# The Potential of Dark Fermentative Bio-hydrogen Production from Biowaste Effluents in South Africa

Patrick T. Sekoai, Michael Olawale Daramola‡

School of Chemical and Metallurgical Engineering, Faculty of Engineering and the Built Environment, University of the Witwatersrand, Wits 2050, Johannesburg, South Africa.

(679314@students.wits.ac.za, Michael.Daramola@wits.ac.za)

‡ Corresponding Author; Michael O. Daramola, University of the Witwatersrand, Johannesburg, South Africa,

Tel: +27 11 717 7536,

Fax: +27 11 717 7591, michael.daramola@wits.ac.za

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**Abstract-** Dark fermentation process technology could play an essential role towards the implementation of clean and sustainable energy markets, especially when it is produced from cost-effective processes. In recent years, South Africa has been experiencing a huge crisis in waste disposal due to the high level of urbanization and industrialization in the country. Landfills and incinerators are the most common waste disposal methods and are reported to have serious detrimental effect on the environment. However, biowaste materials of agricultural, municipal, and industrial effluents are highly considered as suitable substrates for dark fermentative biohydrogen production due to their accessibility and nutritional content. In 2012, 22.9 million tons of biowaste (agricultural, municipal, and industrial effluents) was produced in South Africa and the amount increased to 26.2 million tons in 2014. Over the next decades in South Africa, an increase of 11 million tons/year has been predicted due to high level of infrastructure development in the country. This review, therefore, provides an outlook of South Africa's energy sector and discusses the need for intensification of alternative energy resources in order to reduce the country's reliance on coal energy along with environmental pollution. It evaluates the feasibility of using biowaste effluents for dark fermentative biohydrogen production processes. And assesses the environmental consequences associated with their disposal. It examines the state-of-the-art and advancements in biohydrogen process infrastructure in South Africa. Finally, it reviews the challenges facing dark fermentative biohydrogen scale-up studies and recent advances used to improve its process yields from these feedstocks.

**Keywords** Dark fermentation, Biowaste effluents, Renewable energy, South Africa, Biohydrogen.

## 1. Introduction

The continual use of conventional fuels has resulted in severe challenges of greenhouse gas emissions, environmental concerns, and escalating energy demands [1]. Besides, the United Nations predicted a global population of 6.8 billion in 2009 and expects this value to increase by 47% in 2050, which corresponds to 8.9 billion people [2]. The estimated population will aggravate the problems of climate change along with energy demands. Furthermore, energy agencies have shown that the global carbon dioxide emissions reached a staggering 35.7 billion tons in 2015 [3]. Similar reports have indicated that the current $CO_2$ levels exceed 390 ppm, and the $CO_2$ concentrations have been increasing by more than 3.30 ppm per year over the past decade [4]. Thus, if no effective measures are taken, the amounts of atmospheric $CO_2$ could reach 500 ppm in 2035 causing an alarming temperature increase of 5 °C [5].

The effects of climate change are also being felt in South Africa i.e. there's been a drastic decline in the country's agricultural outputs due to low rainfall seasons and temperature rise [6]. Many parts of the country are experiencing drought and therefore are no longer suitable for commercial farming. Climatologists have warned that climate change will have serious consequences on the following: (i) South Africa's coastal regions are expected to have an atmospheric temperature rise of 2ºC in 2050 and 4ºC by 2100, (ii) the country's interior regions are also expected to increase by 4ºC in 2050 and 7ºC in 2100, (iii) This will affect the country's food security, (iv) Alien invasive plants might increase and negatively affects the country's water resources, (v) This will likely exacerbate the health issues due to droughts and floods. Diseases such as malaria and cholera have been linked to extreme weather patterns, (vi) Bushlands and various commercial plantations will be vulnerable to wildfires [6]. Therefore, diversification of energy fuels is an important requirement in the present global energy scenario [7]. Recent analysis in the world energy outlook suggests that renewable based technologies will provide a huge contribution to global energy provision within the next decades; currently they are only contributing about 15% of global energy supply [8]. Hence, this highlights a crucial need to promote their acceleration in order to boost the global energy supply and mitigate environmental pollution.

Hydrogen is a promising energy option due to its characteristics which include high energy yield of 122 kJ/g and its carbon-neutral abilities [9]. These properties make it an attractive fuel that can be used to reduce the heavy reliance on fossil fuel economy [10-11]. Presently, there are more than 400 projects globally that focuses on the implementation of hydrogen-producing technologies. These initiatives form part of a global plan to boost energy security while mitigating environmental pollution by intensifying the hydrogen markets [12]. Hydrogen-producing technologies are also envisioned to increase significantly from 6% in 2020 to 50% in 2050. During this period, hydrogen infrastructures are expected to develop and become progressively more important in decarbonizing the current energy systems [13]. Hydrogen is commercially produced from thermochemical, photochemical, electrochemical, photocatalytic, and photo-electrochemical processes [14]. The drawback about these processes is that they are expensive, contributes to greenhouse gas emissions, and uses high energy [15]. One attractive avenue for production of hydrogen is through the biological methods. Biological hydrogen methods are advantageous because they are environmental benign and cost-effective, thus being more competitive to thermochemical processes [16-17]. The biological hydrogen routes include photosynthetic and dark fermentation process. However, dark fermentation is a preferred process because it can be conducted at moderate temperatures and pressure; it can use diverse feedstocks and microorganism for its process. Moreover, dark fermentation process development has gained a tremendous impetus and governmental support in more than 30 countries worldwide [18].

Therefore, this review provides an outlook on energy sector in South Africa and highlights the need for implementation of clean and sustainable energy fuels. It comprehensively assesses the potential of using biowaste materials of agricultural, municipal and industrial process effluents for dark fermentative biohydrogen production in South Africa, while confronting their negative impacts on the environment. In addition, it critically evaluates the state-of-the-art and advancements in biohydrogen process infrastructure in South Africa. Finally, it discusses the technical challenges facing dark fermentative biohydrogen process economy and strategies that have been recommended for its scale-up.

## 2. Hydrogen Energy

### *2.1. Its importance, application, and production methods*

Reducing the reliance on hydrocarbon fuels and minimizing environmental pollution can only be realized by introducing clean and sustainable energy resources. Over the past few decades, hydrogen has captured increasing global attention as an alternative to fossil fuels owing to its several merits which include (i) zero-carbon emissions, (ii) high energy yield, (iii) abundance, and (iv) diverse storage forms (e.g. gaseous, liquid, or coupled with metal hydrides). Most developed countries have therefore realized the future role of hydrogen and thus the concept of a "Hydrogen Driven Economy" was proposed by international hydrogen endorsement energy agencies such as the US Department of Energy (US DOE), European Hydrogen Association (EHA), and the International Partnership for Hydrogen Economy (IPHE) in efforts to intensify and commercialize its production [19]. The US DOE indicated in 2015 that it aims to invest about 35 million US dollars towards hydrogen infrastructure development projects as the country plans to reduce its dependence on foreign oil [19]. Hydrogen gas is extensively used in various industrial applications i.e. ammonia synthesis, methanol production, used in oil refineries for removal of impurities, used in processing of steel, electronic devices, and in desulfurization and reformation of gasoline. Furthermore, car manufacturers have now started to create vehicles that are powered by hydrogen fuel cells and are reported to be effective than gasoline powered engines [19]. The global annual production of hydrogen is currently projected at 62 million tons, and has an annual growth rate of 8-10% [19]. Amongst the industrial hydrogen production processes, steam reforming is an extensively used method. It produces nearly up to 50% of hydrogen; oil reforming produces nearly 30% of hydrogen, coal gasification yields about 18%, 3.9% comes from water electrolysis, and 0.1% from other methods [20]. However, these processes present a major challenge because they are energy intensive and contribute to greenhouse gas emissions. To alleviate the negative effects of fossil fuel utilization, hydrogen needs to be produced from clean and sustainable methods. In the past few decades, researchers have started to look into biotechnological hydrogen producing approaches such as dark and photo-fermentation methods to yield cleaner hydrogen energy. The hydrogen producing methods are summarized in Fig. 1.

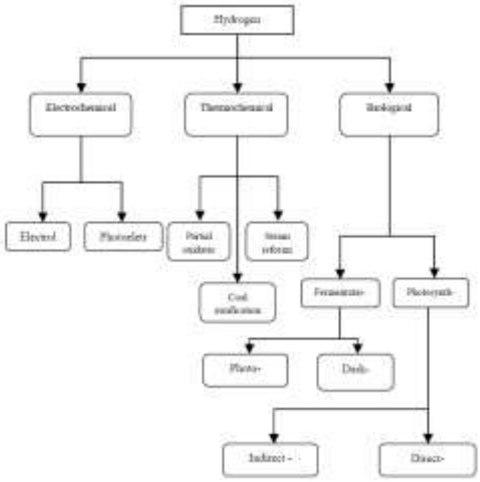


**Fig. 1.** Hydrogen producing methods [20].

## 3. An Outlook on Energy Sector in South Africa

### *3.1. Coal as a primary energy resource*

South Africa is dependent on coal as its main energy source while the rest of the world is dependent on crude oil. Data from BP South Africa (Pty) Ltd showed that coal supplies approximately 72% of energy, followed by crude oil at 22% [21]. Other sources of energy such as nuclear, gas, and renewable fuels are only contributing less than 10% of total energy supply as illustrated in Fig. 2 [21]. Moreso, coal is used by both the private and government sector for generation of electricity. There are five major companies that use more than 80% of the country's coal i.e. BHP Billiton, Anglo-American, Sasol, Exxaro, and Xstrata. The South African power parastatal Eskom which is the largest producer of electricity in Africa and ranked amongst the top energy utilities in the world [22], accounts for 70% of coal that is used for supplying the country's electricity (Fig. 3). The extensive use of coal as a primary energy fuel is due to its widespread availability. South Africa has 19 coal mines that are situated in the provinces of Eastern Cape, North West, Limpopo, KwaZulu-Natal, Free State, Mpumalanga, and Gauteng [23]. However, some of these mines have been abandoned due to depletion of coal reserves (see Fig. 4) [23].

### *3.2. Shortcomings of coal energy*

Several reports have highlighted that South Africa's dependence on coal will cause these reserves to be exhausted sooner than it is anticipated. For example, de Jager [24] postulated these reserves at 58.4 billion tons. Thereafter, Bredell [25] forecasted them at 55.3 billion tons. The Department of Mineral Resources projected them at 33.8 billion tons in 2000. A further decline was confirmed by Hartnady [26]; they were predicted at 15 billion tons. South Africa produces significant amount of carbon dioxide i.e. it generated about 1.4% of $CO_2$ globally and 40% of $CO_2$ within the continent in 2011, therefore making it the highest in Africa and 14$^{th}$ in the world [27]. Moreso, the country's energy consumption has drastically increased the levels of $CO_2$ emissions by 18% from 2001 to 2011 [26]. South Africa's power parastatal (Eskom) has been facing an immense pressure as a result of the country's escalating energy demands. The power utility is presently functioning at near full-scale i.e. it has a production capacity of 40 gigawatts whereas the country's peak demand is 36 gigawatts [27]. This caused persistent power shortages and blackouts which resulted in an economic decline of approximately 282 million US dollars [28]. This crisis is exacerbated by fact that the country's coal stations are old and thus regularly needs maintenance and also have a small capacity [29].

### *3.3. South Africa's alternative energy policy*

South Africa has massive clean alternative energy resources like biomass, wind, solar, and marine energy that could be used in the mitigation of carbon dioxide emissions, and improve the country's energy security [30]. Therefore, the Department of Energy emphasized the need to diversify the country's energy mix in order to curb the problems associated with energy derived from coal. This triggered off the formation of South African National Energy Development Institute (SANEDI), which is an organization formed in 2008. The main purpose of SANEDI is to implement and propose strategic policies and frameworks for development of alternative and sustainable energy development in South Africa by collaborating with various stakeholders such as private, government and academic institutions. In addition, its role is to ensure that South Africa has the necessary skills, expertise, and resources for implementation of alternative energy based technologies to address the country's economic, environmental, and social needs [30].

