

BATCH FERMENTATION SYSTEM FOR BIOHYDROGEN PRODUCTION BY
Klebsiella sp. ABZ11 ISOLATED FROM ANTARCTICA

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DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

The study of cold-adapted bacteria for biohydrogen production has attracted much interest in the last few decades due to the lower energy input required during the fermentation process. However, an extended lag phase of growth and slow metabolic rate of the bacteria remain the obstacles for the process to be feasible, particularly for obligate psychrophilic and psychrotolerant bacteria. Bacteria with the oxygen-tolerant ability are also favourable for large-scale fermentation. Thus, there is a need to find oxygen-tolerant bacteria capable of producing biohydrogen at mesophilic temperature. In this study, Antarctic soil and seawater samples were used for bacterial isolation, before being screened for biohydrogen production ability. Twelve bacteria were successfully isolated and six were found capable of producing biohydrogen. The bacterium with the highest biohydrogen production was characterised. The optimum physicochemical parameters, such as temperature, pH and carbohydrate concentration were determined using one-factor-at-a-time (OFAT) approach. Appropriate nitrogen source, temperature tolerance and the effects of dissolved oxygen on the growth and biohydrogen productivity were also investigated. Precise optimal factors for biohydrogen productivity were then examined using the three-level factorial design of Response Surface Methodology (RSM). Identification of bacterium with the highest biohydrogen production showed that it was closely related to *Klebsiella pneumoniae* with 99% similarity based on the 16S rRNA analysis. The bacterium was therefore designated as *Klebsiella* sp. ABZ11. It was a Gram-negative bacillus, with no capsule detected and grew at a temperature range of 20-40°C, and exhibited 95% uptake of dissolved oxygen in two hours. Screening using OFAT suggested that the optimum conditions for biohydrogen production were 30°C, an initial pH of 6.5, and with glucose supplemented with concentration of 10 g/L. The bacterium utilised various types of carbon and nitrogen sources for biohydrogen production but preferred glucose as the carbon source and beef extract as the nitrogen source. Further optimisation using RSM revealed that the highest biohydrogen productivity (110.15 mol/L) was obtained at 33.5°C, with an initial pH of 6.75 and glucose concentration of 9.15 g/L. For each gram of glucose supplied, the yield for biohydrogen and cell-biomass was 122 mol/L/g and 0.87 g, respectively. Kinetics showed that the bacterium used more of the glucose for biohydrogen production than for biomass formation in the fermentation process. A scale-up culture using the optimised conditions recorded a biohydrogen production of 137.56 mol/L in 36 h with a cumulative yield of 533.51 mol/L. In conclusion, batch fermentation using *Klebsiella* sp. ABZ11 under mesophilic temperature was found to have decreased lag phase of growth and increased metabolic rate, thereby influencing faster and higher biohydrogen production.

ABSTRAK

Kajian bakteria adaptasi sejuk untuk pengeluaran biohidrogen telah menarik minat banyak pihak sejak beberapa dekad yang lalu berikutan input tenaga yang rendah diperlukan semasa proses fermentasi. Walaubagaimanapun, fasa lamban pertumbuhan bakteria yang panjang dan kadar metabolisma yang perlahan menjadi halangan utama bagi proses ini dilaksanakan, terutamanya bagi bakteria psikrofilik obligat dan psikrotoleran. Bacteria dengan keupayaan toleransi oksigen juga lebih sesuai untuk fermentasi berskala besar. Oleh itu, terdapat keperluan untuk mencari bakteria toleransi oksigen yang mampu menghasilkan biohidrogen pada suhu mesofilik. Dalam kajian ini, sampel tanah dan air laut Antartika digunakan untuk pengasingan bakteria, sebelum disaring berdasarkan keupayaan penghasilan biohidrogen. Dua belas bakteria telah berjaya diasingkan dan enam didapati mampu menghasilkan biohidrogen. Bacteria yang menghasilkan biohidrogen tertinggi dicirikan dan parameter fizikokimia optimum, seperti suhu, pH dan kepekatan karbohidrat ditentukan menggunakan kaedah satu-faktor-pada-satu-masa (OFAT). Sumber nitrogen yang sesuai, toleransi suhu dan kesan oksigen terlarut terhadap pertumbuhan dan produktiviti biohidrogen juga dikaji. Faktor optimum yang tepat untuk produktiviti biohidrogen turut diperiksa dengan menggunakan reka bentuk faktorial tiga-tahap Kaedah Gerakbalas Permukaan (RSM). Bacteria yang menghasilkan biohidrogen tertinggi didapati berkait rapat dengan *Klebsiella pneumoniae* sebanyak 99% persamaan berdasarkan analisis 16S rRNA. Bacteria itu kemudiannya dinamakan sebagai *Klebsiella* sp. ABZ11. Bacteria ini adalah basilus Gram-negatif, tanpa kapsul dan hidup dalam julat suhu 20-40°C, dengan kadar pengambilan oksigen terlarut sebanyak 95% dalam masa dua jam. Penyaringan OFAT mencadangkan bahawa keadaan optimum untuk penghasilan biohidrogen ialah 30°C, pada pH awal 6.5 dan kepekatan glukosa 10 g/L. Bacteria ini menggunakan pelbagai jenis sumber karbon dan nitrogen untuk penghasilan biohidrogen, tetapi lebih memilih glukosa sebagai sumber karbon dan ekstrak daging sebagai sumber nitrogen. Pengoptimuman lanjut menggunakan RSM menunjukkan bahawa penghasilan biohidrogen tertinggi (110.15 mol/L) diperoleh pada 33.5°C, dengan pH awal 6.75 dan kepekatan glukosa 9.15 g/L. Kajian kinetik menunjukkan bahawa bakteria ini lebih banyak menggunakan glukosa untuk menghasilkan biohidrogen berbanding untuk membentuk biojisim dalam proses fermentasi. Sebanyak 122 mol/L/g biohidrogen dan 0.87 g biojisim dihasilkan bagi setiap gram glukosa. Pada kesimpulannya penyelidikan skala besar pada keadaan yang optimum menunjukkan penghasilan biohidrogen sebanyak 137.56 mol/L dalam 36 jam dengan hasil kumulatif sebanyak 533.51 mol/L. Fermentasi kelompok menggunakan *Klebsiella* sp. ABZ11 di bawah suhu mesofilik membuktikan dapat memendekkan fasa lamban pertumbuhan dan meningkatkan kadar metabolisma, sehingga mempengaruhi pengeluaran biohidrogen yang lebih cepat dan tinggi.

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LIST OF ABBREVIATIONS

DO	-	Dissolve oxygen
CO ₂	-	Carbon dioxide
CO	-	Carbon monoxide
NOX	-	Nitrogen oxide
CCD	-	Central Composite Design
SO ₂	-	Sulphur oxide
CH ₄	-	Methane
PEM	-	Proton Exchange Membrane
ATP	-	Adenosine Triphosphate
GC/TCD	-	Gas Chromatography/ Thermal Conductivity Detector
SEM	-	Scanning Electron Microscopy
DCW	-	Dry Cell Weight
OD ₆₀₀	-	Optical Density
DNA	-	Dioxyribonucleic acid
ANOVA	-	Analysis of Variance
SPSS	-	Statistical Analysis
rRNA	-	Ribosomal ribonucleic acid
kb	-	Kilobyte
BLAST	-	Basic Local Alignment Search

LIST OF SYMBOLS

δ	-	Minimal error
D, d	-	Diameter
F	-	Force
v	-	Velocity
p	-	Pressure
I	-	Moment of Inertia
r	-	Radius
Re	-	Reynold Number

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

Energy is very important owing to its role as critical input in the creation of goods and services and in other human activities. Before the emergence of industrialised societies, the balance energy going into and out of the atmosphere was at equilibrium, as it was mainly recirculated between the naturally occurring plants and animals. This balance, however, was altered as a result of man's reliance on fossil fuels (coal, petroleum, and natural gas) as the major sources of energy for domestic and industrial uses (see figure 1). This energy imbalance is brought about due to the increasing emission of toxic gases such as CO₂, NO_x, CO and sulphur into the atmosphere (Bächtold, 2018). This indicates that global energy sources are largely dependent on coal, petroleum and natural gas.

Excessive reliance on these sources exposed the environment to dangers such as ozone depletion and drought. It also emphasizes the need to explore biological sources of renewable energy that are cost-effective and pose a minimum possible danger to the environment. Figure 1.1 presents the various energy sources and their percentage contribution to the global energy supply: Biohydrogen production and associated sources of energy supply constitute an insignificant 1% of the global energy supply. This status quo is not due to lack of potentials in the use of hydrogen as a fuel source, but due to the emergent state of research and development in the field. There is, therefore, the need for upscaling research and development activities in this field of enquiry.

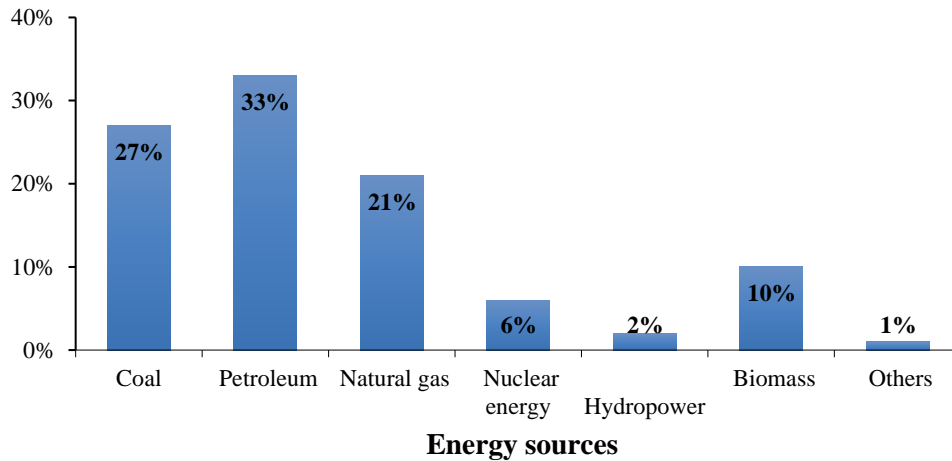


Figure 1.1 Energy sources and their contribution to global supply

At present, the world satisfies 80% of its energy needs from fossil sources that are associated with problems of greenhouse gas emission, climate change and environmental pollution (Xu et al, 2018). Gasification of coal and natural gas, as well as the burning of petroleum oil, release large quantities of greenhouse gases (e.g., black carbon and ozone) into the atmosphere. These gases are toxic and heat up the atmosphere. Also, the greenhouse gases return as sulphur back to the ecosystem in the form of acid rain, damaging building-roofs and other iron-containing material by making them rust.

