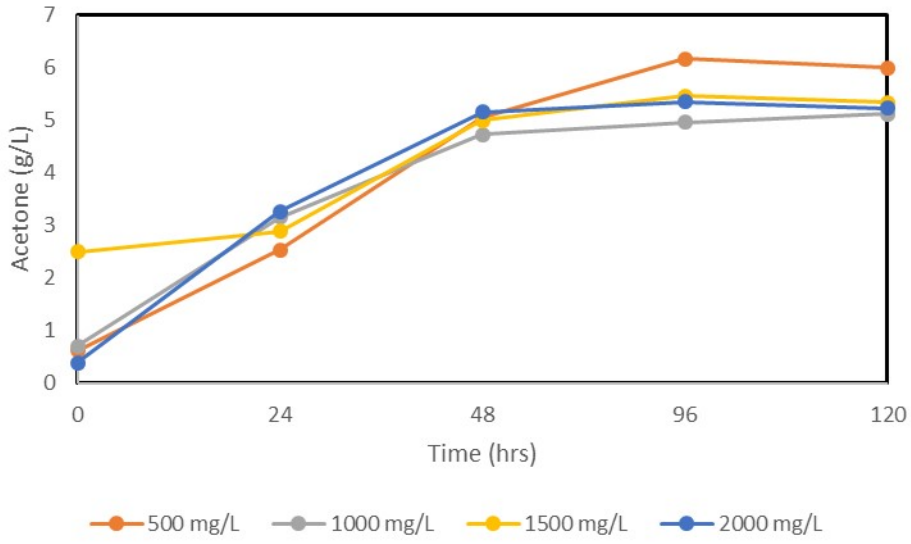

Figure 6: Variation of acetone production with time at high Na^+ concentrations

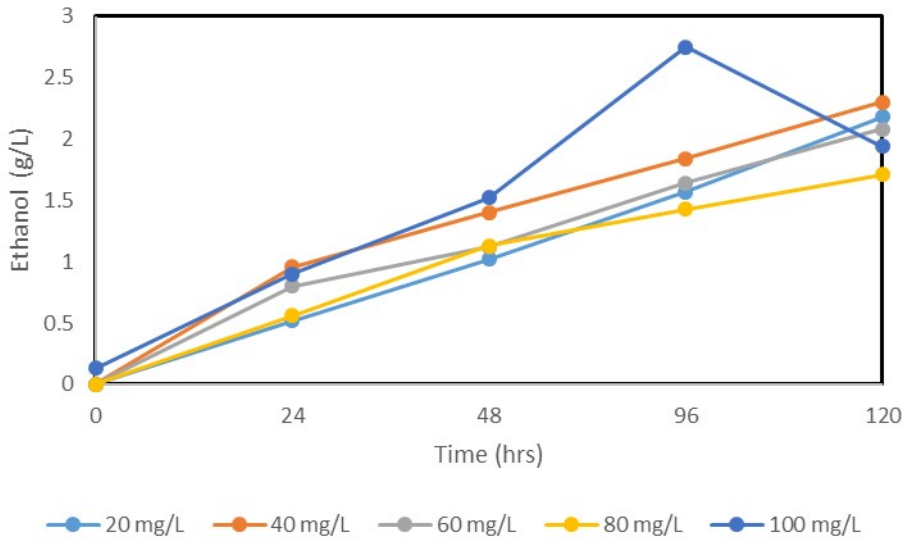

Figure 7: Variation of ethanol production with time at low Na^+ concentrations

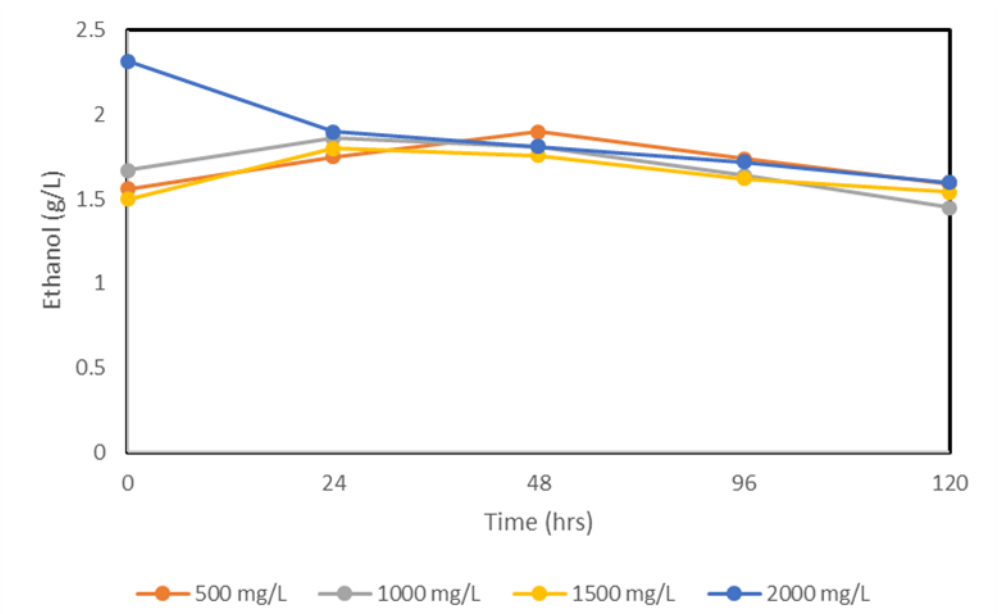

Figure 8: Variation of ethanol production with time at high Na^+ concentrations

Final butanol production (g/L) upon 120 hrs of anaerobic fermentation for the low concentrations (20, 40, 60, 80 and 100 mg/L) of Na^+ tested were 4.34, 4.97, 6.28, 5.17 and 4.49 respectively. Highest and lowest butanol productions were recorded in 60 mg/L and 20 mg/L respectively (Figure 3). Increasing butanol production was observed within 20 - 60 mg/L after which production declines (Figure 4). Differences in butanol production at these extreme levels were around 0.4 g/L between the highest and lowest butanol production (for extreme levels). Thus, no appreciable biobutanol production difference existed amongst the elevated concentrations tested. Thus, elevated Na^+ concentrations are unnecessary and not favourable for butanol production. This also shows that 60 mg/L is the highest Na^+ concentration needed to achieve maximum butanol production. Researchers are so encouraged not to exceed 100 mg/L Na^+ fermentation medium supplementation. This is because 100 mg/L Na^+ also gave an appreciable butanol production compared with low concentrations reported by many authors.

Acetone production in the ABE fermentation process has been a major issue for researchers in this field. High concentrations of acetone remain the major challenge for increased butanol concentration by solventogenic strains of *Clostridium* in the ABE fermentation. Little success has been achieved regarding reducing the acetone yield during ABE fermentation. *C. sporogenesis*, a non-neurotoxigenic *Clostridial* strain produces ethanol and butanol as final ABE products, excluding acetone (Gottumukkala *et al.*, 2013). Although *C. sporogenesis* is not considered an efficient producer of butanol in comparison with other *Clostridial* strains, it produced 5.52 g/ L of butanol under optimized conditions