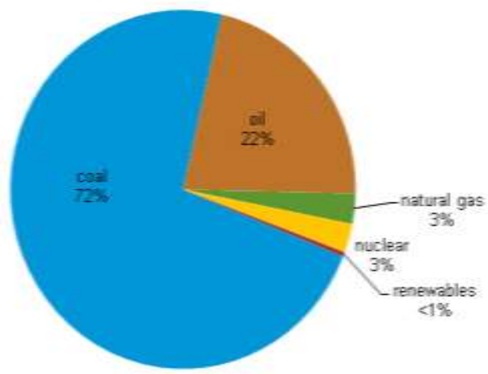


**Fig. 2.** Energy utilization in South Africa [21].

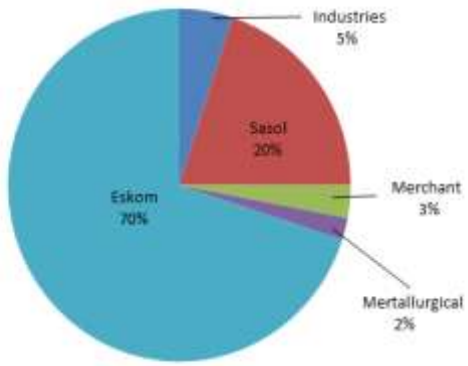


**Fig. 3.** Coal usage in South Africa [30].

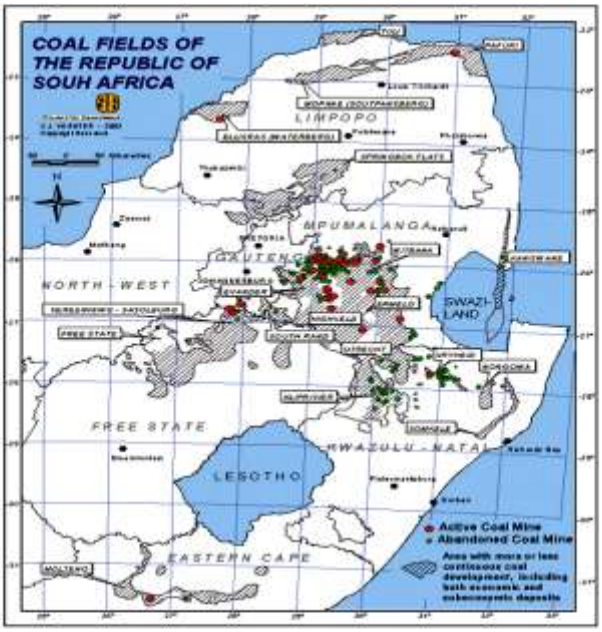


**Fig. 4.** Map showing the distribution of coal mines in South Africa.Active mines are indicated with red dots and abandoned mines are indicated with green dots [31].

*3.4. Types of alternative energy used in South Africa*

South Africa is currently using various forms of renewable energy resources which include nuclear, wind, and solar energy. These technologies are elaborated below.

*3.4.1. Solar energy*

Solar resources include solar water heaters for hot water supply and solar power for generating electricity. The potential for solar water heaters is huge in South Africa, studies show that approximately 400 000 homes are installed with solar water heaters every year [32]. It has been shown that about 4% of residential electricity consumption results from heating of geysers. Moreover, their application is motivated by the socio-economical needs for energy security, environmental sustainability, and reducing the usage of electricity. This technology is currently being applied in other countries such as China (Rizhao) whereby 99% of households are reported to be using solar water heaters [33]. The Department of Energy in South Africa proposed a 5 million long-term plan of installing solar water heaters across the country by 2020. With regards to the utilization of solar power for electricity generation, Eskom installed a 25 kW solar panel as part of the initiatives from the South African government to assess this technology. Besides, Eskom joint collaboration with the University of Stellenbosch resulted in the construction of the SKA Meerkat Radio Telescope Array (Northern Cape, South Africa) which began in 2012 [33].

*3.4.2. Wind energy*

In recent years, development of wind projects has been increasing in South Africa. In 2014, the country launched one of its biggest wind farms in Africa i.e. the Jeffrey's Bay Wind Farm located near Humansdorp in the province of Eastern Cape was built by the British based company Globleleq (Pty) Ltd. The farm comprises of 60 (80 metre high) wind turbines which spread over 3700 hectares and can produce up to 138 megawatts of electricity (Banks and Schaffler, 2006). Other projects include the Klipheuwel Wind Energy Demonstration Facility (KWEDF) which has a total capacity of 3.2 megawatts [33].

*3.4.3. Nuclear energy*

The South African government is in the process of building new nuclear power plants in the country. Two nuclear reactors which are currently operating in Koeberg Power Stations accounts for 4% of the country's electricity supply. However, the country intends to generate 9600 megawatts from the new nuclear power plants that are about to be constructed [34].

## 4. Integration of Biofuel into South African Energy Mix

South Africa aims to strengthen its alternative energy options in order to cope with high energy demands and reduce its carbon footprints. Diversification of alternative energy resources will assist the country to reduce the high costs of imported petroleum oil. Thus, biofuel production technologies have the potential to expand and diversify South Africa's energy supply, which will in turn reduce the country's dependence on dwindling coal reserves and intensify its energy supply. Furthermore, biofuel development initiatives are gaining increasing momentum in developing countries like South Africa and are foreseen as a catalyst for (i) infrastructural development projects, (ii) reducing high international oil prices, (iii) boosting the country's energy sector, (iv) and creation of employment opportunities [35].

## 5. Biofuel Energy Development Initiatives in South Africa

Biofuels contributes up to 14% of energy in South Africa [36]. Biomass derived energy is extensively used by rural and other low-income urban households to generate fuel that is used for cooking and heating. It is also used in boilers by various South African industries to generate electricity [36]. The Department of Energy announced in 2013 that it aims to begin a regulatory blending process of diesel and petrol with biofuels as from 2015; this is intended to stabilize the country's biofuel sector thereby reducing its reliance on hydrocarbon fuel [36]. In addition, it also proposed a five-year pilot-phase plan which is aimed at achieving 2-5% of biofuels. To date, five companies have been granted licenses to produce bioethanol and biodiesel in South Africa. Analysis of potential feedstocks that can be used reveals that sorghum is suitable for bioethanol production while soybeans are potential feedstocks for biodiesel production [36]. However, maize has been excluded from these feedstocks because it's one of the country's stable foods and this may affects the country's food security. Other biofuel development initiatives include the Bronkhorstspruit Biogas Plant which is located in Pretoria and is owned by the Bio2Watt Company. The company is the leading commercial-scale biogas producer in South Africa and uses approximately 120 000 tons of biowaste effluents to generate biogas [37]. It has partnered with a leading car manufacturer (BWW, South Africa) which uses the biogas in their production plant. Moreover, as South Africa is experiencing a huge influx of biomass generated from the agricultural, municipal, and industrial sector; other potential biofuel options such as dark fermentative biohydrogen production will contribute enormously in the intensification of cleaner energy production in the country.

## 6. Biohydrogen Production Potential in South Africa

### *6.1. The potential of dark fermentative biohydrogen production in South Africa*

Recently, South Africa has been focusing on the implementation of other biofuel options such as dark fermentation process because of its non-polluting and waste beneficiation characteristics [29]. Dark fermentation from biowaste effluents is advantageous in South Africa because the country is experiencing an enormous burden with regards to its waste management methods i.e. thus the concept of "waste-to-energy" has been gaining increasing support from various stakeholders within the country. Secondly, dark fermentation can be generated from diverse biowaste effluents (e.g. agricultural, industrial, and municipal) which are abundantly available and are causing a disposal challenge. The utilization of these effluents makes this process economically viable in contrast to other energy generating methods. Other biohydrogen producing methods include photo-fermentation, direct, and indirect biophotolysis [38]. However, dark fermentation is a highly favoured process because of its simplicity, cost-effectiveness, and sustainability. Moreso, researchers throughout the world are focusing on this process because its uses naturally available microorganisms that are found on various habitats (sewage treatment works, soil, etc) and convert these effluents into hydrogen energy [14, 39-40].

### *6.2. State-of-the-art and biohydrogen process advancement in South Africa*

Hydrogen based infrastructures is undergoing serious consideration in South Africa in efforts to develop cleaner, reliable and sustainable energy fuels. A ten year innovation plan was proposed by the Department of Science and Technology in 2008. This strategic plan involved the development of alternative energy resources that would assist in reducing carbon emissions and also contributes to the country's high energy demands. Therefore, Hydrogen South Africa (HySA) was established in the same year [50]. The purpose of HySA is to develop innovation towards the implementation of hydrogen technologies in South Africa. It consists of three centres of competence which are HySA Infrastructure, HySA Catalysts, and HySA Systems. These research centres are co-hosted by five institutions namely Mintek, University of Cape Town, North West University, University of Western Cape, and Council for Scientific and Industrial Research (CSIR) [50].

The HySA Infrastructure focuses on the development of hydrogen production technologies through small and medium-scale hydrogen producing reactor prototypes [50]. The group is also researching on hydrogen storage materials. HySA Catalysts is joint research collaboration between Mintek and the University of Cape Town; they are responsible for developing industrial value chain catalysts that will enhance hydrogen fuel cell technologies. The HySA Systems aims to develop and improve hydrogen-based technologies and is chaired by the University of the Western Cape. Its objectives are to (i) develop hydrogen fuelled vehicles system prototypes, and (ii) conduct validation and hybrid processes within the HySA research centres which are (i) combined heat and power, (ii) miniaturized bioprocess systems, and (iii) hydrogen fuelled cars [51]. Therefore, establishment of HySA could pave a way for the advancement of hydrogen markets in South Africa.

Despite the biohydrogen development initiatives that have been carried out by various research institutions, this technology is still under research and development (R & D) stages in most countries including South Africa, implying that most biohydrogen production studies have been carried out at bench-scale by various researchers across South Africa [41-49]. This prompts the need for extensive large-scale processes in order to fully understand the process dynamics (e.g. setpoint conditions, partial pressure, heat transfer, mass transfer, etc) involved during its production and this will provide reliable scalable data that could be used towards its industrialization.

## 7. Bio-waste Production in South Africa

Over the past years, South Africa witnessed a drastic increase in waste production due to the high level of urbanization and industrialization as mentioned earlier. The total waste distribution data for South Africa in 2014 is shown in Table 1; an estimation of 7.80 million tons of waste was produced by the municipal sector. The agricultural sector generated 2.95 million tons, whereas the industrial sector generated 12.1 million tons of waste [52]. The amount of biowaste generated by each province is also presented in Table 2. It is apparent from this data that South Africa is experiencing a significant growth in waste volumes. As a result, 42.3 million tons of organic municipal waste was generated in 1997 and this value increased to 69 million in 2014. During this period, the production of biowaste rose up to 63.1%. Data from the Department of Environmental Affairs have also indicated that waste volume in South Africa increases by approximately 11 million tons each year [52]. Therefore, biowaste materials will have enormous burden on the environment and people if is not properly managed. Waste beneficiation approaches such as dark fermentative biohydrogen production processes will significantly assist to curb environmental pollution while generating clean and sustainable energy.