Moreover, the gases have long-term adverse effects on human health, including respiratory, cardiovascular and cerebrovascular infections such as asthma, lung, colon and breast cancer, and heart diseases (Patz et al, 2005; Haines et al, 2009). In addition, toxic gases from fossil fuel exacerbate climate change problems directly linked to drought and famine. Currently, 800 million people have been estimated to be malnourished as a result of climate change-induced problems (McMichael, 2017). To mitigate the foregoing environmental, economic, and social problems, scientists are on the relentless search for better and safer sources of energy. One source that has so far shown promising potentials but has not been fully investigated is hydrogen.

Hydrogen is a timely option for fossil fuel owing to its high energy yield per unit mass. It yields 122kJ per gram, which is 2.75 times higher than the conventional fossil fuels (Singh and Wahid, 2015). Moreover, fermentative hydrogen production as

energy source generates water (H₂O) and a small amount of NO_x as by-products. The more fossil fuels are replaced with hydrogen, the greater will be the reduction of greenhouse gas emission (Huang and Tan, 2014). In fact, science has moved from a justification of hydrogen as an energy source to looking into ways hydrogen could be produced in commercial quantities and used on a sustainable basis. It is to contribute to this global scientific drive that this study was carried out. We investigated and reported on hydrogen production through microbial fermentation using bacteria isolated from a naturally low temperature ($\pm 5^{\circ}\text{C}$) environment.

Hydrogen as biofuel can be produced through microbial fermentation of naturally available waste materials. These materials are diverse, cheaper and eco-friendlier compared to the energy from fossil fuel sources. Thus, biological methods of generating hydrogen as biofuel offer a better solution to a wide range of environmental problems associated with the conventional methods that rely on fossil sources. Biohydrogen production is not only a credible alternative to energy-need satisfaction but also a potent environmental conservation strategy for the reduction of wastes, a safer way for the degradation of many toxic organic substrates, and the promotion of a healthier atmosphere.

The success of biohydrogen generation through biological process is much dependent on the efficient microorganism in the system. Different types of bacteria from various environments have been evaluated for biohydrogen production. However, the efficiency of these bacteria in hydrogen production remains a challenge. This is due to their slow metabolic rate which negatively affects their capability to breakdown substrates for hydrogen production. This is evident in a prolonged carbohydrate uptake and hydrogen production under fermentative process demonstrated by this strain of bacteria (Gupta et al, 2016). Thus, studies are still focusing on the search for highly efficient bacteria from different environments for biofuel production.

The polar environment is inhabited by many organisms including bacteria, yeasts, fungi and algae. These microorganisms have undergone physiological adaptation and acquired specific enzymes needed to survive in the harsh Antarctic

environment. This adaptability greatly boosts their biochemical efficiency and activity similar to mesophilic microorganisms (De Maayer et al, 2014). They are often explored for commercial production of drugs, detergents and fertilizer due to possession of enzymes such as proteases, lipases, α -amylases, cellulases and β -galactosidase (Saxena, 2015). However, less attention has been given to the use of cold-adaptive bacteria in the generation of energy as an alternative replacement for fossil fuels. The increasing dangers of climate change and its potentially disturbing values warrants concerted effort at exploiting every available and environmentally friendly alternative energy source. Polar bacteria offer such an alternative

The potential of polar bacteria in hydrogen production is strategic in renewable energy production. Thus, they are viewed as a good biological source of renewable energy. Most importantly, the energy-saving potential and activity under low temperature associated with polar bacteria are characteristics that could enhance renewable energy production and its sustainability. Only a few psychrophilic bacteria from the polar environment have been investigated for biohydrogen production. However, the slow metabolic rate and carbohydrate uptake by psychrophilic bacteria reported remains problematic in fermentative hydrogen production. Specifically, psychrophilic bacteria are known for prolonged production start-up and carbohydrate take-up (Alvarez-Guzmán et al, 2016). Hence, the need for a cold-active bacterium with hydrogen production potential under moderate temperature for improved metabolic activity.

Generally, microorganisms grow very slowly at low temperatures. Low temperatures affect the metabolic rate and substrate degradation capabilities of microorganisms, resulting in low biogas yield in a typical fermentation process. Studies have shown that temperature does not only affect bacterial growth but will also affect the timeframe for growth. For instance, Dobrić and Bååth, (2018) investigated the effect of temperature on lag period and exponential growth of bacteria. They found that the lag phase was around 12 hours at 25°C and 30°C. However, the lag phase increased to almost 200 hours at 0°C. Since growth involved substrate intake to build cell components, this implies that temperature also impacts the rate of substrate breakdown and metabolic rate of enzymes involved in the process.

Similarly, Alvarez-Guzman et al, (2016) observed lag-phase of 20 h, 50 h and 20 h for Antarctic psychrophilic G088 strain with hydrogen production starting after 43 h, 21 h and 34 h for glucose, fructose and sucrose respectively. This prolonged growth lag-phase and hydrogen production start-up are linked to the fermentation at 20°C that affected the metabolic rate and breakdown of the substrates. Moderate temperature would have more influence on biogas productivity than low temperature since every 10°C increase in temperature has been shown to double the microbial growth and their metabolic rate (Robador et al, 2016). In support of this, Deepanraj, et al, (2015) reported high biogas production of 7556 ml with better biodegradation efficiency and reduced lag phase at 50°C compared to 30°C and 40°C in their investigation.

Biogas yield under a temperature above 20°C would be more favourable for biogas production due to the increase in the doubling time. Thus, ambient temperature condition can stimulate rapid production of hydrolytic enzymes by psychrotolerant bacteria for fast substrate degradation in order to generate more energy in the fermentative process (Morgan-Kiss et al, 2018; Saratale et al, 2018). This means that moderate temperature can be used to increase the productive capabilities of cold-active bacteria. Microorganisms with such capabilities are the psychrotolerant strains that proliferate in the polar environment and in seawater where the influx and ingestion of organic matter are greater. However, psychrophiles may not have such ability due to a mean cell turnover of about 1 year and a restricted growth temperature (Wang et al, 2018; Robador et al, 2016).

Psychrotolerant bacteria can thrive at a mesophilic temperature because of their natural physiological characteristics that give them the capability to survive at various temperature conditions and oxygen concentration. Thus, the exploration of hydrogen productivity of psychrotolerant bacteria is important because of their bioactivity at moderate temperature, which has been shown to inactivate psychrophilic strain in fermentative process. It is obvious that they will improve biohydrogen production by greatly enhancing hydrogenase activity compared to psychrophiles due to the influence of the moderate fermentation temperature. Hence, contributing to improvement of low

biohydrogen yield that has been the main constrain for its industrialization process (Xiao et al, 2013).

Psychrotolerant bacteria survive the harsh condition of polar environment and more abundant in that habitat. Oxygen tolerance (Sandle et al, 2013), ability to degrade vast nutrients and grow in vast pH condition, exchange of traits with mesophilic bacteria through plasmid (Dziewit and Bartosik, 2014). These potentials have not been intensively tapped in fermentative hydrogen production. This study is aimed at tapping these potentials of psychrotolerant bacteria to improve biohydrogen production through utilisation for biogas production at ambient condition. It is expected that these potentials will contribute significantly to production of hydrogen through utilisation of this strain of bacteria for production.

1.2 Problem Statement

Reliance on fossil fuels for energy supply has contributed immensely to global warming, environmental pollution and acid rain due to the enormous greenhouse gases often emitted into the atmosphere following the use of fossil fuel. Fermentative biohydrogen production through thermophilic bacteria is unsustainable renewable energy generation process owing to the high energy required.

The activity of psychrophilic microorganisms at low temperature has positive influence on biofuel production owing to the energy-saving and the sustainability properties. However, frequently reported slow metabolism and growth within a narrow temperature range (0-20°C) delay their biosynthetic characteristics.

In view of the foregoing, therefore, using psychrotolerant bacteria in hydrogen production offers better potentials as input in alternative energy production. This potential is feasible due to the improved substrate uptake of psychrotolerant bacteria and their adaptation to a wider temperature range (0-40°C). Since the temperature has been identified as a major factor that influences enzyme activity, this study relies on the temperature adaptability of the of psychrotolerant bacteria in tapping the maximum

biohydrogen producing potentials of these bacteria. Further, the bacteria rely on its adaptability characteristic to bring about improved substrate degradation through rapid metabolic activity, thereby improving their biohydrogen production.

1.3 Research Objectives

- 1 To isolate and characterise psychrotolerant bacteria isolated from Antarctica for biohydrogen production.
- 2 To evaluate the physicochemical conditions for biohydrogen production and determine the oxygen uptake capability of bacterium.
- 3 To optimise biohydrogen production using Central Composite Design of Response Surface Methodology (RSM).

1.4 Scope of the Study

The study covered the isolation of facultative psychrotolerant bacteria from Antarctic seawater. The bacteria obtained were screened for hydrogen production and the potential bacterium identified. Selected biochemical tests and screening were carried out to determine the virulent properties and oxygen take-up capability of the bacterium. Effects of different carbohydrate and nitrogen sources on the biohydrogen productivity of the bacterium were studied. Then optimization for biohydrogen production was finally examined by Central Composite Design (CCD) component of the Response Surface Methodology (RSM) design expert.