which is one of the highest reported for C. sporogenesis (Gottumukkala *et al*., 2013). Also, a synthetic 2, 3-butanediol synthesis pathway for *Clostridium acetobutylicum* can act as an NADH-compensating module by eliminating acetone without manipulating any of the solventogenesis genes and without causing significant undesired effects (Croux *et al*., 2016). From this study, final acetone production (g/L) upon 120 hrs of anaerobic fermentation for the low concentrations (20, 40, 60, 80 and 100 mg/L) of Na^+ tested were 7.31, 8.08, 11.63, 9.46 and 6.37, respectively. Highest and lowest butanol productions were recorded in 60 mg/L and 100 mg/L, respectively. Increasing acetone production was observed within 20 - 60 mg/L after which production declines (Figure 5). This result gave an interesting approach of achieving highest butanol and acetone at the same concentration (60 mg/L). Indeed, a biochemical tussle must have occurred to have the acetone butanol ratio to be less than 2 (<2) despite not making use of acetone free inoculum. This is indeed a success as previous experiments conducted by us gave a higher acetone butanol ratio. Final acetone production (g/L) for 500, 1000, 1500 and 2000 mg/L of Na+ tested were 6.00, 5.13, 5.34 and 5.23, respectively (Figure 6). This showed that high Na^+ concentrations proved capable of achieving almost 50% reduction in acetone production in the ABE fermentation when compared with the highest recorded at 60 mg/L.

Final ethanol production (g/L) upon 120 hrs of anaerobic fermentation for the low concentrations of Na^+ tested was within a range of 1.71 to 2.30 (Figure 7). Ethanol production for the high concentrations of Na^+ tested was within a range of 1.45 to 1.49 (Figure 8). It can be concluded that higher concentrations of Na^+ does not exhibit a major effect on ethanol production has observed for biohydrogen, butanol and acetone.