**Table 1.** Total waste distribution data in South Africa [52]

| Waste type | Produced | Recycled | Disposed | % Recycled |
|---|---|---|---|---|
| Municipal | 7 800 328 | - | 7 800 328 | 0 |
| Agricultural | 2 954 461 | 1 034 061 | 1 920 400 | 35 |
| Industrial | 12 120 783 | 9 255 376 | 2 865 407 | 76 |
| Saltwater | 4 166 129 | - | 4 166 129 | - |
| Fly ash and dust | 31 420 488 | 1 885 229 | 29 535 259 | 6 |
| Bottom ash | 5 385 968 | - | 5 385 968 | - |
| Slag | 5 000 150 | 2 500 075 | 2 500 075 | 50 |
| Mineral | 335 000 | - | 335 000 | - |
| Electronic | 66 321 | 6 975 | 59 346 | 11 |
| Sewage sludge | 657 963 | 125 013 | 493 472 | 19 |
| Miscellaneous | 327 250 | - | 327 250 | - |
| Construction | 4 735 142 | 766 017 | 3 969 125 | 16 |
| Paper | 1 649 257 | 938 900 | 710 357 | 57 |
| Plastic | 1 287 701 | 240 162 | 1 047 539 | 19 |
| Glass | 938 769 | 301 218 | 637 551 | 32 |
| Metals | 3 181 213 | 2 466 960 | 714 253 | 78 |
| Tyres | 245 633 | 9 866 | 235 767 | 4 |
| Other | 36 161 137 | - | 36 161 137 | 0 |

-: data not available

**Table 2.** Biowaste generated by the nine provinces of South Africa [52]

| | 1997 | | 2014 | | 1997-2014 | 1997-2014 |
|---|---|---|---|---|---|---|
| Province | Tons | % | Tons | % | Total growth % | Annual average growth % |
| Eastern Cape | 2 382 000 | 5.6 | 3 215 929 | 4.6 | 35.0 | 2.9 |
| Free State | 1 667 000 | 9.9 | 3 887 381 | 5.6 | 133.2 | 7.3 |
| Gauteng | 17 899 000 | 42.3 | 26 085 304 | 37.8 | 45.7 | 3.2 |
| KwaZulu-Natal | 4 147 000 | 9.8 | 5 754 823 | 8.3 | 38.7 | 3.2 |
| Limpopo | 3 781 000 | 8.9 | 11 320 317 | 16.4 | 199.4 | 9.6 |
| Mpumalanga | 738 200 | 1.7 | 986 392 | 1.4 | 33.6 | 2.4 |
| Northern Cape | 1 487 321 | 3.5 | 2 482 874 | 3.6 | 66.9 | 4.4 |
| North West | 1 652 100 | 3.9 | 2 289 499 | 3.3 | 38.6 | 3.0 |
| Western Cape | 8 553 000 | 20.2 | 12 989 885 | 18.8 | 51.9 | 3.6 |
| **Total** | **42 306 621** | **100** | **69 012 404** | **100** | **63.1** | **4.3** |

## 8. Elemental Composition of South African Bio-waste Effluents

As a preliminary investigation, we conducted CHNS/O analysis of carbon, hydrogen, nitrogen, sulphur, and oxygen elements contained in South African biowaste effluents in our laboratory using a Flash 2000 CHNS/O Analyzer (Thermo Scientific, USA). Oxygen (wt. %) was calculated by the difference of C, H, N, S, which was subtracted from 100. The chosen effluents are highly abundant in South Africa and form a huge fraction of the country's biowaste materials. Thus, organic composition of these effluents is crucial because they affect the overall microbial conversion yields of dark hydrogen producing bioprocesses. Furthermore, these elements also affect the activity of biohydrogen-producing hydrogenase enzymes [53]. The C, H, N, S, and O composition is shown on Table 3.

**Table 3.** CHNSO composition of South African biowaste effluents analyzed

| Elemental composition (%) | C | H | N | S | O |
|---|---|---|---|---|---|
| Apple | 42.58 | 6.51 | 0.36 | - | 50.55 |
| Bread | 40.47 | 5.94 | 1.81 | - | 51.78 |
| Brewery | 42 | 6.19 | 2.01 | - | 49.8 |
| Cabbage | 39.46 | 5.45 | 3.42 | 1.05 | 50.62 |
| Corn-cob | 42.8 | 5.88 | 0.49 | - | 50.83 |
| Kitchen | 43.8 | 6.53 | 2.36 | - | 47.31 |
| Mango | 53.74 | 8.05 | 1.03 | - | 37.18 |
| Pear | 41.89 | 6.45 | 0.33 | - | 51.33 |
| Potato | 40.45 | 5.86 | 0.3 | - | 53.39 |
| Sugarcane | 42.22 | 6.37 | 0.3 | - | 51.11 |

-: not detected.

## 9. Disposal Challenges Associated with Bio-waste Effluents in South Africa

In South Africa, major cities are experiencing increasing population growth due to high level of urbanization and industrialization as highlighted in this review. Furthermore, there is a rapid infrastructure development occurring in these cities in order to cater for the needs of its inhabitants. As a result, there has been a sporadic increase in the generation of biowaste materials. Biowaste materials of agricultural, municipal, and industrial effluent pose serious health risks on people living in these sites. Landfill sites have been underlined as the possible cause of birth defects and respiratory illnesses such as asthma (Broomfield et al., 2004). Incinerators have also been linked to these illnesses. Moreover, composting and material recycling facilities have been linked to odours and lung related diseases such as bronchitis [54]. The Department of Health has raised concerns about the disposal of these effluents because they attract disease vectors such as mosquitoes, flies, and rats to breed in landfills and spread diseases [55].

From an environmental standpoint, biochemical decompositions reactions produce substantial amounts of greenhouse gases (e.g. methane and carbon dioxide) on landfills and are released into the atmosphere. It has also been reported that other toxic gases such as ammonia are formed during biodegradation of biowaste materials [56-57]. A study by Viitez et al. [58] indicated that the biological conversion of biowaste on landfills occurs on a slow rate and it could take years to complete i.e. the authors reported that anaerobic digestion reactions on landfills may extend up to 20-40 years and this poses serious detrimental effects on the environment [58]. The United Nations has indicated that the disposal of these effluents will significantly increase in developing nations like South Africa than in less developed regions, due to rapid infrastructure development that is occurring in these regions [54].

In other related studies, Devesa-Rey et al. [59] showed that the costs of recycling these effluents and the penalties imposed on companies have increased significantly in recent years, often reaching millions of dollars. These fines are sometimes combined with other penalties, such as the obligation to decontaminate polluted areas which can involve considerable expenses for companies. In this regard, the South African Environmental Legislation mandates government municipalities and industries to dispose their effluents in a manner that will not cause a threat to people and the environment. Nonetheless, the current waste disposal methods do not comply with these regulations, implying that new and innovative approaches for biowaste management are needed to address these challenges.

## 10. Feasibility of Bio-waste Effluents for Dark Fermentation in South Africa

Studies in literature have assessed the potential of various carbon sources such as glucose [60], sucrose [61-62], and xylose [63-64] on dark fermentative bioprocess yields. Even though this process is well researched from these sugars, utilization of these substrates is too expensive to support the dark fermentative "biohydrogen driven" economy in South Africa i.e. the cost of substrates account for approximately 60% of the overall bioprocess costs [65]. Therefore, the use of biowaste effluents for its production will significantly enhance its process economics because these feedstocks are readily available, considered waste materials, and possess high hydrogen efficiency. Feedstocks such food materials are highly favoured substrates because they are rich in nutritional composition i.e. 80-95% volatile solids, and 75-85% moisture, thus favouring the enumeration of dark biohydrogen-producing bacteria during dark fermentation processes [66-69].

The latent energy present in these effluents can be recovered via microbial bioprocesses to produce biohydrogen. The potential of using these effluents for dark fermentation processes is highly documented in literature [17, 70, 71, 63, 69, 70]. Examples of dark fermentation yields reported are 138 ml $H_2$/g VS, 92 ml $H_2$/g TVS, 126.9 ml $H_2$/g TVS, 183 ml H2/g TVS, 189 ml $H_2$/g COD, and 78 ml $H_2$/g COD respectively. These studies were conducted at different operational set-point conditions of temperature (30-48 $^oC$) and pH (5-6), deemed favourable for biohydrogen fermentation studies [68-69]. In addition, other associated substrates such as wastewaters from food processing industries have a great potential for dark fermentation due to their nutritional content. For example, South Africa is listed amongst the top seven wine producers in the world, and therefore the wine industry yields large quantities of wastewater each year. Approximately one billion litres of wastewater are produced from more than three thousand wine distillers in South Africa [72]. Wastewater from wine industries is rich in COD (300 - 60 000 mg/l), has a pH range of 3 - 8, and consists of various trace elements (Ca, K, Na, and Mg) which makes it an ideal substrate for dark fermentation processes [73]. Other huge sectors such as the sugarcane industry (generates up to 20.6 million tons of sugarcane per annum) produce large volumes of molasses which has a high concentration of fermentable sugars and COD (50–100 g/l) [74]. Several researchers assessed the biohydrogen production potential from wastewaters; Lin et al. [75] studied the effect of food processing wastewaters of fructose and molasses on dark fermentation, and obtained a biohydrogen yield of 167 ml H2/g COD for wastewater of fructose and 187 ml H2/g COD for wastewater of molasses respectively. Van Ginkel et al. [76] investigated dark biohydrogen production from different wastewaters (potato, apple pomace, and confectioners), and reported a high yield of 210 ml $H_2$/g COD from potato wastewater. These studies present a viable approach towards an economically feasible dark fermentative biohydrogen production process based on the beneficiation of waste. Table 4 shows various studies that have utilized agricultural, municipal, and industrial biowaste materials for dark fermentative biohydrogen fermentation processes. The biohydrogen production yields varied due to several factors such as (i) inoculum type, (ii) operational conditions, (iii) bioreactor design, (iv) type of substrate, and (v) working volume. Hence, this review presents strategies for optimizations of dark hydrogen fermentations from these biowaste effluents which are discussed in section 14.2.