1.5 Significance of the Study

Cold-active bacteria have become attractive microorganisms for hydrogen production owing to their potential energy-saving activity at ambient-temperature

REFERENCES

- Adessi, A., Venturi, M., Candelieri, F., Galli, V., Granchi, L. and De Philippis, R. (2018). Bread wastes to energy: Sequential lactic and photo-fermentation for hydrogen production. *International Journal of Hydrogen Energy*, 43 (20), 9569-9576.
- Agrawal, D.P.K. and Agrawal, S. (2013). Characterization of *Bacillus* sp. strains isolated from rhizosphere of tomato plants (*Lycopersicon esculentum*) for their use as potential plant growth promoting rhizobacteria. *International Journal of Current Microbiological Applied Sciences* 2 (10), 406-417.
- Ainala, S.K., Seol, E., Kim, J.R. and Park, S. (2016). Effect of culture medium on fermentative and CO-dependent H₂ production activity in *Citrobacter amalonaticus* Y19. *International Journal of Hydrogen Energy*, 41(16), 6734-6742.
- Åkerberg, C. and Zacchi, G., (2000). An economic evaluation of the fermentative production of lactic acid from wheat flour. *Bioresource Technology*, 75 (2), 119-126.
- Alalayah, W.M., Al-Zahrani, A., Edris, G. and Demirbas, A., (2017). Kinetics of biological hydrogen production from green microalgae *Chlorella vulgaris* using glucose as initial substrate. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, 39(12), 1210-1215.
- Alvarado, P., Huang, Y., Wang, J., Garrido, I. and Leiva, S., (2018). Phylogeny and bioactivity of epiphytic Gram-positive bacteria isolated from three co-occurring antarctic macroalgae. *Antonie van Leeuwenhoek*, 1-13.
- Alvarado-Cuevas, Z. D., López-Hidalgo, A. M., Ordoñez, L. G., Ocegüera-Contreras, E., Ornelas-Salas, J. T. and De León-Rodríguez, A. (2015). Biohydrogen production using psychrophilic bacteria isolated from Antarctica. *International Journal of Hydrogen Energy*. 40 (24), 7586-7592.
- Alvarez-Guzmán, C.L., Ocegüera-Contreras, E., Ornelas-Salas, J.T., Balderas-Hernández, V.E. and De León-Rodríguez, A. (2016). Biohydrogen production by the psychrophilic G088 strain using single carbohydrates as substrate. *International Journal of Hydrogen Energy*, 41(19), 8092-8100.

- An, D., Li, Q., Wang, X., Yang, H. and Guo, L. (2014). Characterization on hydrogen production performance of a newly isolated *Clostridium beijerinckii* YA001 using xylose. *International Journal of Hydrogen Energy*, 39(35), 19928-19936.
- Andrews, S.C., Berks, B.C., McClay, J., Ambler, A., Quail, M.A., Golby, P. and Guest, J.R., (1997). A 12-cistron *Escherichia coli* operon (hyf) encoding a putative proton-translocating formate hydrogenlyase system. *Microbiology*, 143(11), 3633-364
- Arslan, C., Sattar, A., Ji, C., Sattar, S., Yousaf, K. and Hashim, S. (2015). Optimizing the impact of temperature on bio-hydrogen production from food waste and its derivatives under no pH control using statistical modelling. *Biogeosciences*, 12 (21), 6503-6514.
- Awasthi, S., Srivastava, P., Singh, P., Tiwary, D. and Mishra, P.K. (2017). Biodegradation of thermally treated high-density polyethylene (HDPE) by *Klebsiella pneumoniae* CH001. *3 Biotechnology*, 7(5), 332.
- Babu, P., Chandel, A.K. and Singh, O.V. (2015). *Extremophiles and their Applications in Medical Processes*. Springer International Publishing.
- Bächtold, M. (2018). How should energy be defined throughout schooling?. *Research in Science Education*, 48(2), 345-367.
- Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y. and Tan, T. (2015). Challenges and opportunities in improving the production of bio-ethanol. *Progress in Energy and Combustion Science*, 47, 60-88.
- Bakermans, C. (2018). Adaptations to marine versus terrestrial low temperature environments as revealed by comparative genomic analyses of the genus *Psychrobacter*. *FEMS Microbiology Ecology*, 94 (7), 102.
- Barbosa, M.J., Rocha, J.M., Tramper, J. and Wijffels, R.H. (2001). Acetate as a carbon source for hydrogen production by photosynthetic bacteria. *Journal of Biotechnology*, 85(1), 25-33.
- Barbosa, T.M., Serra, C.R., La Ragione, R.M., Woodward, M.J. and Henriques, A.O. (2005). Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Applied and Environmental Microbiology*, 71 (2), 968-978.
- Berndes, G., Hoogwijk, M. and Van den Broek, R. (2003). The contribution of biomass in the future global energy supply: a review of 17 studies. *Biomass and Bioenergy*, 25(1), 1-28.

- Berney, M., Greening, C., Conrad, R., Jacobs, W.R. and Cook, G.M., (2014). An obligately aerobic soil bacterium activates fermentative hydrogen production to survive reductive stress during hypoxia. *Proceedings of the National Academy of Sciences*, 111(31), 11479-11484.
- Bialek-Davenet, S., Criscuolo, A., Ailloud, F., Passet, V., Jones, L., Delannoy-Vieillard, A.S., Garin, B., Le Hello, S., Arlet, G., Nicolas-Chanoine, M.H. and Decré, D. (2014). Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. *Emerging infectious diseases*, 20 (11), 1812.
- Bisaillon, A., Turcot, J. and Hallenbeck, P.C. (2006). The effect of nutrient limitation on hydrogen production by batch cultures of *Escherichia coli*. *International Journal of Hydrogen Energy*, 31(11), 1504-1508.
- Boodhun, B.S.F., Mudhoo, A., Kumar, G., Kim, S.H. and Lin, C.Y. (2017). Research perspectives on constraints, prospects and opportunities in biohydrogen production. *International Journal of Hydrogen Energy*, 42 (45), 27471-27481.
- Botheju, D. and Bakke, R. (2011). Oxygen effects in anaerobic digestion-a review. *The Open Waste Management Journal*, 4, 1-19
- Bowman, J.P. (2001). Methods for psychrophilic bacteria. *Methods in Microbiology*, 30, 591-614.
- Brat, K., Sedlacek, I., Sevcikova, A., Merta, Z., Laska, K. and Sevcik, P. (2016). Imported anthropogenic bacteria may survive the Antarctic winter and introduce new genes into local bacterial communities. *Polish Polar Research*, 37 (1), 89-104.
- Budiman, P.M. and Wu, T.Y. (2018). Role of chemicals addition in affecting biohydrogen production through photofermentation. *Energy Conversion and Management*, 165, 509-527.
- Carter, G.R., and Cole Jr J. R. (2012). Diagnostic procedure in veterinary bacteriology and mycology. Academic Press.
- Castinel, A., Grinberg, A., Pattison, R., Duignan, P., Pomroy, B., Rogers, L. and Wilkinson, I. (2007). Characterization of *Klebsiella pneumoniae* isolates from New Zealand sea lion (*Phocarctos hookeri*) pups during and after the epidemics on Enderby Island, Auckland Islands. *Veterinary Microbiology*, 122 (1-2), 178-184.

- Cavicchioli, R., Siddiqui, K.S., Andrews, D. and Sowers, K.R. (2002). Low-temperature extremophiles and their applications. *Current Opinion in Biotechnology*, 13(3), 253-261.
- Chandrasekhar, K., Lee, Y.J. and Lee, D.W. (2015). Biohydrogen production: strategies to improve process efficiency through microbial routes. *International Journal of Molecular Sciences*, 16(4), 8266-8293.
- Chen, C.Y., Lu, W.B., Liu, C.H. and Chang, J.S. (2008). Improved phototrophic H₂ production with *Rhodospseudomonas palustris* WP3-5 using acetate and butyrate as dual carbon substrates. *Bioresource Technology*, 99 (9), 3609-3616.
- Chen, C.Y., Lu, W.B., Wu, J.F. and Chang, J.S. (2007). Enhancing phototrophic hydrogen production of *Rhodospseudomonas palustris* via statistical experimental design. *International Journal of Hydrogen Energy*, 32(8), 940-949.
- Chen, X., Sun, Y., Xiu, Z., Li, X. and Zhang, D. (2006). Stoichiometric analysis of biological hydrogen production by fermentative bacteria. *International Journal of Hydrogen Energy*, 31(4), 539-549.
- Cheng, J., Song, W., Xia, A., Su, H., Zhou, J. and Cen, K. (2012). Sequential generation of hydrogen and methane from xylose by two-stage anaerobic fermentation. *International Journal of Hydrogen Energy*, 37(18), 13323-13329.
- Chookaew, T., Sompong, O. and Prasertsan, P. (2012). Fermentative production of hydrogen and soluble metabolites from crude glycerol of biodiesel plant by the newly isolated thermotolerant *Klebsiella pneumoniae* TR17. *International Journal of Hydrogen Energy*, 37(18), 13314-13322.
- Chu, L.B., Zhang, X.W., Li, X. and Yang, F.L. (2005). Simultaneous removal of organic substances and nitrogen using a membrane bioreactor seeded with anaerobic granular sludge under oxygen-limited conditions. *Desalination*, 172(3), 271-280.
- Cirne, D.G., Paloumet, X., Björnsson, L., Alves, M.M. and Mattiasson, B. (2007). Anaerobic digestion of lipid-rich waste—effects of lipid concentration. *Renewable Energy*, 32 (6), 965-975.
- Connaughton, S., Collins, G. and O’Flaherty, V. (2006). Psychrophilic and mesophilic anaerobic digestion of brewery effluent: a comparative study. *Water Research*, 40(13), 2503-2510.

- Connelly, T.L., Tilburg, C.M. and Yager, P.L. (2006). Evidence for psychrophiles outnumbering psychrotolerant marine bacteria in the springtime coastal Arctic. *Limnology and Oceanography*, 51 (2), 1205-1210.
- Coyle, E.L. and Evans, J.W., EVACO LLC, (2018). Method and apparatus for a low cost and carbon free point of use dissociation of water into elemental gases and production of hydrogen related power. U.S. Patent 9,994,450.
- Cvetkovska, M., Hüner, N.P. and Smith, D.R. (2017). Chilling out: the evolution and diversification of psychrophilic algae with a focus on Chlamydomonadales. *Polar Biology*, 40 (6), 1169-1184.
- da Silva Veras, T., Mozer, T.S. and da Silva César, A. (2017). Hydrogen: Trends, production and characterization of the main process worldwide. *International Journal of Hydrogen Energy*, 42(4), 2018-2033.
- Dabrock, B., Bahl, H. and Gottschalk, G., (1992). Parameters affecting solvent production by *Clostridium pasteurianum*. *Applied and Environmental Microbiology*, 58(4), 1233-1239
- Dahbia, A.A., Hakim, L., Nadia, A., and Nabil, M. (2014). Dark fermentative hydrogen production rate from glucose using facultative anaerobe bacteria *E. coli*. International Conference on Control, Engineering and Information Technology (CEIT'14), *Proceedings Engineering and Technology*. ISSN 2356-5608.
- Das, D. (2009). Advances in biohydrogen production processes: an approach towards commercialization. *International Journal of Hydrogen Energy*, 34(17), 7349-7357.
- Das, D., Dutta, T., Nath, K., Kotay, S.M., Das, A.K. and Veziroglu, T.N. (2006). Role of Fe-hydrogenase in biological hydrogen production. *Current Science*, 1627-1637.
- De la Cueva, S.C., Guzmán, C.L.A., Hernández, V.E.B. and Rodríguez, A.D.L. (2017). Optimization of biohydrogen production by the novel psychrophilic strain N92 collected from the Antarctica. *International Journal of Hydrogen Energy*. 43, 13798-13809.
- De Vrije, T., Budde, M.A., Lips, S.J., Bakker, R.R., Mars, A.E. and Claassen, P.A. (2010). Hydrogen production from carrot pulp by the extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. *International journal of Hydrogen Energy*, 35(24), 13206-13213.