Scholarly works from previous authors in the ABE fermentation studies have reported different concentrations and yield. The fact still remains that all new studies in this field, irrespective of the production concentrations reported provides either insight into areas in which new researches can be channeled or components that needs to be excluded from fermentation medium (i.e reduces butanol production concentration). Most authors employ biotechnologically engineered microbial strains in their studies to improve butanol production. However, this research made use of an inoculum (*C. pasteuranium* DSM 525) with butanol and biohydrogen producing potential was employed and to give the two bioproducts equal opportunities to compete for an increased production concentration. Zinc (which is the main element favouring butanol production) widely employed in fermentation media by almost all authors in ABE fermentation study was specifically left out in the fermentation medium to fully understand the effect of Na^+ on ABE fermentation and biohydrogen production. In addition, protein source like meat extract was excluded from the fermentation medium. Only little amount (0.6 g/L) of yeast extract was employed. This was done to determine if the fig hydrolysate was enough to act as sugar and protein sources to the inoculum employed in the study. This was done to further compare the possibilities of an improved ABE fermentation and biohydrogen production with published work that employed yeast extract (3.0 g/ L) and meat extract (10.0 g/ L) as constituents in the fermentation medium (Abibu and Karapinar, 2024).

From this research, the mechanism of action of low Na^+ concentrations in the biosynthesis of butanol can be equated to the role of methyl viologen. Methyl viologen is detrimental to the hydrogenase enzyme (hydrogen-producing enzymes) by competing and overpowering reduced ferredoxin (FdH_2) for the active site of the hydrogenase enzyme, thus, providing an enabling condition for increased production of butanol. In addition, low Na^+ concentrations accelerate substrate utilization by microorganisms, thus, enhancing the release of enough fermentable sugar for butanol formation when compared with high Na^+ concentrations studied in this research. The possibility of NADH increase at low Na^+ concentrations cannot be ignored. Electron mediators like methyl viologen and neutral red also achieve high butanol concentrations by increasing NADH supply to inoculum involved in the biochemical pathway (Utesch *et al*., 2019), hence, the possibility of Na^+ as a crucial cofactor for enzymes involved in the metabolism of organic acids and solvents for the ABE fermentation process. Thus, this present work shows the significance of low Na^+ concentrations in achieving high concentrations of ABE products. It can be inferred that sodium ion naturally present in fig which gave a similar result with those obtained at high Na^+ concentrations produce ABE concentrations better than those reported by some authors. However, fermentation medium supplementation with Na^+ within a range of 40 - 100 mg/L stands the chance of achieving 600% increase in butanol when compared with the control. Thus, external addition of Na^+ is needed.

CONCLUSIONS

Production of valuable biofuels from lignocellulosic substrates has been well documented. However, its production from fig has not been extensively researched. Acetone, butanol, ethanol and biohydrogen are bioproducts capable of being produced from fig. Cations have been found to have effects on the aforementioned bioproducts. Sodium ion is one example of such cation significantly having a direct and inverse involvement in butanol and biohydrogen production respectively. Thus, it is important to study its effect at low and elevated concentrations. The results obtained from this study show that low sodium ion levels support increased production of ABE products when compared with the control experiment (>600 %) but inhibited biohydrogen production. High sodium ion levels gave results almost similar with the control experiment. Thus, elevated sodium ion levels are not encouraged when considering fermentation medium supplementation with sodium ion for improved ABE products. Sodium concentration of 60 mg/L has been recommended to future researchers as the optimum concentration to have high results of butanol, which is the most important bioproduct of the ABE fermentation process.

REFERENCES

Abibu, W.A., Karapinar, I. 2023. Optimization of pretreatment conditions of fig (Ficus carica) using autoclave and microwave treatments. *Biomass Conversion and Biorefinery,* 13: 11229-11243

Abibu, W.A., Karapinar, I. 2024. Effect of metals on simultaneous ABE fermentation and biohydrogen production from fig (Ficus carica) via Plackett-Burman experimental design. *Biomass Conversion and Biorefinery*, 14: 1305–1315. https://doi.org/10.1007/s13399-023-04886-3

Bao, T., Hou, W., Wu, X., Lu, L., Zhang. X., Yang, S.T. 2021. Engineering Clostridium cellulovorans for highly selective n-butanol production from cellulose in consolidated bioprocessing. *Biotechnology Bioengineering*, 118(7): 2703-2718. doi: 10.1002/bit.27789.