**Table 4.** Biohydrogen fermentation processes from various biowaste materials

| Inoculum | Substrate | $H_2$ yield | % $H_2$ | Reference |
|---|---|---|---|---|
| Anaerobic sludge | Food waste | 38 ml $H_2$/g VS | 49.1 | [77] |
| Seed sludge | Food waste | 70.7 ml $H_2$/g TVS | - | [78] |
| *Bacillus* sp. | Organic waste | 61 ml $H_2$/g VS | - | [79] |
| *Aspergillus Awamori* | Bread waste | 7.4 $H_2$ L/Ld | - | [80] |
| Anaerobic sludge | Potato waste | 171.1 ml $H_2$/g VS | - | [81] |
| Anaerobic sludge | Paper waste | 140 ml $H_2$/g total sugar | - | [82] |
| Anaerobic sludge | Potatoes | 106 ml $H_2$/g VS | 41-55 | [17] |
| Anaerobic sludge | Lettuce | 50 ml $H_2$/g VS | 37-67 | [17] |
| Mixed cultures | Rice waste | 2.14 mol $H_2$/mol hexose | 53-61 | [83] |
| Sewage sludge | Biosolids | 10 ml $H_2$/g COD | - | [84] |
| *Clostridium + Enterobacter* | Sweet potato (5%) | 7.0 mol $H_2$/mol glucose | - | [85] |
| *Clostridium + Enterobacter* | Sweet potato (2%) | 4.5 mol $H_2$/mol glucose | - | [85] |
| Mixed cultures | Fructose wastewater | 166.8 ml $H_2$/g COD | - | [75] |
| Mixed cultures | Molasses wastewater | 187 ml $H_2$/g COD | - | [75] |
| Sewage sludge | Food waste | 205 ml $H_2$/g VS | 52-56 | [86] |
| Sewage sludge | Mixed organic waste | 52.5-71.3 $H_2$ L/kg VS | - | [87] |
| Anaerobic sludge | Food waste | 97 ml $H_2$/g VS | - | [70] |
| Anaerobic digester | Food waste | 1-2.3 mol $H_2$/mol hexose | 43.9-51.4 | [88] |
| Anaerobic digester | Food waste | 96-114 ml $H_2$/g VS | - | [89] |
| Anaerobic sludge | Mixed organic waste | 76 ml $H_2$/g COD | - | [69] |
| *Caldicellulosiruptor* | Maize leaves | 18 ml $H_2$/g TVS | - | [90] |
| *Caldicellulosiruptor* | Sweet sorghum | 32.4 ml $H_2$/g VS | - | [90] |
| *Caldicellulosiruptor* | Bagasse | 19.6 ml $H_2$/g VS | - | [90] |
| Anaerobic sludge | Cabbage | 26.3-61.7 ml $H_2$/g TVS | - | [91] |
| Anaerobic sludge | Carrot | 44.9-70.7 ml $H_2$/g TVS | - | [91] |
| Anaerobic sludge | Rice | 19.3-96.0 ml $H_2$/g TVS | - | [91] |
| Digested sludge | Potato | 30 L $H_2$/kg TS | 45 | [92] |
| Mixed cultures | Wheat starch | 92 mol $H_2$/g starch | - | [93] |
| Anaerobic sludge | Sugarbeet juice | 1.9 mol $H_2$/mol hexose | - | [94] |
| Mixed cultures | Sweet sorghum | 10.4 L $H_2$/kg TS | - | [95] |
| Heat treated soil | Apple processing waste | 0.9 L $H_2$/g COD | - | [76] |
| Heat treated soil | Potato processing waste | 2.8 L $H_2$/g COD | - | [76] |
| Digested sludge | Cheese whey | 0.83 mol $H_2$/mol glucose | - | [96] |
| Anaerobic sludge | Cassava waste | 851.84 ml $H_2$/h | - | [97] |
| Activated sludge | Cornstalk | 126.22 ml $H_2$/g CS | - | [98] |
| Dairy manure | Corncob | 757.7 ml $H_2$/g COD | - | [99] |
| *Clostridium butyricum* | Sugarcane bagasse | 1.73 mol $H_2$/mol TS | - | [100] |
| Anaerobic sludge | Corn starch | 0.51 mol $H_2$/mol glucose | - | [101] |

-: data not available

## 11. Classification of Biohydrogen-producing Bio-waste Effluents

### *11.1. Agricultural waste*

Agricultural residues consist mainly of lignocellulose materials which are abundantly available in South Africa. They are economically feasible because they are cheaper and easily accessible feedstocks [44]. However, these waste materials create a disposal challenge in most countries including South Africa because most of them have a slow degradation process and contains high mineral content. Hence, they are mostly burnt which increases air pollution and jeopardize human health. The plant biomass of these substrates consists of lignin, cellulose, and hemicellulose which must undergo vigorous pretreatments to release the fermentable sugars (e.g. glucose, galactose, etc). Examples include bean husks, grasses, corn cobs, wheat straw, and other materials [11].

### *11.2. Organic fraction of municipal solid waste*

Food waste consists of a huge percentage of Organic Fraction of Municipal Solid Waste (OFMSW); it is rich in nutritional content (85-95% volatile solids and 75-85% moisture). And its nutritional characteristics make it an ideal substrate for dark hydrogen bioprocesses [15]. It also comprises of other fermentable rich materials that are found in raw and cooked food products that are discarded on recycle bins and landfills. However, it poses an environmental challenge because it generates odour and pests [15].

### *11.3. Industrial waste*

Industrial waste includes effluents from sugar refineries, cereals, cheese, brewery, paper, and beverage processing companies. These industries produce large quantities of wastewater which contains sugars and starch that are high in carbohydrates. Thus, this favours the production of dark biohydrogen production which is generated by a series of biochemical pathways manifested by acidogenic-producing bacterial species such as *Clostridium* and *Bacillus* species [15]. The exploitation of wastewater for dark fermentative biohydrogen production process provides a platform for generation of clean energy while removing contaminants in water [17]. Moreover, utilization of wastewaters for energy production is advantageous because it does not generate environmental pollution, and there's simultaneous energy recovery [17].

### *11.4. Other types of bio-waste substrates*

Besides the abovementioned substrates, other feedstocks that have been used in dark fermentative biohydrogen production processes include:

- Livestock manure [102-103].
- Perennial grasses [44, 104].
- Algal biomass [105-106].
- Waste sludge [75, 84].

All of these biowaste feedstocks are classified in Fig.5. Municipal and industrial effluents are ideal substrates for biohydrogen producing bacteria because they contain low lignin content and they're also rich in carbohydrate composition as compared to agricultural waste, which requires various pretreatment methods in order to access the fermentable sugars.

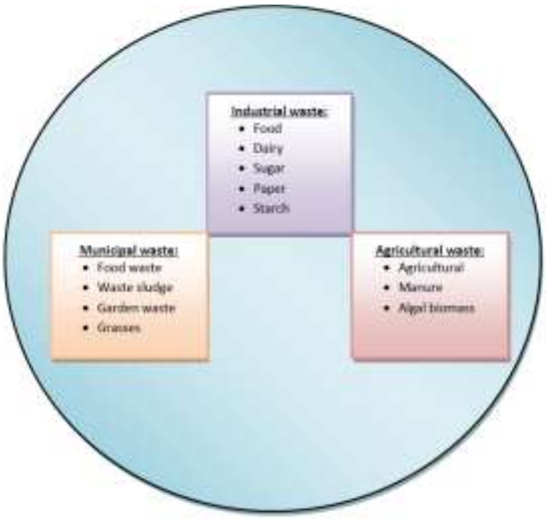


**Fig. 5.** Classification of biohydrogen producing bio-waste substrates

## 12. Dark Fermentation as a Process

### *12.1. Advantages, limitations and potentials*

Dark fermentation has been repeatedly highlighted as a promising renewable source of energy, and has received considerable attention in recent years due to its social, economic and environmental merits [18]. In addition, it provides an avenue for effective disposal and beneficiation of biowaste materials such as agricultural, municipal and industrial effluents [107-108]. Nonetheless, the realization of a dark fermentation driven economy has been hindered by its low production yield. A theoretical analysis reveals that the maximum yield by Clostridium species on glucose is 4 mol $H_2$/mol glucose when acetate is produced (see equation (1)) or 2 mol $H_2$/mol glucose when butyrate is produced as shown in equation (2) [109].

Acetate: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ (1)

Butyrate: $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ (2)

The accomplishment of higher bioprocess yields is still a crucial research issue in dark fermentation bioprocess technology [48]. Currently, microbial dark fermentation processes can only produce 2-3 mol $H_2$/mol glucose, resulting in 80-90% of initial chemical oxygen demand (COD) remaining in solution in the form of various volatile organic acids and solvents [110], this phenomenon is referred to as the dark fermentation “process barrier”. They are several “process barriers” that affects the overall dark fermentative biohydrogen yields; these include hydrogenotrophic methanogens, homoacetogens, nitrate-reducing bacteria, sulphate-reducing bacteria, organic acids, and other end-products. Thus, to improve the process economics of dark fermentation from biowaste; various strategies such as metabolic engineering, two-stage fermentation processes, application of optimization tools, and pre-treatment methods are pivotal in dark fermentation process technology (these strategies are discussed in section 14.2). In addition, more nutrient-rich substrates need to be exploited for its process development.

The utilization of biowaste effluents for dark fermentation processes is scantly reported in most African countries. Thus, this impedes initiatives for development of renewable and sustainable energy production within the continent. In addition, as a response to the Millennium Development Goal (MDG), devising better waste management options could promote environmental security and sustainability in the continent. A report from the United Nations has shown that proper waste management facilities are still lacking in Africa [2]. Hence, there’s widespread dumping of waste in water bodies and landfills which in turn aggravates the challenges of sanitation. Other contributing factors include urbanization which is said to be on the rise in Africa i.e. Africa is estimated to have an urban growth of 3.5% per annum which is the highest in the world [2]. Thus, several practices have been proposed and widely accepted in most countries in order to combat this challenge. Among these, conversion of “waste-to-energy” is highly encouraged as the continent faces the energy crisis and climate change.

Dark fermentative biohydrogen production from biowaste effluents has the potential to become a cost competitive energy generating process owing to their nutritional composition and accessibility. Furthermore, South Africa will increasingly generate more waste due to the high level of urbanization and industrialization as emphasized earlier. Therefore, the production of biohydrogen from these waste materials will have a significant contribution to the generation of clean fuel, mitigation of environmental pollution, and reduce their disposal costs. As the maximum theoretical yield of biohydrogen production on pure glucose substrate is low (4 mol $H_2$/mol glucose), dark fermentation from these waste effluents may enhance the overall biohydrogen production rates and yields.

### *12.2. Synergy between dark fermentation and other biohydrogen processes*

The need for hybrid processes is highly emphasized in dark fermentation studies in order to improve the overall biohydrogen conversion efficiency from biowaste substrates. The fermentation residual/effluent from dark fermentation process can be used as a substrate in other biohydrogen producing processes such as photo-fermentation, Microbial Fuel Cell (MFC), Microbial Electrolysis Cell (MEC), and biogas production as indicated in Table 5 and Fig.6 respectively. Chen et al. [111] reported a COD removal efficiency of 90% in a hybrid process of dark and photo-fermentation process. Lalaurette et al. [112] also reported a 90% COD removal efficiency in a two-stage process of dark fermentation and Microbial Electrolysis Cell (MEC). Meanwhile, Massanet-Nicolau et al. [113] reported a hydrogen increase of 13.4% in a two-stage process of biohydrogen and biomethane production. Hybrid processes of dark and photo-fermentation are encouraged due to high conversion efficiency. Photo-fermentative biohydrogen producing bacteria can utilize the organic acids (acetic, butyric, propionic, valeric acid) found in dark fermentation effluents (equation (3)) for further biohydrogen conversion. For example, 8 mols of biohydrogen can be generated from acetate-rich effluents as shown in equation (4) [114]. However, the process of photo-fermentation has its own limitations such as the need for an (i) external light source, (ii) maintenance of photo-fermentative bacteria, (iii) high risks of contamination, and (iv) the process will be expensive at large-scale [115-117].

Dark fermentation (Stage1): $C_6H_{12}O_6 + 2H_2O \rightarrow$

$$2CH_3COOH + 2CO_2 + 4H_2 \quad (3)$$

Photo-fermentation (Stage 2): $2CH_3COOH + 4H_2O \rightarrow 8H_2 +$

$$4CO_2 \quad (4)$$

Other biological hydrogen production processes (e.g. direct and indirect biophotolysis) are presented in Table 6. Among these processes, dark fermentation is highly favoured due to its several process advantages such as, (i) utilization of diverse carbon sources including the treatment of waste materials, (ii) utilization of diverse microorganisms which include bacterial species from sludge, soil samples, industrial and municipal sites, (iii) this process can be carried out at ambient temperature, (iv) there's less contamination problems, (v) this process can be integrated with other biohydrogen production processes as shown in Fig. 6. Nonetheless, this process has its own constrains such as low biohydrogen yields as a result of metabolites and thermodynamic limitations. Thus, optimization strategies are highly essential in dark fermentative biohydrogen process development, and they are elaborated in section 14.2.