- Dębowski, M., Korzeniewska, E., Filipkowska, Z., Zieliński, M. and Kwiatkowski, R. (2014). Possibility of hydrogen production during cheese whey fermentation process by different strains of psychrophilic bacteria. *International Journal of Hydrogen Energy*, 39(5), 1972-1978.
- Deepanraj, B., Sivasubramanian, V. and Jayaraj, S. (2015). Kinetic study on the effect of temperature on biogas production using a lab scale batch reactor. *Ecotoxicology and Environmental Safety*, 121, 100-104.
- Deming, J.W. (2002). Psychrophiles and Polar Regions. *Current Opinion in Microbiology*, 5 (3), 301-309.
- Deppe, U., Richnow, H.H., Michaelis, W. and Antranikian, G. (2005). Degradation of crude oil by an arctic microbial consortium. *Extremophiles*, 9 (6), 461-470.
- Devi, L., Ptasiński, K.J. and Janssen, F.J. (2003). A review of the primary measures for tar elimination in biomass gasification processes. *Biomass and Bioenergy*, 24 (2), 125-140.
- Dincer, I. and Acar, C. (2018). Potential energy solutions for better sustainability. In *Exergetic, Energetic and Environmental Dimensions*, 3-37.
- Dobrić, S. and Bååth, E. (2018). Temperature affects lag period and growth of bacteria in soil according to a Ratkowsky (square root) model after a drying/rewetting episode. *Soil Biology and Biochemistry*, 124, 32-37.
- Dong, J., Zhao, W., Gasmalla, M.A., Sun, J., Hua, X., Zhang, W., Han, L., Fan, Y., Feng, Y., Shen, Q. and Yang, R. (2015). A novel extracellular cold-active esterase of *Pseudomonas* sp. TB11 from glacier No. 1: Differential induction, purification and characterisation. *Journal of Molecular Catalysis B: Enzymatic*, 121, 53-63.
- Dziewit, L. and Bartosik, D. (2014). Plasmids of psychrophilic and psychrotolerant bacteria and their role in adaptation to cold environments. *Frontiers in Microbiology*, 5, 596.
- Eroglu, E. and Melis, A. (2016). Microalgal hydrogen production research. *International Journal of Hydrogen Energy*, 41(30), 12772-12798.
- Eroğlu, İ., Tabanoğlu, A., Gündüz, U., Eroğlu, E. and Yücel, M. (2008). Hydrogen production by *Rhodobacter sphaeroides* OU 001 in a flat plate solar bioreactor. *International Journal of Hydrogen Energy*, 33(2), 531-541.
- Faloye, F.D., Kana, E.G. and Schmidt, S., (2014). Optimization of biohydrogen inoculum development via a hybrid pH and microwave treatment technique–

- Semi pilot scale production assessment. *International Journal of Hydrogen Energy*, 39(11), 5607-5616.
- Fangkum, A. and Reungsang, A. (2011). Biohydrogen production from sugarcane bagasse hydrolysate by elephant dung: effects of initial pH and substrate concentration. *International Journal of Hydrogen Energy*, 36(14), 8687-8696.
- Fareleira, P., Santos, B.S., António, C., Moradas-Ferreira, P., LeGall, J., Xavier, A.V. and Santos, H. (2003). Response of a strict anaerobe to oxygen: survival strategies in *Desulfovibrio gigas*. *Microbiology*, 149(6), 1513-1522.
- Ferchichi, M., Crabbe, E., Hintz, W., Gil, G.H. and Almadidy, A. (2005). Influence of culture parameters on biological hydrogen production by *Clostridium saccharoperbutylacetonicum* ATCC 27021. *World Journal of Microbiology and Biotechnology*, 21 (6-7), 855–862
- Ferreira, T.B., Rego, G.C., Ramos, L.R., Soares, L.A., Sakamoto, I.K., de Oliveira, L.L., Varesche, M.B.A. and Silva, E.L. (2018). Selection of metabolic pathways for continuous hydrogen production under thermophilic and mesophilic temperature conditions in anaerobic fluidized bed reactors. *International Journal of Hydrogen Energy* [43 \(41\)](#), 18908-18917
- Finneran, K.T., Johnsen, C.V. and Lovley, D.R. (2003). *Rhodoferrax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (III). *International Journal of Systematic and Evolutionary Microbiology*, 53 (3), 669-673.
- Franzmann, P.D., Liu, Y., Balkwill, D.L., Aldrich, H.C., De Macario, E.C. and Boone, D.R. (1997). *Methanogenium frigidum* sp. nov., a psychrophilic, H₂-using methanogen from Ace Lake, Antarctica. *International Journal of Systematic and Evolutionary Microbiology*, 47 (4), 1068-1072.
- Fritsch, J., Lenz, O. and Friedrich, B. (2013). Structure, function and biosynthesis of O₂-tolerant hydrogenases. *Nature Reviews Microbiology*, 11(2), 106.
- Fukunaga, N. and Russell, N.J. (1990). Membrane lipid composition and glucose uptake in two psychrotolerant bacteria from Antarctica. *Microbiology*, 136 (9), 1669-1673.
- Gao, H., Obraztova, A., Stewart, N., Popa, R., Fredrickson, J.K., Tiedje, J.M., Nealson, K.H. and Zhou, J. (2006). *Shewanella loihica* sp. nov., isolated from iron-rich microbial mats in the Pacific Ocean. *International Journal of Systematic and Evolutionary Microbiology*, 56 (8), 1911-1916.

- García-Depraect, O. and León-Becerril, E. (2018). Fermentative biohydrogen production from tequila vinasse via the lactate-acetate pathway: Operational performance, kinetic analysis and microbial ecology. *Fuel*, 234, 151-160.
- Geller, D.P., Das, K.C., Bagby-Moon, T., Singh, M., Hawkins, G. and Kiepper, B.H. (2018). Biomass productivity of snow algae and model production algae under low temperature and low light conditions. *Algal Research*, 33, 133-141.
- Georges F., Emmanuel N., Jean L.A., Mohamed A., Etienne B., Sabine G and Charles G. (1996). Enzymes from psychrophilic organisms. *FEMS Microbiology Reviews*, 18,189-202.
- Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P.N. and Esposito, G. (2015). A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. *Applied Energy*, 144, 73-95.
- Ghosh, S. and Bandyopadhyay, P.K. (2018). Molecular characterization of newly identified Klebsiella PKBSG14 and analysis of its effect on immune response and cell cycle progression using common catfish (*Channa punctatus*) as a model. *Microbial Pathogenesis*.
- Gillis, R.J., Al-Ali, K. and Green, W.H. (2018). Thermochemical production of hydrogen from hydrogen sulfide with iodine thermochemical cycles. *International Journal of Hydrogen Energy*, 43, 12939-12942
- Ginkel, S.V., Sung, S. and Lay, J.J. (2001). Biohydrogen production as a function of pH and substrate concentration. *Environmental Science & Technology*, 35(24), 4726-4730.
- Giudice, A.L., Casella, P., Caruso, C., Mangano, S., Bruni, V., De Domenico, M. and Michaud, L. (2010). Occurrence and characterization of psychrotolerant hydrocarbon-oxidizing bacteria from surface seawater along the Victoria Land coast (Antarctica). *Polar Biology*, 33 (7), 929-943.
- Glazer, A.N. and Nikaido, H. (2007). *Microbial biotechnology: fundamentals of applied microbiology*. Cambridge University Press.
- Gunsalus, I.C. and STANIER, R.Y. (1961). The Bacteria. A Treatise on Structure and Function. Vol. II. Metabolism. *The Bacteria. A Treatise on Structure and Function. Vol. II. Metabolism*.

- Guo, X.M., Trably, E., Latrille, E., Carrere, H. and Steyer, J.P. (2010). Hydrogen production from agricultural waste by dark fermentation: a review. *International Journal of Hydrogen Energy*, 35 (19), 10660-10673.
- Gupta, N., Pal, M., Sachdeva, M., Yadav, M. and Tiwari, A. (2016). Thermophilic biohydrogen production for commercial application: the whole picture. *International Journal of Energy Research*, 40 (2), 127-145.
- Habib, S., Ahmad, S.A., Johari, W.L.W., Shukor, M.Y.A., Alias, S.A., Khalil, K.A. and Yasid, N.A. (2018). Evaluation of conventional and response surface level optimisation of n-dodecane (n-C12) mineralisation by psychrotolerant strains isolated from pristine soil at Southern Victoria Island, Antarctica. *Microbial cell Factories*, 17(1), 44.
- Hakobyan, M., Sargsyan, H. and Bagramyan, K. (2005). Proton translocation coupled to formate oxidation in anaerobically grown fermenting *Escherichia coli*. *Biophysical Chemistry*, 115(1), 55-61.
- Hallenbeck, P.C. and Benemann, J.R. (2002). Biological hydrogen production; fundamentals and limiting processes. *International Journal of Hydrogen Energy*, 27(11-12), 1185-1193.
- Hamdan, A. (2018). Psychrophiles: Ecological significance and potential industrial application. *South African Journal of Science*, 114(5-6), 15-20.
- He, D., Bultel, Y., Magnin, J.P., Roux, C. and Willison, J.C. (2005). Hydrogen photosynthesis by *Rhodobacter capsulatus* and its coupling to a PEM fuel cell. *Journal of Power Sources*, 141 (1), 19-23.
- Held, T.K., Jendrike, N.R., Rukavina, T., Podschun, R. and Trautmann, M. (2000). Binding to and Opsonophagocytic Activity of O-Antigen-Specific Monoclonal Antibodies against Encapsulated and Nonencapsulated *Klebsiella pneumoniae* Serotype O1 Strains. *Infection and Immunity*, 68(5), 2402-2409.
- Helmke, E. and Weyland, H. (2004). Psychrophilic versus psychrotolerant bacteria- occurrence and significance in polar and temperate marine habitats. *Cellular and Molecular Biology*, 50 (5), 553-561
- Heyndrickx, M. (2011). The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. *Applied and Environmental Soil Science*, ID 561975, 1-11.