Cao, X., Zhao, Y. 2009. The influence of sodium on biohydrogen production from food waste by anaerobic fermentation. *Journal of Material Cycles and Waste Management*, 11(3): 244–250. doi:10.1007/s10163-009-0237-5

Croux, C., Lee, J., Raynaud, C., Saint-Prix, F., Gonzalez-Pajuelo, M., Meynial-Salles, I., Soucaille, P. 2016. Construction of a restriction-less, marker-less mutant useful for functional genomic and metabolic engineering of the biofuel producer Clostridium acetobutylicum. *Biotechnology Biofuels*, 9: 23.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.T., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356

Eker, S., Erkul, B. 2018. Biohydrogen production by extracted fermentation from sugar beet. *International Journal of Hydrogen Energy*, 43: 10645-10654

Ercisli, S., Tosun, M., Karlidag, H., Dzubur, A., Hadziabulic, S., Aliman, Y. 2012. *Plant Food and Human Nutrition*, 67: 271

Gottumukkala, L.D., Parameswaran, B., Valappil, S.K., Mathiyazhakan, K. 2013. Butanol production from rice straw by a non- acetone producing Clostridium sporogenes BE01. *Bioresource Technology*, 145:182-187

Goyal, Y., Kumar, M., Gayen, K. 2013. Metabolic engineering for enhanced hydrogen production: a review. *Canadian Journal of Microbiology*, 59(2): 59–78.

Hao, X., Zhou, M., Yu, H., Shen, Q., Lei, L. 2006. Effect of Sodium Ion Concentration on Hydrogen Production from Sucrose by Anaerobic Hydrogen-producing Granular Sludge. *Chinese Journal of Chemical Engineering*, 14(4): 511-517

Kumar, M., Goyal, Y., Sarkar, A., Gayen, K. 2012. Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. *Applied Energy*, 93: 193–204.

Liu, W., Zha, W., Yin, H., Yang, C., Lu, K., Chen, J. 2023. Integration of rare earth element stimulation, activated carbon adsorption and cell immobilization in ABE fermentation for promoting biobutanol production. *Chemical Engineering Processing - Process Intensification*. 184: 109306. 10.1016/j.cep.2023.109306

Mukherjee, M., Sarkar, P., Goswami, G., Das, D. 2019. Regulation of butanol biosynthesis in Clostridium acetobutylicum ATCC 824 under the influence of zinc supplementation and magnesium starvation. *Enzyme and Microbial technology*, 129: 109352. https://doiorg/101016/jenzmictec201905009

Taheri, A., Mohammadi, S., Rezayati-Zad, Z. 2018. Ultrasensitive and selective non-enzymatic glucose detection based on Pt electrode modified by carbon nanotubes@graphene oxide/ nickel hydroxide-

Nafion hybrid composite in alkaline media. *Progress in Chemical and Biochemical Research*, 1: 1-10. 10.29088/SAMI/PCBR.2018.1.110.

Tosuner, Z.V., Taylan, G.G., Ozmihci, S. 2019. Effects of rice husk particle size on biohydrogen production under solid state fermentation. *International Journal of Hydrogen Energy*, 44: 18785-18791

Utesch, T., Sabra, W., Prescher, C., Baur, J., Arbter, P., Zeng, A.P. 2019. Enhanced electron transfer of different mediators for strictly opposite shifting of metabolism in *Clostridium pasteurianum* grown on glycerol in a new electrochemical bioreactor. *Biotechnology and Bioengineering*, 116 (7): 1627–1643

Veza, I., Said, M.F.M., Latiff, Z.A. 2019. Progress of Acetone-Butanol-Ethanol (ABE) as biofuel in gasoline and diesel engine: a review. *Fuel Processing Technology*, 196: 106179

Wang, J., Yin, Y. 2021. Clostridium species for fermentative hydrogen production: An overview. *International Journal of Hydrogen Energy*, 46 (70): 34599–34625. doi:10.1016/j.ijhydene.2021.08.052.

Zabihi, R., Mowla, D., Karimi, G., Setoodeh, P. 2019. Examination of the impacts of salinity and culture media compositions on *Clostridium acetobutylicum* NRRL B-591 growth and acetone-butanol-ethanol biosynthesis. *Journal of Environmental Chemical Engineering*. https://doi.org/10.1016/j.jece.2018.102835.

Zhao, X., Condruz, S., Chen, J., Jolicoeur, M. 2016. A quantitative metabolomics study of high sodium response in *Clostridium acetobutylicum* ATCC 824 acetone-butanol-ethanol (ABE) fermentation. Scientific Reports, 6(1). doi:10.1038/srep28307

(Manuscript received: 4th April, 2024; accepted: 20th June, 2024).