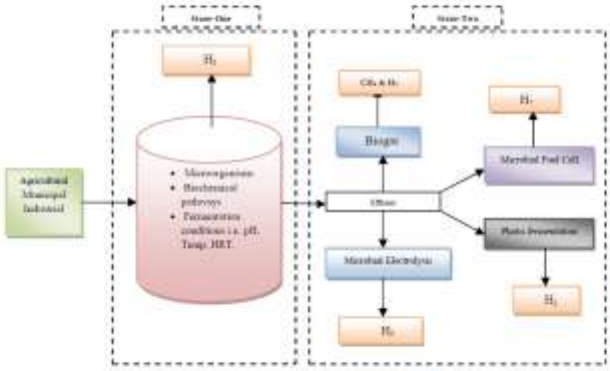

**Fig. 6.** Dark fermentation process integrated with other biohydrogen processes.

**Table 5.** Two-stage processes involving dark fermentation process and other biohydrogen production processes

| First stage | Second stage | Microorganism for 2nd stage | Fermentation conditions | COD recovery (%) | $H_2$ yield | Reference |
|---|---|---|---|---|---|---|
| Dark-fermentation | Photo-fermentation | *Rhodopseudomonas palustris* WP3-5 | 32 °C, pH 7.1, 100 rpm, light intensity of ca. 95 W/m$^2$ | 72 | 10.02 mol $H_2$/mol sucrose | [111] |
| Dark-fermentation | Biomethane production | Unpretreated anaerobic sludge | 35 °C, pH 7.5, OLR of 20-35 kg COD/m$^3$ d | 98 | - | [118] |
| Dark-fermentation | Microbial Fuel Cell | Mixed sludge | 20 °C, pH 7.0, OLR of 0.99-3.13 kg COD/m$^3$ day | 84.6 | - | [119] |
| Dark-fermentation | Microbial Electrolysis Cell | Domestic wastewater | 25 °C, pH 7.0, voltage of 25 mV | 23 | 33.2 mmol $H_2$/g COD | [120] |

**Table 6.** Biological hydrogen producing processes with their advantages and limitations [117].

| Biohydrogen process | General reaction of the process | Advantages | Process limitations |
|---|---|---|---|
| Dark fermentation process | $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ | Uses diverse substrates<br>Uses diverse bacteria<br>Minimum sterility required<br>It's a cost-effective process | Low $H_2$ yields<br>Low substrate conversion efficiency<br>Metabolites inhibits $H_2$ production<br>Gas mixtures containing $CO_2$ requires separation |
| Photo-fermentation | $CH_3COOH + 2H_2O + Light \rightarrow 4H_2 + 2CO_2$ | Uses diverse substrates<br>Uses diverse light source | Requirement for an external light source<br>Light conversion is low<br>Low $H_2$ yields due to poor light source |
| Direct biophotolysis | $2H_2O + light \rightarrow 2H_2 + O_2$ | Uses diverse waste materials | Requirement for an external light source<br>Low light conversion<br>Hydrogenase enzyme sensitive to $O_2$ |
| Indirect biophotolysis | $12H_2O + light \rightarrow 12H_2 + 6O_2$ | Can produce $H_2$ from water<br>Can fix $N_2$ from the atmosphere | Low $H_2$ yields due usage of hydrogenase<br>Requirement for an external light source<br>Enzyme inhibition by $O_2$ |

## 13. Economic Evaluation of Dark Fermentation from Bio-waste Effluents

Despite the extensive research that has been carried out over the past decade, few researchers have evaluated the economic potential of industrial-scale biohydrogen production processes. Classen et al. [121] conducted a cost evaluation on biohydrogen-producing dark-fermenter (total volume = 95 000 L) and photo-fermenter (total volume = 300 000 L). The production capacity for these vessels was 39 kg $H_2$/h, and the overall costs were estimated at US \$3.65 $kg^{-1}$ $H_2$. However, the costs of biomass, construction, and labour were not included. A hydrogen production rate of 425 000 L $H_2$ $h^{-1}$ was postulated from the process and this corresponded to an energy equivalent of 5.4 GJ $h^{-1}$ [118]. Meanwhile, Benemann [122] conducted an initial cost analysis for algal biohydrogen production system. The reactor had a capacity of 25 694 kg $H_2$/day which corresponded to 3600 GJ/day. The costs for the algal reactor were projected at US \$43 million, whereas the annual operating costs were US \$12 million/year. In this evaluation, the capital costs accounted for 90% the overall costs [119].

deVrije and Classen [123] also conducted the cost analysis of biohydrogen fermentation process using lignocellulose materials. The plant capacity was 910 kg $H_2$ day-1 and consisted of 95 000 litres thermo-bioreactor for dark fermenter which was coupled to a 300 000 L photo-fermenter. The production costs were estimated at US \$3 dollar per kg $H_2$, without taking into accounts the cost of hydrolysis. Therefore, all the above cost analyses are based on assumptions and aimed to assess the economic feasibility of the process on a commercial-scale. Nonetheless, more R&D should be invested in dark fermentative biohydrogen process because this technology is more expensive as compared to other fuel options due to its process complexities. This implies that many technical and engineering challenges need to be tackled before this technology can be implemented on an industrial-scale.

## 14. Challenges and the way Forward in Dark Fermentation Process

### *14.1. Technical challenges facing dark fermentation scale-up studies from bio-waste*

A critical challenge facing scale-up studies of biohydrogen production from biowaste is the low biohydrogen conversion efficiency [16, 66]. This is attributed to the accumulation of hydrogen inhibiting reactions such as solventogenic and methanogenesis processes during biohydrogen production [62, 47]. Biohydrogen production intermediates such as volatile fatty acids, propionate, ethanol, carbon dioxide, and biohydrogen-consuming bacteria (like homoacetogens and methanogens), lowers the overall biohydrogen yield [71]. Hitherto, the maximum biohydrogen yield reported in literature is 2.91 mol $H_2$/mol hexose [124]; from pure strain of *Clostridium* species. Nonetheless, this process is still not commercially viable.

Moreover, a study conducted by Sekoai and Gueguim Kana [48] highlighted some limitations about the utilization of biowaste effluents for biohydrogen fermentation processes: (i) these feedstocks consists of many compounds and thus some may have an inhibitory effects on dark fermentation pathways; (ii) these effluents are usually dispersed, and this might escalates their collection costs (iii) the lignin structure on biowaste materials is hard to penetrate, thus pretreatment strategies such as mechanical, physical, chemical and biological procedures are often adopted to break down the lignocellulose content thereby enhancing the release of soluble sugars and accessibility to microorganisms during fermentation. However, these pretreatments methods are energy-intensive and expensive [125-126].

### *14.2. Strategies for optimization of dark fermentation process yields from bio-waste*

Several optimization strategies have been proposed in dark fermentative biohydrogen production processes for enhancing its conversion efficiency from biowaste effluents. These strategies are discussed below:

- Glucose is an ideal substrate in dark fermentation process but it’s too costly to support its large-scale production. Thus, utilization of carbohydrates-rich biowaste substrates is a viable approach to overcome some of the economic constrains of dark fermentative biohydrogen process development.
- Cost-effective pretreatments of biowaste materials are necessary to improve the biohydrogen conversion efficiency because some of these substrates contain high amounts of lignocellulose.
- The use of optimization tools such as response surface methodology (RSM) and Artificial Neural Network (ANN) may significantly improve the overall yields because these statistical methods determine the synergistic optimum parameters that are favourable for biohydrogen fermentation processes [48].
- There is a need for bioreactor designs with high level of parallelization coupled with online monitoring devices for detecting the critical fermentation conditions during biohydrogen processes. The development of micro-sensors in bioreactors is essential in order to provide real-time and reliable bioprocess data and also to determine suitable parameter setpoints for maximum biohydrogen production [48].
- Integration of hybrid bioprocesses is vital in order to enhance the overall biohydrogen conversion efficiency. These include, (i) dark fermentation and biomethane production, (ii) dark fermentation and Microbial Fuel Cells (MFCs), (iii) dark fermentation and Microbial Electrolysis Cell (MECs), and (iv) dark and photo-fermentation process [127].
- Cost-effective pretreatment methods of the inoculum are necessary for the enumeration of dark biohydrogen-producing bacteria (e.g. *Clostridium* sp., *Bacillus* sp.) while suppressing dark biohydrogen-consuming methanogens.
- Utilization of co-substrates has been shown to improve the dark fermentation process yields. For instance, Zhu et al. [92] observed that the combination of the substrates (food waste + primary sludge + waste activated sludge) enhanced the overall yields as compared to individual substrates. Meanwhile, Sekoai and Gueguim Kana [47] reported a 3.8% in hydrogen increase from organic fraction of municipal solid waste comprising of apple waste, orange waste, cabbage waste, potato waste, bread waste, and paper waste respectively. Therefore, these wastes provide a desirable carbon and nitrogen (C/N) ratio for dark fermentation process [47].
- Metabolic engineering has also gained much attention over the past few years and it could potentially improve the biohydrogen yields. Efforts have been focusing on redirection, identification and engineering of oxygen tolerant hydrogenases (Sinha and Pandey, 2011). Studies have also focused on metabolic pathways to regulate the biohydrogen-producing reactions and biohydrogen-producing microorganisms. However, some reports in literature have highlighted a need for an extension of substrates in metabolic studies of biohydrogen-producing bacteria because these organisms are fastidious [128].
- Another technology that has received much attention is immobilization of biohydrogen-producing inoculum. It offers process advantages such as high metabolic activity, increases cell density, easier handling, re-use of cells, better solid/liquid separation efficiency, and better operation stability [11]. It is used in various reactor prototypes such as continuous stirred tank reactor [129], fluidized bed reactor [63], carrier induced granular sludge bed [127], up-flow anaerobic sludge bed reactor [130], and trickling biofilter [106]. The immobilization methods include granulation [131], biofilm [132], gel-entrapment [90], ceramics or glass beads [133], cellulosic materials [134], and polyacrylamide gels [135-136]. Some of the abovementioned optimization strategies are summarized in Fig. 7.

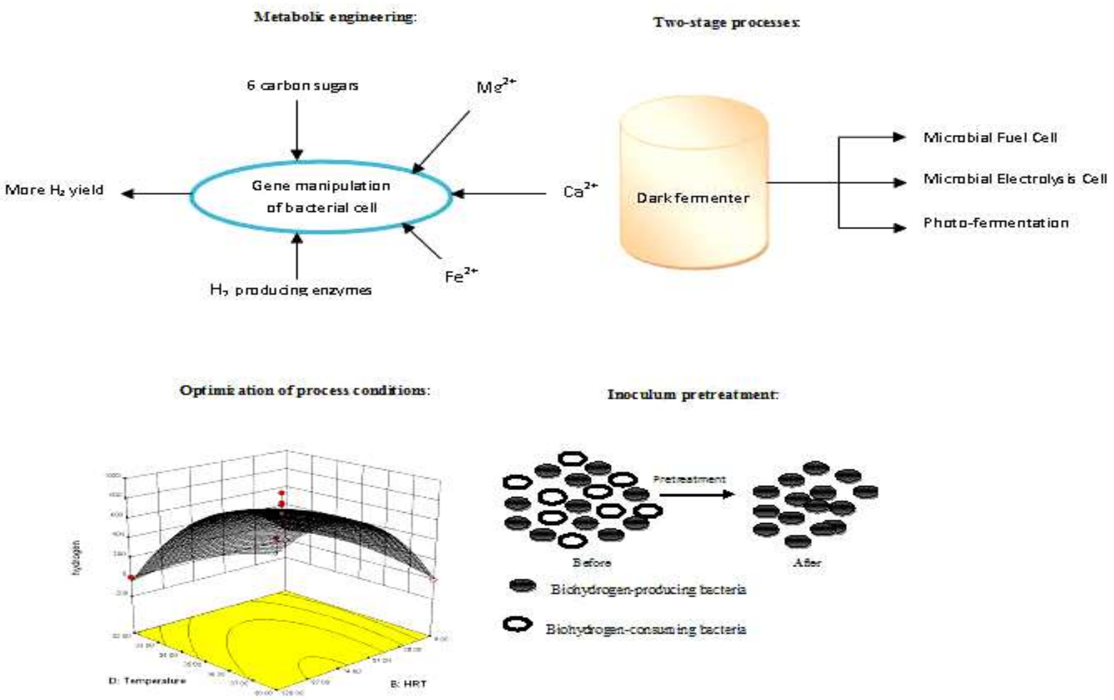


**Fig. 7.** Optimization strategies for dark fermentative biohydrogen production process

## 15. Conclusions

Dark fermentative biohydrogen production from bio-waste effluents demonstrates the possibilities of generating alternative and sustainable energy fuels that are environmentally friendly and reliable in South Africa. In addition, availability of biohydrogen as a clean alternative source of energy could pave the way to meeting the country’s escalating energy demands. Furthermore, the use of bio-waste which is abundantly present in South Africa for biohydrogen production, will significantly improve the process economics of the process. However, to fully realize the commercialization of biohydrogen production in South Africa and the rest of the world, it is imperative for both the government and private sector to invest enormously on technological development and technical expertise pertaining to biohydrogen fermentation processes. The economic analysis of dark fermentation process shows that the unit price of biohydrogen production will be more expensive at industrial-scale as compared to energy derived from hydrocarbon fuels due to its process complexities such as low conversion efficiency, accumulation of by-products that competes with biohydrogen pathways, the need for optimum bioreactor designs, the need for hydrogen purification methods, and the requirements for hydrogen storage systems. Nonetheless, biohydrogen is still a preferred energy fuel when taking into account the adverse effects of climate change, dwindling fossil reserves, and escalating energy prices.