- Ho, K.L., Chen, Y.Y. and Lee, D.J. (2010). Biohydrogen production from cellobiose in phenol and cresol-containing medium using *Clostridium* sp. R1. *International Journal of Hydrogen Energy*, 35 (19), 10239-10244.
- Holt, J.G., Williams, S.T. and Holt, (1989). *Bergey's Manual of Systematic Bacteriology, Vol. 4*. Lippincott Williams & Wilkins.
- Höök, M. and Tang, X. (2013). Depletion of fossil fuels and anthropogenic climate change—A review. *Energy Policy*, 52, 797-809.
- Hoover, R.B. and Pikuta, E.V. (2010). Psychrophilic and psychrotolerant microbial extremophiles in polar environments. 1-42.
- Horner, D.S., Heil, B., Happe, T. and Embley, T.M. (2002). Iron hydrogenases—ancient enzymes in modern eukaryotes. *Trends in Biochemical Sciences*, 27(3), 148-153.
- Hu, W., Niu, H., Murata, K., Wu, Z., Hu, M., Kojima, T. and Zhang, D. (2018). Bacteria in atmospheric waters: Detection, characteristics and implications. *Atmospheric Environment*. 179, 201-221
- Hue, N.T.H., Ngoc, P.D., Hanh, T.M., Tien, N.A. and Ha, B.T.V. (2016). Effects of Environmental Parameters on Hydrogen Production of Strain *Clostridium beijerinckii* CB3 Isolated in North of Vietnam under Anaerobic Condition. *VNU Journal of Science: Natural Sciences and Technology*, 32 (1S), 258-268
- Hung, C.H., Cheng, C.H., Guan, D.W., Wang, S.T., Hsu, S.C., Liang, C.M. and Lin, C.Y. (2011). Interactions between *Clostridium* sp. and other facultative anaerobes in a self-formed granular sludge hydrogen-producing bioreactor. *International Journal of Hydrogen Energy*, 36(14), 8704-8711.
- Imlay, J.A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nature Reviews Microbiology*, 11 (7), 443.
- Islam, R., Cicek, N., Sparling, R. and Levin, D. (2006). Effect of substrate loading on hydrogen production during anaerobic fermentation by *Clostridium thermocellum* 27405. *Applied Microbiology and Biotechnology*, 72 (3), 576.
- Jamil, Z., Annuar, M.S.M., Ibrahim, S. and Vikineswary, S. (2009). Optimization of phototrophic hydrogen production by *Rhodospseudomonas palustris* PBUM001 via statistical experimental design. *International Journal of Hydrogen Energy*, 34 (17), 7502-7512.

- Jiang, L., Hu, S., Syed-Hassan, S.S.A., Xu, K., Shuai, C., Wang, Y., Su, S. and Xiang, J. (2018). Hydrogen-Rich Gas Production from Steam Gasification of Lignite Integrated with CO₂ Capture Using Dual Calcium-Based Catalysts: An Experimental and Catalytic Kinetic Study. *Energy and Fuels*, 32 (2), 1265-1275.
- Jo, J.H., Kennedy, E.A. and Kong, H.H. (2016). Research techniques made simple: bacterial 16S ribosomal RNA gene sequencing in cutaneous research. *Journal of Investigative Dermatology*, 136(3), 23-27.
- Kalil, M.S., Alshiyab, H.S. and Yusoff, W.M.W. (2008). Effect of nitrogen source and carbon to nitrogen ratio on hydrogen production using *C. acetobutylicum*. *American Journal of Biochemistry and Biotechnology*, 4(4), 393-401.
- Kamalaskar, L.B., Dhakephalkar, P.K., Meher, K.K. and Ranade, D.R. (2010). High biohydrogen yielding *Clostridium* sp. DMHC-10 isolated from sludge of distillery waste treatment plant. *International Journal of Hydrogen Energy*, 35(19), 10639-10644.
- Kargi, F., Eren, N.S. and Ozmihci, S. (2012). Hydrogen gas production from cheese whey powder (CWP) solution by thermophilic dark fermentation. *International Journal of Hydrogen Energy*, 37(3), 2260-2266.
- Keskin, T. and Hallenbeck, P.C. (2012). Hydrogen production from sugar industry wastes using single-stage photofermentation. *Bioresource Technology*, 112, 131-136.
- Kimata, S., Mochizuki, D., Satoh, J., Kitano, K., Kanesaki, Y., Takeda, K., Abe, A., Kawasaki, S. and Niimura, Y. (2018). Intracellular free flavin and its associated enzymes participate in oxygen and iron metabolism in *Amphibacillus xylanus* lacking a respiratory chain. *FEBS Open Biology*, 947-961.
- Koller, M. (2016). Recent Advances in Biotechnology Volume, 1: Microbial Biopolyester Production, Performance and Processing Microbiology, Feedstocks, and Metabolism (Vol. 1). Bentham Science Publishers.
- Kracke, F., Vassilev, I. and Krömer, J.O. (2015). Microbial electron transport and energy conservation—the foundation for optimizing bio-electrochemical systems. *Frontiers in Microbiology*, 6, 575.
- Kulkarni, H.M., Swamy, C.V. and Jagannadham, M.V. (2014). Molecular characterization and functional analysis of outer membrane vesicles from the

- antarctic bacterium *Pseudomonas syringae* suggest a possible response to environmental conditions. *Journal of Proteome Research*, 13 (3), 1345-1358.
- Kumar, V., Kothari, R., Pathak, V.V. and Tyagi, S.K. (2016). Optimization of Simple Sugars and Process pH for Effective Biohydrogen Production Using *Enterobacter Aerogens*: An Experimental Study, *Journal of Scientific & Industrial Research*, 75,626-631
- Kurahashi, M. and Yokota, A. (2002). A preliminary report of phylogenetic diversity of bacterial strains isolated from marine creatures. *The Journal of General and Applied Microbiology*, 48 (5), 251-259.
- Laurinavichene, T.V. and Tsygankov, A.A. (2001). H₂ consumption by *Escherichia coli* coupled via hydrogenase 1 or hydrogenase 2 to different terminal electron acceptors. *FEMS Microbiology Letters*, 202(1), 121-124.
- Lavin, P.L., Yong, S.T., Wong, C.M. and De Stefano, M. (2016). Isolation and characterization of Antarctic psychrotroph *Streptomyces* sp. strain INACH3013. *Antarctic Science*, 28 (6), 433-442.
- Lee, K.S., Lin, P.J. and Chang, J.S. (2006). Temperature effects on biohydrogen production in a granular sludge bed induced by activated carbon carriers. *International Journal of Hydrogen Energy*, 31 (4), 465-472.
- Leiba, J., Sabra, A., Bodinier, R., Marchetti, A., Lima, W.C., Melotti, A., Perrin, J., Burdet, F., Pagni, M., Soldati, T. and Lelong, E., (2017). Vps13F links bacterial recognition and intracellular killing in *Dictyostelium*. *Cellular Microbiology*, 19(7), 12722.
- Lettinga, G., Rebac, S. and Zeeman, G. (2001). Challenge of psychrophilic anaerobic wastewater treatment. *TRENDS in Biotechnology*, 19(9), 363-370.
- Levin, D.B., Islam, R., Cicek, N. and Sparling, R. (2006). Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. *International Journal of Hydrogen Energy*, 31 (11), 1496-1503.
- Li, C. and Fang, H.H., (2007). Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. *Critical Reviews in Environmental Science and Technology*, 37(1), 1-39.
- Li, D. and Chen, H., (2007). Biological hydrogen production from steam-exploded straw by simultaneous saccharification and fermentation. *International Journal of Hydrogen Energy*, 32(12), 1742-1748.

- Li, R., Zhi, Z. and Wang, H. (2014). Influence of carbon/nitrogen ratio on the anaerobic fermentative hydrogen production with cow dung compost. *Journal of Renewable and Sustainable Energy*, 6 (3), 033139.
- Li, R.Y. and Fang, H.H. (2008). Hydrogen production characteristics of photoheterotrophic *Rubrivivax gelatinosus* L31. *International Journal of Hydrogen Energy*, 33 (3), 974-980.
- Li, Y., Zhang, Q., Deng, L., Liu, Z., Jiang, H. and Wang, F. (2018). Biohydrogen production from fermentation of cotton stalk hydrolysate by *Klebsiella* sp. WL1316 newly isolated from wild carp (*Cyprinus carpio* L.) of the Tarim River basin. *Applied Microbiology and Biotechnology*, 102(9), 4231-4242.
- Licciano, M., Terlizzi, A., Giangrande, A., Cavallo, R.A. and Stabili, L. (2007). Filter-feeder macroinvertebrates as key players in culturable bacteria biodiversity control: a case of study with *Sabella spallanzanii* (Polychaeta: Sabellidae). *Marine Environmental Research*, 64 (4), 504-513.
- Lima, W.C., Pillonel, T., Bertelli, C., Ifrid, E., Greub, G. and Cosson, P. (2018). Genome sequencing and functional characterization of the non-pathogenic *Klebsiella pneumoniae* KpGe bacteria. *Microbes and Infection*, 20 (5), 293-301.
- Lin, C.Y. and Lay, C.H. (2004). Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora. *International Journal of Hydrogen Energy*, 29(1), 41-45.
- Liu, B.F., Ren, N.Q., Xie, G.J., Ding, J., Guo, W.Q. and Xing, D.F. (2010). Enhanced bio-hydrogen production by the combination of dark-and photo-fermentation in batch culture. *Bioresource Technology*, 101 (14), 5325-5329.
- Liu, F. and Fang, B. (2007). Optimization of bio-hydrogen production from biodiesel wastes by *Klebsiella pneumoniae*. *Biotechnology Journal: Healthcare Nutrition Technology*, 2(3), 374-380.
- Liu, H., Tan, H., Liu, Y., Liu, Z., Ma, L., Pourkashanian, M. and Xu, T. (2011). Study of the layered structure of deposit in a biomass-fired boiler (case study). *Energy and Fuels*, 25 (6), 2593-2600.
- Liu, H., Wang, G., Zhu, D. and Pan, G. (2012). Improvement of hydrogen production by transposon-mutagenized strain of *Pantoea agglomerans* BH18. *International Journal of Hydrogen Energy*, 37 (10), 8282-8287.

- Liu, P., Ji, J., Wu, Q., Ren, J., Wu, G., Yu, Z., Xiong, J., Tian, F., Zafar, Y. and Li, X. (2018). *Klebsiella pneumoniae* sp. LZU10 degrades oil in food waste and enhances methane production from co-digestion of food waste and straw. *International Biodeterioration and Biodegradation*, 126, 28-36.
- Lo, Y.C., Chen, W.M., Hung, C.H., Chen, S.D. and Chang, J.S. (2008). Dark H₂ fermentation from sucrose and xylose using H₂-producing indigenous bacteria: feasibility and kinetic studies. *Water Research*, 42 (4-5), 827-842.
- Lu, L., Yang, G., Zhu, B. and Pan, K. (2017). A comparative study on three quantitating methods of microalgal biomass, *Indian Journal of Geo Marine Sciences*, 46, 2265-2272.
- Madigan, M.T., Kempfer, M.L., Bender, K.S., Sullivan, P., Sattley, W.M., Dohnalkova, A.C. and Joye, S.B. (2017). Characterization of a cold-active bacterium isolated from the South Pole "Ice Tunnel". *Extremophiles*, 21(5), 891-901.
- Maeda, I., Miyasaka, H., Umeda, F., Kawase, M. and Yagi, K. (2003). Maximization of hydrogen production ability in high-density suspension of *Rhodovulum sulfidophilum* cells using intracellular poly (3-hydroxybutyrate) as sole substrate. *Biotechnology and Bioengineering*, 81(4), 474-481.
- Maintinguer, S.I., Fernandes, B.S., Duarte, I.C., Saavedra, N.K., Adorno, M.A.T. and Varesche, M.B.A. (2011). Fermentative hydrogen production with xylose by *Clostridium* and *Klebsiella* species in anaerobic batch reactors. *International Journal of Hydrogen Energy*, 36(21), 13508-13517.
- Mand, T.D., Kulkarni, G. and Metcalf, W.W. (2018). Genetic, biochemical, and molecular characterization of *Methanosarcina barkeri* mutants lacking three distinct classes of hydrogenase. *Journal of Bacteriology*, JB-00342.
- Margesin, R. (2009). Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. *Extremophiles*, 13(2), 257-262.
- Mars, A.E., Veuskens, T., Budde, M.A., Van Doeveren, P.F., Lips, S.J., Bakker, R.R., De Vrije, T. and Claassen, P.A. (2010). Biohydrogen production from untreated and hydrolysed potato steam peels by the extreme thermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga neapolitana*. *International Journal of Hydrogen Energy*, 35 (15), 7730-7737.

- Martínez-Rosales, C. and Castro-Sowinski, S. (2011). Antarctic bacterial isolates that produce cold-active extracellular proteases at low temperature but are active and stable at high temperature. *Polar Research*, 30(1), 7123.
- Matsumura, Y., Sato, K., Al-saari, N., Nakagawa, S. and Sawabe, T. (2014). Enhanced hydrogen production by a newly described heterotrophic marine bacterium, *Vibrio tritonius* strain AM2, using seaweed as the feedstock. *International Journal of Hydrogen Energy*, 39(14), 7270-7277.
- Mei, Y.Z., Huang, P.W., Liu, Y., He, W. and Fang, W.W. (2016). Cold stress promoting a psychrotolerant bacterium *Pseudomonas fragi* P121 producing trehalase. *World Journal of Microbiology and Biotechnology*, 32 (8), 134.
- Miller, G.L., (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31 (3), 426-428.
- Minnan, L., Jinli, H., Xiaobin, W., Huijuan, X., Jinzao, C., Chuannan, L., Fengzhang, Z. and Liangshu, X. (2005). Isolation and characterization of a high H₂-producing strain *Klebsiella oxytoca* HP1 from a hot spring. *Research in Microbiology*, 156 (1), 76-81.
- Mishra, P., Thakur, S., Singh, L., Krishnan, S., Sakinah, M. and Ab Wahid, Z. (2017). Fermentative hydrogen production from indigenous mesophilic strain *Bacillus anthracis* PUNAJAN 1 newly isolated from palm oil mill effluent. *International Journal of Hydrogen Energy*, 42(25), 16054-16063.
- Montes, M.J., Mercadé, E., Bozal, N. and Guinea, J. (2004). *Paenibacillus antarcticus* sp. nov., a novel psychrotolerant organism from the Antarctic environment. *International Journal of Systematic and Evolutionary Microbiology*, 54 (5), 1521-1526.
- Moosvi, S.A., McDonald, I.R., Pearce, D.A., Kelly, D.P. and Wood, A.P. (2005). Molecular detection and isolation from Antarctica of methylotrophic bacteria able to grow with methylated sulfur compounds. *Systematic and Applied Microbiology*, 28 (6), 541-554.
- Mu, Y., Wang, G. and Yu, H.Q. (2006). Kinetic modeling of batch hydrogen production process by mixed anaerobic cultures. *Bioresource Technology*, 97(11), 1302-1307
- Mu, Y., Zheng, X.J., Yu, H.Q. and Zhu, R.F. (2006). Biological hydrogen production by anaerobic sludge at various temperatures. *International Journal of Hydrogen Energy*, 31(6), 780-785.

- Myka, K.K., Allcock, D.J., Eloë-Fadrosh, E.A., Tryfona, T., Haag, A.F., Lauro, F.M., Bartlett, D.H. and Ferguson, G.P. (2017). Adaptations of Cold-and Pressure-Loving Bacteria to the Deep-Sea Environment: Cell Envelope and Flagella. In *Microbial Ecology of Extreme Environments* (pp. 51-80). Springer, Cham.
- Najafpour, G., Younesi, H. and Mohamed, A.R. (2004). Effect of organic substrate on hydrogen production from synthesis gas using *Rhodospirillum rubrum*, in batch culture. *Biochemical Engineering Journal*, 21 (2), 123-130.
- Nedwell, D.B. and Rutter, M. (1994). Influence of temperature on growth rate and competition between two psychrotolerant Antarctic bacteria: low temperature diminishes affinity for substrate uptake. *Applied and Environmental Microbiology*, 60(6), 1984-1992.
- Nevot, M., Deroncelé, V., Messner, P., Guinea, J. and Mercadé, E. (2006). Characterization of outer membrane vesicles released by the psychrotolerant bacterium *Pseudoalteromonas Antarctica NF3*. *Environmental Microbiology*, 8 (9), 1523-1533.
- Nikolaidis, P. and Poullikkas, A. (2017). A comparative overview of hydrogen production processes. *Renewable and Sustainable Energy Reviews*, 67, 597-611.
- Nishijima, M., Adachi, K., Katsuta, A., Shizuri, Y. and Yamasato, K. (2013). *Endozoicomonas numazuensis* sp. nov., a gamma proteo-bacterium isolated from marine sponges, and emended description of the genus *Endozoicomonas* Kurahashi and Yokota 2007. *International Journal of Systematic and Evolutionary Microbiology*, 63 (2), 709-714.
- Niu, K., Zhang, X., Tan, W.S. and Zhu, M.L., (2010). Characteristics of fermentative hydrogen production with *Klebsiella pneumoniae* ECU-15 isolated from anaerobic sewage sludge. *International Journal of Hydrogen Energy*, 35(1), 71-80.
- Noor, I. Mohamad, P., Nur S. Y. S. and Tami, A. U. (2018). Feasibility study and Kinetics of Biohydrogen Production by *Escherichia coli* from Hydrolyzed Sago Wastewater. *Journal of Advanced Research in Biofuel and Bioenergy* 2(1)1-8.
- Nuttall, M., Christensen, T.R. and Siegert, M.J. (2018). Introduction: locating the Polar Regions. In *The Routledge Handbook of the Polar Regions* (pp. 27-42). Routledge.

- Oh, Y.K., Seol, E.H., Kim, M.S. and Park, S. (2004). Photoproduction of hydrogen from acetate by a chemoheterotrophic bacterium *Rhodospseudomonas palustris* P4. *International Journal of Hydrogen Energy*, 29 (11), 1115-1121.
- Olorunnisola, A.O. (2018). Energy Production and Consumption for Sustainable Development. *Capacity Building for Sustainable Development*, 192.
- Pan, C.M., Fan, Y.T., Zhao, P. and Hou, H.W., (2008). Fermentative hydrogen production by the newly isolated *Clostridium beijerinckii* Fanp3. *International Journal of Hydrogen Energy*, 33(20), 5383-5391.
- Pan, N. and Imlay, J.A. (2001). How does oxygen inhibit central metabolism in the obligate anaerobe *Bacteroides thetaiotaomicron*. *Molecular Microbiology*, 39 (6), 1562-1571.
- Park, J.H., Choi, M.A., Kim, Y.J., Kim, Y.C., Chang, Y.K. and Jeong, K.J. (2017). Engineering of *Klebsiella oxytoca* for production of 2, 3-butanediol via simultaneous utilization of sugars from a *Golenkinia* sp. hydrolysate. *Bioresource Technology*, 245, 1386-1392.
- Parthasarathy, P. and Narayanan, K.S. (2014). Hydrogen production from steam gasification of biomass: influence of process parameters on hydrogen yield—a review. *Renewable Energy*, 66, 570-579.
- Peeters, K., Ertz, D. and Willems, A. (2011). Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. *Systematic and Applied Microbiology*, 34 (5), 360-367.
- Peix, A., Ramírez-Bahena, M.H. and Velázquez, E. (2009). Historical evolution and current status of the taxonomy of genus *Pseudomonas*. *Infection, Genetics and Evolution*, 9 (6), 1132-1147.
- Peng, J., Wegner, C.E. and Liesack, W. (2017). Short-term exposure of paddy soil microbial communities to salt stress triggers different transcriptional responses of key taxonomic groups. *Frontiers in Microbiology*, 8, 400.
- Penniston, J. and Gueguim Kana, E.B. (2018). Impact of medium pH regulation on biohydrogen production in dark fermentation process using suspended and immobilized microbial cells. *Biotechnology and Biotechnological Equipment*, 32 (1), 204-212.