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**References**

[1] G. Davila-Vazqueza, F. Alatriste-Mondragón, A. de Leo´n-Rodrı´guez, and E. Razo-Flores, “Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: influence of initial substrate concentration and pH", *Int. J. Hydrogen Energ.*, vol. 33, no. 19, pp. 4989-4997, Oct. 2008.

[2] Economic and social council. Africa Review Report on Waste Management. https://sustainabledevelopment.un.org/content/documents/AfricanReviewReport-on wastemanagementsummary.pdf. (accessed on 18 March 2016).

[3] Netherlands Environmental Agency. http://edgar.jrc.ec.europa.eu/newsdocs/jrc-2015-trends-in-global-co2-emissions-2015-report-98184.pdf. (accessed 18 March 2016).

[4] Framework Convention on Climate Change. Paris, France. http://unfccc.int/meetings/parisnov2015/meeting/8926.php. (accessed 18 March 2016).

[5] International Energy Agency. https://www.iea.org/publications/freepublications/pu

blication/KeyWorldStatistics2015.pdf. (accessed 18 March 2016).

[6] Climate change conference. http://www.cop17-cmp7durban.com/en/south-africa-on-climate-change/effects-of-climate-change-on-south-africa.html. (accessed 18 March 2016).

[7] M.R. Nouni, "Hydrogen energy and fuel cell technology: Recent development and future prospects in India", *Ren. Energy Akshay Urja,* vol. 55, no. 5, pp. 10-14, Apr. 2012.

[8] BP Statistical Review of World Energy, http://www.bp.com/content/dam/bp/pdf/statistical-review/statisticalreviewof worldenergy2013.pdf . (accessed 18 March 2016).

[9] X.Y. Cheng, and C.Z. Liu, "Hydrogen production via thermophilic fermentation of cornstalk by *Clostridium thermocellum*", *Energy Fuels,* vol. 25, no. 4, 1714-1720, Mar. 2011.

[10] O. Elsharnouby, H. Hafez, G. Nakhla, and M.H. El Naggar, "A critical literature review on biohydrogen production by pure cultures", *Int. J. Hydrogen Energ.,* vol. 38, no. 12, pp. 4945-4966, Apr. 2013.

[11] S.Y. Wu, C.H. Hung, C.N. Lin, H.W. Chen, A.S. Lee, and J.S. Chang, "Fermentative hydrogen production and bacterial community structure in high-rate anaerobic bioreactors containing silicone-immobilized and self-flocculated sludge", *Biotechnol. Bioeng.,* vol. 93, no. 5, pp. 934-946, Apr. 2006.

[12] Energy Information Administration. http://www.eia.gov/. (accessed 18 March 2016).

[13] M.L. Barry, H. Steyn, and A. Brent, "Selection of renewable energy technologies for Africa: Eight case studies in Rwanda, Tanzania and Malawi", *Renewable Energy,* vol. 36, no. 11, pp. 2845-2852, Nov. 2011.

[14] I.Z. Boboescu, V.D. Gherman, G. Lakatos, B. Pap, and T.B.G. Mariti, "Surpassing the current limitations of biohydrogen production systems: The case for a novel approach", *Bioresour. Technol.,* vol. 204, pp. 192-201, Mar. 2016.

[15] S.K. Han, and H.S. Shin, "Biohydrogen production by anaerobic fermentation of food waste", Int. J. Hydrogen Energ., vol. 29, no. 6, pp. 569-577, May 2004.

[16] D. Das, and T.N. Veziroglu, "Hydrogen production by biological processes: a survey of literature", *Int. J. Hydrogen Energ.,* vol. 26, no. 1, pp. 13-28, Jan. 2001.

[17] L. Dong, Y. Zhenhong, S. Yongming S, K. Xiaoying, and Z. Yu, "Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation", *Int. J. Hydrogen Energ.,* vol. 34, no. 2, pp. 812-820, Jan. 2009.

[18] S. Meher Kotay, and D. Das, "Biohydrogen as a renewable energy resource-prospects and potentials", *Int. J. Hydrogen Energ.,* vol. 33, no. 1, pp. 258-263, Jan. 2008.

[19] United States Department of Energy. https://www.hydrogen.energy.gov/production.html. (accessed 18 March 2016).

[20] B. Rausch, M.D. Symes, G. Chisholm, and C. Leroy, "Decoupled catalytic hydrogen evolution from a molecular metal oxide redox mediator in water splitting", *Science,* vol. 345, no. 6202, pp. 1326-1330, Sep. 2014.

[21] BP Statistical Review of World Energy. http://www.bp.com/content/dam/bp/pdf/statistical-review/statisticalreviewofworldenergy2013.pdf . (accessed 18 March 2016).

[22] Eskom Integrated Results. http://www.eskom.co.za/OurCompany/MediaRoom/Documents/IRpresentation2014.pdf. (accessed 18 March 2016).

[23] L.S. Jeffrey, "Characterization of the coal resources of South Africa", *J. South African Inst. Min. Metall.,* vol. 3, pp. 95-102, Feb. 2005.

[24] F.S.J. de Jager, "Coal Reserves of the Republic of South Africa", Geological Survey, *Bulletin,* vol. 74, no. 9-10, pp. 289-330, 1978.

[25] J.H. Bredell, South African coal resources explained and analysed, Geological Survey of South Africa, 1987.

[26] C. Hartnady, "South Africa's Diminishing Coal Reserves", South African J. Sci. vol. 106, no. 9-10, pp. 18-22, Sep. 2010.

[27] International Energy Agency, $CO_2$ Emissions From Fuel Combustion Highlights IEA/OECD, Pairs, 2013.

[28] Energy Information Administration. https://www.eia.gov/beta/international/analysisinclu des/countrieslong/SouthAfrica/southafrica.pdf. (accessed 18 March 2016).

[29] P.T. Sekoai, and M.O. Daramola, "Biohydrogen production as a potential energy fuel in South Africa", *Biofuel Res. J.,* vol. 6, pp. 223-226, Jun. 2015.

[30] A.A. Eberhard, "The future of South African coal: market, investment, and policy challenges, Stanford University: Program and Energy and Sustainable Development", working paper no. 100, 2011.

[31] C.J.S. Fourie, S.J. du Plessis, and G. Henry, "New airborne geophysical data from the Waterberg Coalfield –South Africa's major future energy source", 2006.

[32] T. Fluri, "The potential of concentrating solar power in South Africa", Energy Policy, vol. 37, no. 12, pp. 5075-5080, 2009.

[33] H. Winkler, "South Africa's renewable energy policy roadmaps, Energy Research Centre", University of Cape Town, 2010.

[34] S. Brodski, "South Africa's REFIT Programme Starts to Take", Dewey & LeBoeuf for Energy Indaba, 2009.

[35] P.T. Sekoai, K.O. Yoro, Biofuel development Initiatives in Sub-Saharan Africa: Opportunities and Challenges", *Climate*, vol. 4, no. 33, pp. 1-13, Jun. 2016.

[36] The potential contribution of renewable energy in South Africa. http://earthlife.org.za/www/wp-content/uploads/2009/04/potential-of-re-in-sa-feb06.pdf. (accessed 18 March 2016).

[37] Bio2Watt. http://www.bio2watt.com/bio2watt%E2%80%99s-bronkhorstspruit-biogas-plant-(pty)-ltd.html. (accessed 18 March 2016).

[38] K. Chandrasekhar, Y.J. Lee, and D.W. Lee, "Biohydrogen Production: Strategies to Improve Effiency through Microbial Routes", *Int. J. Mol. Sci.*, vol. 16, no. 4, pp. 8266-8293, Apr. 2015.

[39] A. Ghimire, L. Frunzo, L. Pontoni, G. d'Antonio, P.N.L. Lens, G. Esposito, and F. Pirozzi, "Dark fermentation of complex waste biomass for biohydrogen production by pretreated thermophilic anaerobic digestate", *J. Env. Manag.*, vol. 152, pp. 43-48, Apr. 2015.

[40] G.R. Kumar, and N. Chowdhary, "Biotechnological and Bioinformatics approaches for augmentation of biohydrogen production: A review", *Renew. Sust. Energ. Rev.*, vol. 56, pp. 1194-1206, Apr. 2016.

[41] F.O. Obazu, M.O. Daramola, and V. Gray, "Biohydrogen Production by Bacterial Granules Adapted to grow at Different Thermophilic Temperatures, Proceedings of the World Congress on Engineering 2015 Volume II WCE", 2015.

[42] F.D. Faloye, E.B. Gueguim Kana, and S. Schmidt, "Optimization of biohydrogen inoculum development via a hybrid pH and microwave treatment technique – Semi pilot scale production assessment", *Int. J. Hydrogen Energ.*, vol. 39, no. 11, pp. 56074-5616, Apr. 2014.

[43] M.B. Hassan, and E.B. Gueguim, "Design, implementation and assessment of a novel bioreactor for fermentative biohydrogen process development", *Int. J. Hydrogen Energ.*, vol. 41, no. 24, pp. 10136-10144, Jun. 2016.

[44] S. Mafuleka, and E.B. Gueguim Kana, "Modelling and optimization of xylose and glucose production from Napier grass using hybrid pre-treatment techniques", *Biomass Bioenergy*, vol. 77, pp. 200-208, Jun. 2015.

[45] P. Moodley, and E.B. Gueguim Kana, "Optimization of xylose and glucose production from sugarcane leaves (*Saccharum officinarum*) using hybrid pretreatment techniques and assessment for hydrogen generation at semi-pilot scale", *Int. J. Hydrogen Energ.*, vol. 40, no. 10, pp. 3859-3867, Mar. 2015.

[46] L. Ngoma, P. Masilela, F.O. Obazu, and V.M. Gray, "The effect of temperature and effluent recycle rate on hydrogen production by undefined bacterial granules", *Bioresour. Technol.*, vol. 102, no. 19, pp. 8986-8991, Oct. 2011.

[47] P.T. Sekoai, and E.B. Gueguim Kana, "Semi-pilot scale production of hydrogen from Organic Fraction of Solid Municipal Waste and electricity generation from process effluents", *Biomass Bioenergy*, vol. 60, pp. 156-163, Jan. 2014.