- Pesciaroli, C., Cupini, F., Selbmann, L., Barghini, P. and Fenice, M. (2012). Temperature preferences of bacteria isolated from seawater collected in Kandalaksha Bay, White Sea, Russia. *Polar Biology*, 35 (3), 435-445.
- Pintucci, C., Giovannelli, A., Traversi, M.L., Ena, A., Padovani, G. and Carlozzi, P. (2013). Fresh olive mill waste deprived of polyphenols as feedstock for hydrogen photo-production by means of *Rhodospseudomonas palustris* 42OL. *Renewable Energy*, 51, 358-363.
- Podschun, R., Pietsch, S., Höller, C. and Ullmann, U. (2001). Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. *Applied and Environmental Microbiology*, 67 (7), 3325-3327.
- Poladyan, A., Trchounian, K., Vassilian, A. and Trchounian, A. (2018). Hydrogen production by *Escherichia coli* using brewery waste: Optimal pretreatment of waste and role of different hydrogenases. *Renewable Energy*, 115, 931-936.
- Pomeroy, L.R. and Wiebe, W.J. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*, 23(2), 187-204.
- Precht, H. (2013). *Temperature and Life*. Springer Science and Business Media.
- Pugazhendhi, A. and Thamaraiselvi, K. (2017). Optimization of Fermentative Hydrogen Production by *Klebsiella pneumoniae* KTSMBNL 11 Isolated from Municipal Sewage Sludge. In *Bioremediation and Sustainable Technologies for Cleaner Environment* (pp. 267-278). Springer, Cham.
- Raghunandan, K., Mchunu, S., Kumar, A., Kumar, K.S., Govender, A., Permaul, K. and Singh, S., (2014). Biodegradation of glycerol using bacterial isolates from soil under aerobic conditions. *Journal of Environmental Science and Health, Part A*, 49(1), 85-92.
- Ren, N.Q., Zhao, L., Chen, C., Guo, W.Q. and Cao, G.L. (2016). A review on bioconversion of lignocellulosic biomass to H₂: key challenges and new insights. *Bioresource Technology*, 215, 92-99.
- Ringeisen, B.R., Ray, R. and Little, B. (2007). A miniature microbial fuel cell operating with an aerobic anode chamber. *Journal of Power Sources*, 165 (2), 591-597.
- Robador, A., Müller, A.L., Sawicka, J.E., Berry, D., Hubert, C.R., Loy, A., Jørgensen, B.B. and Brüchert, V., (2016). Activity and community structures of sulfate-

- reducing microorganisms in polar, temperate and tropical marine sediments. *The ISME Journal*, 10(4), 796.
- Roh, Y., Gao, H., Vali, H., Kennedy, D.W., Yang, Z.K., Gao, W., Dohnalkova, A.C., Stapleton, R.D., Moon, J.W., Phelps, T.J. and Fredrickson, J.K. (2006). Metal reduction and iron biomineralization by a psychrotolerant Fe (III)-reducing bacterium, *Shewanella* sp. strain PV-4. *Applied and Environmental Microbiology*, 72 (5), 3236-3244.
- Russell, N.J. (2006). Antarctic microorganisms: coming in from the cold. *Culture*, 27 (965), 989.
- Safari, A., Jafari, S. and Assadi, M. (2018). Role of Gas-Fuelled Solutions in Support of Future Sustainable Energy World; Part I: Stimuluses, Enablers, and Barriers. In *Sustainable Energy Technology and Policies* (pp. 1-33). Springer, Singapore.
- Sahly, H., Ofek, I., Podschun, R., Brade, H., He, Y., Ullmann, U. and Crouch, E. (2002). Surfactant protein D binds selectively to *Klebsiella pneumoniae* lipopolysaccharides containing mannose-rich O-antigens. *The Journal of Immunology*, 169(6), 3267-3274.
- Salerno, M.B., Park, W., Zuo, Y. and Logan, B.E. (2006). Inhibition of biohydrogen production by ammonia. *Water Research*, 40 (6), 1167-1172.
- Salmon, J.D. (2018). Hydrogen Energy: Political and Social Impacts. In *Hydrogen: Its Technology and Implication* (pp. 79-102). CRC Press.
- Sandle, T. and Skinner, K. (2013). Study of psychrophilic and psychrotolerant microorganisms isolated in cold rooms used for pharmaceutical processing. *Journal of Applied Microbiology*, 114 (4), 1166-1174.
- Schembri, M.A., Blom, J., Krogfelt, K.A. and Klemm, P. (2005). Capsule and fimbria interaction in *Klebsiella pneumoniae*. *Infection and Immunity*, 73(8), 4626-4633.
- Seelert, T., Ghosh, D. and Yargeau, V. (2015). Improving biohydrogen production using *Clostridium beijerinckii* immobilized with magnetite nanoparticles. *Applied Microbiology and Biotechnology*, 99 (9), 4107-4116.
- Shin, H.S., Youn, J.H. and Kim, S.H. (2004). Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. *International Journal of Hydrogen Energy*, 29 (13), 1355-1363.

- Shurong Xiang, Tandong Yao, Lizhe An, Bingliang Xu, and Junxia Wang, (2005). 16S rRNA Sequences and Differences in Bacteria Isolated from the Muztag Ata Glacier at Increasing Depths, *Applied and Environmental Microbiology*, 71(8), 4619-4627
- Siddiqui, K.S. (2015). Some like it hot, some like it cold: Temperature dependent biotechnological applications and improvements in extremophilic enzymes. *Biotechnology Advances*, 33 (8), 1912-1922.
- Siliakus, M.F., van der Oost, J. and Kengen, S.W. (2017). Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. *Extremophiles*, 21(4), 651-670.
- Silva, R.M., Toledo, M.R. and Trabulsi, L.R. (1980). Biochemical and cultural characteristics of invasive *Escherichia coli*. *Journal of Clinical Microbiology*, 11 (5), 441-444.
- Silvaa, A.S., Júniora, A.M.O., de Farias Silvab, C.E. and Abud, A.K.S. (2016). Inhibitors Influence on Ethanol Fermentation by *Pichia Stipitis*. *Chemical Engineering*, 49, 367-372.
- Silva-Illanes, F., Tapia-Venegas, E., Schiappacasse, M.C., Trably, E. and Ruiz-Filippi, G. (2017). Impact of hydraulic retention time (HRT) and pH on dark fermentative hydrogen production from glycerol. *Energy*, 141, 358-367.
- Sitha C., Sunthorn K., Sirima, S. J., Kaemwich, J. Claire, J. and Patricia T. (2017). Using a combined oxygen-supply and substrate-feeding strategy to improve 2,3-butanediol production by metabolically engineered *Klebsiella oxytoca* KMS005, *Journal of Chemical Technology and Biotechnology*, 93(2),1-9
- Sivagurunathan, P., Kumar, G., Mudhoo, A., Rene, E.R., Saratale, G.D., Kobayashi, T., Xu, K., Kim, S.H. and Kim, D.H. (2017). Fermentative hydrogen production using lignocellulose biomass: an overview of pre-treatment methods, inhibitor effects and detoxification experiences. *Renewable and Sustainable Energy Reviews*, 77, 28-42.
- Skonieczny, M.T. and Yargeau, V. (2009). Biohydrogen production from wastewater by *Clostridium beijerinckii*: effect of pH and substrate concentration. *International journal of Hydrogen Energy*, 34(8), 3288-3294.
- Somasegaran, P. and Hoben, H.J. (2012). *Handbook for rhizobia: methods in legume-Rhizobium technology*. Springer Science and Business Media.

- Somasegaran, P. and Hoben, H.J. (2012). *Handbook for rhizobia: methods in legume-Rhizobium technology*. Springer Science and Business Media.
- Song, Z.X., Li, W.W., Li, X.H., Dai, Y., Peng, X.X., Fan, Y.T. and Hou, H.W., (2013). Isolation and characterization of a new hydrogen-producing strain *Bacillus* sp. FS2011. *International Journal of Hydrogen Energy*, 38(8), 3206-3212.
- Sørensen, B. and Spazzafumo, G. (2018). *Hydrogen and fuel cells: emerging technologies and applications*. Academic Press.
- Srirangan, K., Pyne, M.E. and Chou, C.P. (2011). Biochemical and genetic engineering strategies to enhance hydrogen production in photosynthetic algae and cyanobacteria. *Bioresource Technology*, 102 (18), 8589-8604.
- Srivastava, N., Srivastava, M., Kushwaha, D., Gupta, V.K., Manikanta, A., Ramteke, P.W. and Mishra, P.K. (2017). Efficient dark fermentative hydrogen production from enzyme hydrolyzed rice straw by *Clostridium pasteurianum* (MTCC116). *Bioresource Technology*, 238, 552-558.
- Stanbury, P.F., Whitaker, A. and Hall, S.J., (2013). *Principles of fermentation Technology*. Elsevier.
- Stavropoulos, K.P., Kopsahelis, A., Zafiri, C. and Kornaros, M. (2016). Effect of pH on continuous biohydrogen production from end-of-life dairy products (EoL-DPs) via dark fermentation. *Waste and Biomass Valorisation*, 7 (4), 753-764.
- Stephen, A.J., Archer, S.A., Orozco, R.L. and Macaskie, L.E. (2017). Advances and bottlenecks in microbial hydrogen production. *Microbial Biotechnology*, 10 (5), 1120-1127.
- Sterling Jr, M.C., Lacey, R.E., Engler, C.R. and Ricke, S.C. (2001). Effects of ammonia nitrogen on H₂ and CH₄ production during anaerobic digestion of dairy cattle manure. *Bioresource Technology*, 77 (1), 9-18.
- Taifor, A.F., Zakaria, M.R., Yusoff, M.Z.M., Toshinari, M., Hassan, M.A. and Shirai, Y. (2017). Elucidating substrate utilization in biohydrogen production from palm oil mill effluent by *Escherichia coli*. *International Journal of Hydrogen Energy*, 42 (9), 5812-5819.
- Tamagnini, P., Leitão, E., Oliveira, P., Ferreira, D., Pinto, F., Harris, D.J., Heidorn, T. and Lindblad, P. (2007). Cyanobacterial hydrogenases: diversity, regulation and applications. *FEMS Microbiology Reviews*, 31(6), 692-720.

- Tang, J., Wang, X., Hu, Y., Zhang, Y. and Li, Y. (2016). Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. *Waste Management*, 52, 278-285.
- Tanisho, S., Suzuki, Y. and Wakao, N. (1987). Fermentative hydrogen evolution by *Enterobacter aerogenes* strain E. 82005. *International Journal of Hydrogen Energy*, 12(9), 623-627.
- Tao, Y., Chen, Y., Wu, Y., He, Y. and Zhou, Z. (2007). High hydrogen yield from a two-step process of dark-and photo-fermentation of sucrose. *International Journal of Hydrogen Energy*, 32(2), 200-206.
- Tawfik, A. and El-Qelish, M. (2012). Continuous hydrogen production from co-digestion of municipal food waste and kitchen wastewater in mesophilic anaerobic baffled reactor. *Bioresource Technology*, 114, 270-274.
- Todar, K. (2008) Online Textbook of Bacteriology, University of Wisconsin, http://www.textbookofbacteriology.net/nutgro_5.html (accessed 1 September 2012)
- Trchounian, A. (2015). Mechanisms for hydrogen production by different bacteria during mixed-acid and photo-fermentation and perspectives of hydrogen production biotechnology. *Critical Reviews in Biotechnology*, 35(1), 103-113.
- Urbaniec, K. and Bakker, R.R. (2015). Biomass residues as raw material for dark hydrogen fermentation—A review. *International Journal of Hydrogen Energy*, 40 (9), 3648-3658.
- Valdez-Vazquez, I. and Poggi-Varaldo, H.M., 2009. Alkalinity and high total solids affecting H₂ production from organic solid waste by anaerobic consortia. *International Journal of Hydrogen Energy*, 34(9), 3639-3646.
- Veras, D.L., de Souza Lopes, A.C., Vaz da Silva, G., Gonçalves, A., Gazzoni, G., de Freitas, C.F., de Lima, F.C.G., Vieira Maciel, M.A., Feitosa, A.P.S., Alves, L.C. and Brayner, F.A. (2015). Ultrastructural Changes in Clinical and Microbiota Isolates of *Klebsiella pneumoniae* Carriers of Genes blaSHV, blaTEM, blaCTX-M, or blaKPC When Subject to β -Lactam Antibiotics. *The Scientific World Journal*, 1-13
- Vignais, P.M., Billoud, B. and Meyer, J. (2001). Classification and phylogeny of hydrogenases. *FEMS Microbiology Reviews*, 25(4), 455-501.

- von Klein, D., Arab, H., Völker, H. and Thomm, M. (2002). *Methanosarcina baltica*, sp. nov., a novel methanogen isolated from the Gotland Deep of the Baltic Sea. *Extremophiles*, 6(2), 103-110.
- Wang, J. and Wan, W. (2008). Effect of temperature on fermentative hydrogen production by mixed cultures. *International Journal of Hydrogen Energy*, 33(20), 5392-5397.
- Wang, X., Jin, B. and Mulcahy, D. (2008). Impact of carbon and nitrogen sources on hydrogen production by a newly isolated *Clostridium butyricum* W5. *International Journal of Hydrogen Energy*, 33 (19), 4998-5005.
- Wee, Y.J., Kim, S.Y., Yoon, S.D. and Ryu, H.W. (2011). Isolation and characterization of a bacterial celluloseproducing bacterium derived from the persimmon vinegar. *African Journal of Biotechnology*, 10 (72), 16267-16276.
- Weijun, Y. (2015). Analytical accuracy of hydrogen measurement using gas chromatography with thermal conductivity detection. *Journal of Separation Science*, 38(15), 2640-2646.
- Wiebe, W.J., Sheldon, W.M. and Pomeroy, L.R. (1992). Bacterial growth in the cold: evidence for an enhanced substrate requirement. *Applied and Environmental Microbiology*, 58(1), 359-364.
- Wong, Clemente, Heng Tam, Siti Alias, Marcelo González, Gerardo González-Rocha, and Mariana Domínguez-Yévenes (2011). "Pseudomonas and Pedobacter isolates from King George Island inhibited the growth of foodborne pathogens." *Polish Polar Research*, 32, 1: 3-14.
- Wu, K.J., Saratale, G.D., Lo, Y.C., Chen, W.M., Tseng, Z.J., Chang, M.C., Tsai, B.C., Su, A. and Chang, J.S. (2008). Simultaneous production of 2, 3-butanediol, ethanol and hydrogen with a *Klebsiella* sp. strain isolated from sewage sludge. *Bioresource Technology*, 99(17), 7966-7970.
- Wu, X., Wang, X., Yang, H. and Guo, L. (2010). A comparison of hydrogen production among three photosynthetic bacterial strains. *International Journal of Hydrogen Energy*, 35(13), 7194-7199.
- Xia, A., Cheng, J., Ding, L., Lin, R., Song, W., Su, H., Zhou, J. and Cen, K. (2015). Substrate consumption and hydrogen production via co-fermentation of monomers derived from carbohydrates and proteins in biomass wastes. *Applied Energy*, 139, 9-16.

- Xiao, Y., Zhang, X., Zhu, M. and Tan, W. (2013). Effect of the culture media optimization, pH and temperature on the biohydrogen production and the hydrogenase activities by *Klebsiella pneumoniae* ECU-15. *Bioresource Technology*, 137, 9-17.
- Xiong, H., Carter, R. A., Leiner, I. M., Tang, Y.-W., Chen, L., Kreiswirth, B. N. and Pamer, E. G. (2015). Distinct contributions of neutrophils and CCR2+ monocytes to pulmonary clearance of different *Klebsiella pneumoniae* strains. *Infection and Immunology* 83 (9), 3418-3427.
- Xiong, W., Lin, P.P., Magnusson, L., Warner, L., Liao, J.C., Maness, P.C. and Chou, K.J., (2016). CO₂-fixing one-carbon metabolism in a cellulose-degrading bacterium *Clostridium thermocellum*. *Proceedings of the National Academy of Sciences*, 113(46), 13180-13185.
- Xu, Z., Ben, Y., Chen, Z., Jiang, A., Shen, J. and Han, X. (2018). Application and microbial ecology of psychrotrophs in domestic wastewater treatment at low temperature. *Chemosphere*, 191, 946-953.
- Yakimov, M.M., Giuliano, L., Gentile, G., Crisafi, E., Chernikova, T.N., Abraham, W.R., Lünsdorf, H., Timmis, K.N. and Golyshin, P.N. (2003). *Oleispira Antarctica* gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *International Journal of Systematic and Evolutionary Microbiology*, 53 (3), 779-785.
- Yakimov, M.M., Golyshin, P.N., Lang, S., Moore, E.R., Abraham, W.R., Lünsdorf, H. and Timmis, K.N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 48 (2), 339-348.
- Yan, L., Ye, J., Zhang, P., Xu, D., Wu, Y., Liu, J., Zhang, H., Fang, W., Wang, B. and Zeng, G. (2018). Hydrogen sulfide formation control and microbial competition in batch anaerobic digestion of slaughterhouse wastewater sludge: Effect of initial sludge pH. *Bioresource Technology*, 259, 67-74.
- Yu, L., Cao, M.Y., Wang, P.T., Wang, S., Yue, Y.R., Yuan, W.D., Qiao, W.C., Wang, F. and Song, X., (2017). Simultaneous decolorization and biohydrogen production from xylose by *Klebsiella oxytoca* GS-4-08 in presence of azo dyes with sulfonate and carboxyl groups. *Applied and Environmental Microbiology*, 1-13.

- Zakaria, M.A., Yusoff, M.Z.M., Zakaria, M.R., Hassan, M.A., Wood, T.K. and Maeda, T. (2018). Pseudogene product YqiG is important for pflB expression and biohydrogen production in *Escherichia coli* BW25113. *3 Biotechnology*, 8(10), 435.
- Zeng, A.P. and Deckwer, W.D. (1996). Bioreaction techniques under microaerobic conditions: from molecular level to pilot plant reactors. *Chemical Engineering Science*, 51 (10), 2305-2314.
- Zhang, L., Chung, J., Ren, N. and Sun, R. (2015). Effects of the ecological factors on hydrogen production and [Fe–Fe]-hydrogenase activity in *Ethanoligenens harbinense* YUAN-3. *International Journal of Hydrogen Energy*, 40 (21), 6792-6797.
- Zhang, Y. and Shen, J. (2006). Effect of temperature and iron concentration on the growth and hydrogen production of mixed bacteria. *International Journal of Hydrogen Energy*, 31(4), 441-446.
- Zhang, Y., Wei, J., Yuan, Y. and Yue, T. (2019). Diversity and characterization of spoilage-associated psychrotrophs in food in cold chain. *International Journal of Food Microbiology*, 290, 86-95.
- Zhao, B., Cheng, D.Y., Tan, P., An, Q. and Guo, J.S. (2018). Characterization of an aerobic denitrifier *Pseudomonas stutzeri* strain XL-2 to achieve efficient nitrate removal. *Bioresource Technology*, 250, 564-573.
- Zhao, P., Fan, S.Q., Tian, L., Pan, C.M., Fan, Y.T. and Hou, H.W. (2010). Hydrogen production characteristics from dark fermentation of maltose by an isolated strain FP 01. *International Journal of Hydrogen Energy*, 35 (13), 7189-7193.
- Zhao, X., Xing, D., Fu, N., Liu, B. and Ren, N. (2011). Hydrogen production by the newly isolated *Clostridium beijerinckii* RZF-1108. *Bioresource Technology*, 102(18), 8432-8436.
- Zieliński, M., Korzeniewska, E., Filipkowska, Z., Dębowski, M., Harnisz, M. and Kwiatkowski, R. (2017). Biohydrogen production at low load of organic matter by psychrophilic bacteria. *Energy*, 134, 1132-1139.
- Zitomer, D.H. and Shrout, J.D. (1998). Feasibility and benefits of methanogenesis under oxygen-limited conditions. *Waste Management*, 18 (2), 107-116.
- Zogaj, X.; Bokranz, W.; Nimitz, M.; Römling, U. (2003). Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. *Infection and Immunity*, 71, 4151–4158.

Zorin, B., Pal-Nath, D., Lukyanov, A., Smolskaya, S., Kolusheva, S., Didi-Cohen, S., Boussiba, S., Cohen, Z., Khozin-Goldberg, I. and Solovchenko, A. (2017). Arachidonic acid is important for efficient use of light by the microalga *Lobosphaera incisa* under chilling stress. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1862 (9), 853-868.