[48] P.T. Sekoai, and E.B. Gueguim Kana, "Fermentative biohydrogen modelling and optimization in light of miniaturized parallel bioreactors-Review", *Biotechnol. Biotechnol. Equip.*, vol. 27, no. 4, pp. 3901-3908, Apr. 2014.

[49] Y. Sewsynker, and E.B. Gueguim Kana, "Modelling of biohydrogen generation in microbial electrolysis cells (MECs) using a committee of artificial neural networks (ANNs)", *Biotechnol. Biotechnol. Equip.*, vol. 29, no. 6, pp. 1208-1215, Jul. 2015.

[50] Hydrogen South Africa. http://www.hysainfrastructure.org/. (accessed 18 March 2015).

[51] South African Institute for Advanced Materials Chemistry. http://www.uwc.ac.za/Faculties/NS/SAIAMC/Pages/default.aspx. (accessed 18 March 2016).

[52] Department of Environmental Affairs, National waste information, Baseline Report. Pretoria, South Africa, 2014.

[53] I.K. Kapdan, and F. Kargi, "Bio-hydrogen production from waste materials", *Enzyme Microb. Technol.,* vol. 38, no. 5, pp. 569-582, Mar. 2006.

[54] M. Broomfield, J. Davies, and R. Harrison, "Review of Environmental and Health Effects of Management of Municipal Solid Waste", Department for Environment, Food and Rural Affairs. London, United Kingdom, 2004.

[55] Sustainable energy development. https://sustainabledevelopment.un.org/content/documents/AfricanReviewReport-onWasteManagementSummary.pdf. (accessed on 18 March 2016).

[56] B. Eklund, E.P. Anderson, B.L. Walker, and D.B. Burrows, "Characterization of landfill gas composition at the fresh kills municipal solid-waste landfill", *Environ. Sci. Technol.,* vol. 32, no. 15, pp. 2233-2237, Jun. 1998.

[57] C. Kemfert, and W.P. Schill, "An analysis of methane mitigation as a response to climate change, Copenhagen", Consensus Center, Denmark, 2009.

[58] E. Viitez, J. Mosquera, and S. Ghosh, "Kinetics of accelerated solid-state fermentation of organic-rich municipal solid waste", *Water Sci. Technol.,* vol. 41, no. 3, pp. 231-238, Jan. 2000.

[59] R. Devesa-Rey, X. Vecino, J.L. Varela-Alende, M.T. Barral, J.M. Cruz, and A.M. Moldes, "Valorization of winery waste vs. the costs of not recycling", *Waste Manag. Res.,* vol. 31, no.11, pp. 2327-2335, Jul. 2011.

[60] S.W. Van Ginkel, S. Sung, and J.J. Lay, "Biohydrogen production as a function of pH and substrate concentration", *Environ. Sci. Technol.,* vol. 35, no. 24, pp. 4726-4730, Nov. 2001.

[61] C. Chen, C. Lin, and J. Chang, "Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate", *Appl. Microbiol. Biotechnol.,* vol. 57, pp. 56-64, Jul. 2001.

[62] S.K. Khanal, W.H. Chen, L. and Li, S. Sung, "Biological hydrogen production: effects of pH and intermediate products", *Int. J. Hydrogen Energ.,* vol. 29 no. 11, pp. 1123-1131, Sep. 2004.

[63] C.N. Lin, S.Y. Wu, and J.S. Chang, "Fermentative hydrogen production with a draft tube fluidized bed reactor containing silicone-gel-immobilized anaerobic sludge", *Int. J. Hydrogen Energ.,* vol. 3, no. 15, pp. 2200–2210, Dec. 2006.

[64] Y.C. Lo, K.S. Lee, P.J. Lin, and J.S. Chang, "Bioreactors configured with distributors and carriers enhance the performance of continuous dark hydrogen fermentation", *Bioresour. Technol.,* vol. 100, no. 19, pp. 4381-4387, Oct. 2009.

[65] H. Argun, and S. Dao, "Hydrogen gas production from waste peach pulp by dark fermentation and electrohydrolysis", *Int. J. Hydrogen Energ.,* vol. 41, no. 27, pp. 11568-11576, Jul. 2016.

[66] X.M. Guo, E. Trably, E. Latrille, H. Carrere, and J.P. Steyer, "Hydrogen production from agricultural waste by dark fermentation: a review", *Int. J. Hydrogen Energ.,* vol. 35, no. 19, pp. 10660-10673, Oct. 2010.

[67] S.H. Kim, S.K. Han, and H.S. Shin, "Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge", Int. J. Hydrogen Energ., vol. 29, no. 15, pp. 1607-1616, Dec. 2004.

[68] H.S. Shin, S.H. Kim, and B.C. Paik, "Characteristics of hydrogen production from food waste and waste activated sludge", *Water Environ. Technol.,* vol. 1 no. 2, pp. 177-187, Jul. 2003.

[69] P. Zhou, E. Elbeshbishy, and G. Nakhla, "Optimization of Biological Hydrogen Production for Anaerobic Co-digestion of Food Waste and Wastewater Biosolids", *Bioresour. Technol.,* vol. 130, pp. 710-718, Feb. 2013.

[70] E. Elbeshbishy, H. Hafez, B.R. Dhar, and G. Nakhla, "Single and combined effect of various pretreatment methods for biohydrogen production from food waste", *Int. J. Hydrogen Energ.,* vol. 36, no. 17, pp. 11379-11387, Aug. 2011.

[71] J.J. Lay, Y.J. Lee, and T. Noike, "Feasibility of biological hydrogen production from organic fraction of municipal solid waste", *Water Res.,* vol. 33, no. 11, pp. 2579-2586, Aug. 1999.

[72] C.M. Sheridan, D. Glasser, D. Hildebrandt, J. Petersen, and J. Rohwer, "An Annual and Seasonal Characterisation of Winery Effluent in South Africa", *South African J. Enol. Vitic.,* vol. 3, no. 1, pp. 1-8, Oct. 2010.

[73] W.Y. Buys, "Investigating the effect of wine and distillery wastewater on the efficacy of an upflow anaerobic sludge blanket (UASB) and enhancing biomass immobilisation by the addition of magnetisable foam glass particles", MSc Thesis, Stellenbosch University, South Africa, 2015.

[74] A.M. Jimenez, R. Borja, and A. Martin, "A comparative kinetic evaluation of the anaerobic digestion of untreated molasses and molasses previously fermented with *Penicillium decumbens* in batch reactors", *Biochem. Eng. J.,* vol. 18, no. 2, pp. 121-132, May 2004.

[75] C.Y. Lin, S.Y. Wu, P.J. Lin, J.S. Chang, C.H. Hung, and K.S. Lee, "A pilot-scale high-rate biohydrogen production system with mixed microflora", *Int. J. Hydrogen Energ.,* vol. 36, no. 14, pp. 8758-8764, Jul. 2011.

[76] S.W. Van Ginkel, S.E. Oh, and B.E. Logan, "Biohydrogen gas production from food processing and domestic wastewaters", *Int. J. Hydrogen Energ.,* vol. 30, no. 15, pp. 1535-1542, Dec. 2005.

[77] R. Angeriz-Campoy, C.J. Álvarez-Gallego, L.I. Romero-García, "Thermophilic anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW) with food waste (FW): enhancement of bio-hydrogen production", *Bioresour. Technol.,* vol. 194, pp. 291–296, Oct. 2015.

[78] D.E. Algapani, W. Qiao, M. Su, F. di Pumpo, S.M. Wandera, and F. Adani, "Bio-hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process", *Bioresour. Technol.,* vol. 216, pp. 768–777, Sep. 2016.

[79] A.T. Shah, L. Favaro, L. Alibardi, L. Cagnin, A. Sandon, and R. Cossu, "*Bacillus* sp. strains to produce bio-hydrogen from the organic fraction of municipal solid waste", *Appl. Energy,* vol. 176, pp. 116–124, Aug. 2015.

[80] H. Wei, H. Jingang, Z. Hongting, and L. Yongfeng, "Continuous biohydrogen production from waste bread by anaerobic sludge", *Bioresour. Technol.,* vol. 212, pp. 1–5, Jul. 2016.

[81] A. Ghimire, L. Frunzo, L. Pontoni, G. d'Antonio, P.N.L. Lens, and G. Esposito, "Dark fermentation of complex waste biomass for biohydrogen production by pretreated thermophilic anaerobic digestate", *Env. Manag.,* vol. 152, pp. 43-48, Apr. 2015.

[82] S. Eker, and M. Sarp, "Hydrogen gas production from waste paper by dark fermentation: Effects of initial substrate and biomass concentrations", *Int. J. Hydrogen Energ.,* 2016, doi:10.1016/j.ijhydene.2016.04.020.

[83] H. Yu, Z. Zhu, and W. Hu, "Hydrogen production from rice winery wastewater in an upflow anaerobic reactor by using mixed anaerobic cultures", *Int. J. Hydrogen Energ.,* vol. 27, no.11-12, pp. 1359-1365, Nov. 2002.

[84] C.C. Wang, C.W. Chang, C.P. Chu, D.J. Lee, B.V. Chang, and C.S. Liao, "Producing hydrogen from wastewater sludge by *Clostridium bifermentans*", *J. Biotechnol.,* vol. 102, no. 1, pp. 83-92, Apr. 2003.

[85] H. Yokoi, R. Maki, and J. Hirose, "Microbial production of hydrogen from starch-manufacturing wastes", *Biomass Bioenergy,* vol. 22, no. 5, pp. 389-395, May 2002.

[86] C.F. Chu, Y.Y. Li, K.Q. Xu, Y. Ebie, and Y. Inamori, "A pH-and temperature-phased two-stage process for hydrogen and methane production from food waste", *Int. J. Hydrogen Energ.,* vol. 33, no. 18, pp. 4739-4746, Sep. 2008.

[87] X. Gomez, A. Moran, M. Cuetos, and M.E. Sanchez, "The production of hydrogen by dark fermentation of municipal solid wastes and slaughterhouse waste: a two-phase process", *J. Power Sources,* vol. 157, no. 2, pp. 727-732, Jul. 2006.

[88] D.Y. Lee, Y. Ebie, and K.Q. Xu, "Continuous $H_2$ and $CH_4$ production from high-solid food waste in the two-stage thermophilic fermentation process with the recirculation of digester sludge", *Bioresour. Technol.,* vol. 101, no. 1, pp. 42-47, Jan. 2010.

[89] G. Cappai, G. De Gioannis, and G. Giordano, "Energy and material recovery through combined hydrogen-methane production and final composting of different solid and liquid waste, Proceedings of Sardina 2009 12$^{th}$ International Waste Management and Landfill Symposium", Margherita di Pula, Italy, 2009.

[90] G. Ivanova, G. Rakhely, and K.L. Kovacs, "Thermophilic biohydrogen production from energy plants by *Caldicellulosiruptor saccharolyticus* and comparison with related studies", *Int. J. Hydrogen Energ.,* vol. 34, no. 9, pp. 3659–3670, May 2009.

[91] M. Okamoto, T. Miyahara, O. Mizuno, and T. Noike, "Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes", *Water Sci. Technol.,* vol. 41, no. 3, pp. 25-32, Jan. 2000.

[92] H. Zhu, W. Parker, and R. Basnar, "Biohydrogen production by anaerobic codigestion of municipal food waste and sewage sludges", *Int. J. Hydrogen Energ.,* vol. 33, no. 14, pp. 3651–3659, Jul. 2008.

[93] T. Zhang, H. Liu, and H.H.P. Fang, "Biohydrogen production from starch in wastewater under thermophilic condition", *Environ. Manag.,* vol. 69, no. 2, pp. 149-156, Oct. 2003.

[94] F.R. Hussy, R. Hawkes, and R. Dinsdale, "Continuous fermentative hydrogen production

from sucrose and sugarbeet", *Int. J. Hydrogen Energ.,* vol. 34, no. 5, pp. 471-483, Apr. 2005.

[95] G. Antonopoulou, H. Gavala, I. Skiadas, K. Angelopoulos, and G. Lyberatos, "Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass", *Bioresour. Technol.,* vol. 99, no. 1, pp. 110-119, Jan. 2008.

[96] N. Venetsaneas, G. Antonopoulou, K. Stamatelatou, M. Kornaros, and G. Lyberatos, "Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches", *Bioresour. Technol.,* vol. 100, no. 15, pp. 3713-3717, Aug. 2009.

[97] S. Sangyoka, A. Reungsang, and S. Moonamart, "Repeated-batch fermentative for biohydrogen production from cassava starch manufacturing wastewater", Pakistan J. Biol. Sci., vol. 10, no. 11, pp. 1782-1789, Jan. 2007.

[98] Y. Wang, H. Wang, X. Feng, X. Wang, and J. Huang, "Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge", *Int. J. Hydrogen Energ.,* vol. 35, no. 7, pp. 3090–3099, Apr. 2010.

[99] H. Yang, L. Guo, and F. Liu, "Enhanced bio-hydrogen production from corncob by a two–step process: Dark-and photo-fermentation", *Bioresour. Technol.,* vol. 101, no. 6, pp. 2049–2052, Mar. 2010.

[100] S. Pattra, S. Sangyoka, and M. Boonmee, "Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*", *Int. J. Hydrogen Energ.,* vol. 33, no. 19, pp. 5256–5265, Oct. 2008.

[101] M.F. Arooj, S.K. Han, and S.H. Kim, "Continuous biohydrogen production in a CSTR using starch as a substrate", *Int. J. Hydrogen Energ.,* vol. 33, no. 13, pp. 3289–3294, Jul. 2008.

[102] C. Cavinato, A. Giuliano, D. Bolzonella, P. Pavan, and F. Cecchi, "Bio-hythane production from food waste by dark fermentation coupled with anaerobic digestion process: a long-term pilot scale experience", *Int. J. Hydrogen Energ.,* vol. 37, no. 15, pp. 11549–11555, Aug. 2015.

[103] D. Yin, W. Liu, and N. Zhai, "Anaerobic digestion of pig and dairy manure under photo-dark fermentation condition", *Bioresour. Technol.,* vol. 166, pp. 373–80, Aug. 2014.

[104] A.J. Lewis, S. Ren, X. Ye, P. Kim, N. Labbe, and A.P. Borole, "Hydrogen production from switchgrass via an integrated pyrolysis-microbial electrolysis process", *Bioresour. Technol.,* vol. 195, pp. 231-241, Nov. 2015.

[105] J.L. Park, J. Lee, S.J. Sim, and J.H. Lee, "Production of Hydrogen from Marine Macro-algae Biomass Using Anaerobic Sewage Sludge Microflora", *Biotechnol. Biop. Eng.,* vol. 14, pp. 307-315, Jul. 2009.

[106] S. Roy, K. Kumar, S. Ghosh, and D. Das, "Thermophilic biohydrogen production using pre-treated algal biomass as substrate", *Biomass Bioenergy,* vol. 61, pp. 157-166, Feb. 2014.

[107] K. Nath, and D. Das, "Improvement of fermentative hydrogen production: various approaches", *Appl. Microbiol. Biotechnol.,* vol. 65, no. 5, pp. 520–529, Oct. 2004.

[108] K. Pandu, and S. Joseph, "Comparisons and limitations of biohydrogen production processes: a review", *Res. J. Biotech.,* vol. 2, pp. 342–356, May 2012.

[109] M.B. Salerno, W. Park, Y. Zuo, and B.E. Logan, "Inhibition of biohydrogen production by ammonia", *Water Res.,* vol. 40, no. 6, pp. 1167–1172, Mar. 2006.

[110] M. Liu, Y. Yuan, L.X. Zhang, L. Zhuang, S.G. Zhou, and J.R. Ni, "Bioelectricity generation by a Gram-positive *Corynebacterium* sp. strain MFC03 under alkaline condition in microbial fuel cells", *Bioresour. Technol.,* vol. 101, no. 6, pp. 1807-1811, Mar. 2010.

[111] C.Y. Chen, M.H. Yang, K.L. Yeh, C.H. Liu, and J.S. Chang, "Biohydrogen production using sequential two-stage dark and photo fermentation processes", *Int. J. Hydrogen Energ.,* vol. 33, no.18, pp. 4755-4762, Sep. 2008.

[112] E. Lalaurette, S. Thammannagowda, A. Mohagheghi, A. Mohagheghi, P.C. Maness, and B.E. Logan, "Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis", *Int. J. Hydrogen Energ.,* vol. 34, no. 15, pp. 6201-6210, Aug. 2009.

[113] J. Massanet-Nicolau, R. Dinsdale, A. Guwy, and G. Shipley, "Utilising biohydrogen to increase methane production, energy yields and process efficiency via two stage anaerobic digestion of grass", *Bioresour. Technol.,* vol. 189, no. pp. 379-383, Aug. 2015.

[114] K. Bala Amutha, and A.G. Murugesen, "Biological hydrogen production by the algal biomass *Chorella vulgaris* MSU 01 strain isolated from pond sediment", *Bioresour. Technol.,* vol. 102, no. 1, pp. 194-199, Jan. 2011.

[115] S. Ozmihci, and F. Kargi F, “Bio-hydrogen production by photo-fermentation of dark fermentation effluent with intermittent feeding and effluent removal”, *Int. J. Hydrogen Energ.,* vol. 35, no. 13, pp. 6674–6680, Jul. 2010.

[116] B. Uyar, M. Gurgan, E. Ozgur, U. Gunduz, M. Yucel, and I. Eroglu, “Hydrogen production by hup$^{-}$ mutant and wild-type strains of *Rhodobacter capsulatus* from dark fermentation effluent of sugar beet thick juice in batch and continuous photobioreactors”, *Bioprocess Biosyst. Eng.,* vol. 38, no. 10, pp. 1935–1942, Oct. 2015.

[117] N. Khanna, and D. Das, “Biohydrogen production by dark fermentation”, *WIRES Energy Environ*., vol. 2, pp. 401-421, Aug. 2013.

[118] M. Kisielewska, I. Wysocka, M.R. Rynkiewicz, “Continuous Biohydrogen and Biomethane Production from Whey Permeate in a Two-Stage Fermentation Process”, *Environ. Prog. Sustain. Energ.,* vol. 33, no 4, pp. 4111-1418, Nov. 2013.

[119] G. Mohanakrishna, S. Venkata Mohan, P.N. Sarma, “Utilizing acid-rich effluents of fermentative hydrogen production process as substrate for harnessing bioelectricity: An integrative approach”, *Int. J. Hydrogen Energ.,* vol. 35, no. 1, pp. 3440 – 3449, Feb. 2010.

[120] A. Wang, D. Sun, G. Cao, H. Wang, N. Ren, W.M. Wu, B.E. Logan, “Integrated hydrogen production process from cellulose by combining dark fermentation, microbial fuel cells, and a microbial electrolysis cell”, *Bioresour. Technol.,* vol. 102, no. 5, pp. 4137-4143, Mar. 2011.

[121] P.MA. Classen, J.W. van Groenestijn, A.J.H. Janssen, E.W.J. Niel, and R.H. Wijffels, “Feasibility of biological hydrogen production from biomass for utilization in fuel cells”, Proceedings of the 1st World Conference and Exhibition on Biomass for Energy and Industry, Sevilla, Spain, 2000.

[122] J.R. Benemann, “Hydrogen production by microalgae”, *J. Appl. Phycol.,* vol. 12, no. 3, pp. 291-300, Oct. 2000.

[123] T. de Vrije T, and P.M.A. Claasen, “Dark hydrogen fermentation, Biomethane and bio-hydrogen, Dutch Biological Hydrogen Foundation”, The Hague, Netherland, 2003.

[124] J. Masset, M. Calusinska, C. Hamilton, S. Hiligsmann, B. Joris, and P. Thornat, “Fermentative hydrogen production from glucose and starch using pure strains and artificial co-cultures of *Clostridium* spp”, *Biotechnol. Biofuels,* vol. 5, no. 1, pp. 1-15, May 2012.

[125] A.R. Esteghlalian, S.D. Mansfield, and J.N. Saddler, “Cellulose: Agents for fiber modification or bioconversation? The effect of substrates accessibility on cellulose enzymatic hydrolyzability”, *Biotechnol. Pulp Paper Industry,* vol. 21, no. pp. 21-36, Jan. 2002.

[126] G. Zheng, Z. Kang, Y. Qian, and L. Wang, “Enhanced biohydrogen generation from organic wastewater containing $NH4^{+}$ by phototrophic bacteria *Rhodobacter sphaeroides* AR–3”, *Frontiers Environ. Sci. Eng.,* vol. 3, pp. 387-392, Dec. 2009.

[127] H. Argun, F. Kargi, and I.K. Kapdan, “Light fermentation of dark fermentation effluent for bio-hydrogen production by different *Rhodobacter* species at different initial volatile fatty acid (VFA) concentration”, *Int. J. Hydrogen Energ.,* vol. 33, no. 24, pp. 7405–7412, Dec. 2008.

[128] E. Eroglu, U. Gunduz, M. Yucel, and I. Eroglu, “Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive mill wastewater as feedstock”, *Int. J. Hydrogen Energ.,* vol. 35, no. 11, pp. 5293–5300, Jun. 2009.

[129] H.H.P. Fang, H. Liu, and T. Zhang, “Characterization of a hydrogen-producing granular sludge”, *Biotechnol. Bioeng.,* vol. 78, no.1, pp. 44–52, Apr. 2002.

[130] K.S. Lee, J.F. Wu, Y.S. Lo, P.J. Lin, and J.S. Chang, “Anaerobic hydrogen production with an efficient carrier-induced granular sludge bed bioreactor”, *Biotechnol. Bioeng.,* vol. 87, no. 5, pp. 648–657, Apr. 2004.

[131] F.Y. Chang, and C.Y. Lin, “Biohydrogen production using an up-flow anaerobic sludge blanket reactor”, *Int. J. Hydrogen Energ.,* vol. 29, no. 1, pp. 33–39, Jan. 2009.

[132] K.Y. Show, D.J. Lee, and J.S. Chang, “Bioreactor and process design for biohydrogen production”, *Bioresour. Technol.,* vol. 102, no. 18, pp. 8524-8533, Sep. 2011.

[133] P. Perego, A. Converti, E. Palazzi, M.B. Borghi, and M. Ferraiolo, “Fermentation of hardwood hemicellulose hydrolysate by *Pachysolen tannophilus, candida shehatae* and *Pichia stipitis*”, Biop. Eng., vol. 22, pp. 205–213, Nov. 1989.

[134] L.M.D. Goncalves, M.T.O. Barreto, A.M.B.R. Xavier, M.J.T. Carrondo, and J. Klein, “Inert support for lactic acid fermentation. A technological assessment”, *Appl. Microbiol. Biotechnol.,* vol. 38, no. 3, pp. 305–311, Dec. 1992.

[135] E. Bardi, and A.A. Kountinas, “Immobilization of yeast on delignified cellulose material for room and low-temperature wine-making”, *J. Agric. Food Chem.*, vol. 42, no. 1, pp. 221–226, Jan. 1994.

[136] M. Liu, Y. Yuan, L.X. Zhang, L. Zhuang, S.G. Zhou, and J.R. Ni, “Bioelectricity generation by a Gram-positive *Corynebacterium* sp. strain MFC03 under alkaline condition in microbial fuel cells”, *Bioresour. Technol.*, vol. 101, no. 6, pp. 1807-1811, Mar. 2010.