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# Heterotrophic bacterial flora associated with the Conventional Activated sludge plant employed for treating effluent of a commercial flight kitchen

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ABSTRACT

Safe disposal of wastewater, reuse and recycle of the same is mandatory for effective management of wastewater generated and disposed by human activities. In this study wastewater generated from a commercial flight kitchen catering to the needs of different flight services operating from the International Airport, Cochin, India, subjected to treatment by conventional activated sludge plant (CASP) was evaluated for its efficiency and the bacterial diversity associated with CASP. Samples collected over a period of five months were evaluated for the Organic Load Removal Efficiency (OLRE) of the system with regard to the removal of the $BOD_5$, COD, TSS and FOG of the disposed effluent. Total viable counts were made on the samples of the Mixed Liquor Suspended Solids (MLSS) of the conventional activated sludge plant (CASP) and distinct colony types were isolated and identified employing polyphasic taxonomy including molecular taxonomy. The CASP showed more than 50% OLRE. Species of *Bacillus* were the predominant group among the bacteria isolated, and most of them were capable of producing amylase, caseinase, gelatinase, lipase, and cellulase production; biofilm formation; and biosurfactant production. Study indicated that species of *Bacillus* play a significant role in the efficiency of CASP.

## Introduction

Wastewater treatment is an inevitable and mandatory process that directly influences the human life including public health, environmental protection, and aesthetics besides conservation of natural resources, sustainable development and sustainable utilization of available water resources in the context of growing concerns for availability of water in future. Indiscriminate discharge of untreated wastewater is the death knell to the water bodies leading to eutrophication, anoxic condition and biodiversity reduction turning the water resources to dead zones making them even unfit to serve fresh water to humanity (WWAP, 2009; Water, 2013). Hence, irrespective of the source whether domestic or industrial, wastewater must be collected and treated before returning it into the natural water cycle (UNESCO, 2012) which otherwise would contribute to deterioration in environmental health. Globally only 20% of the wastewater receives proper treatment before discharge and the rest reaches water resources untreated (Anbusaravanan, 1993; UNESCO, 2012). Water Act of 1974 and its subsequent amendments in 1988 compel the industries to treat their wastewater to meet the stringent effluent norms set by the Central Pollution Control Board (CPCB) and State Pollution Control Boards (SPCBs) (Walker, 1998).

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Biological treatment of waste water/sewage, for its eco-friendly edge, is the most preferred method of treatment although chemical and physical methods of treatments are also done along with biological methods. Among the different methods of biological treatment systems available, Conventional Activated Sludge plant (CASP) is the most commonly used method for secondary treatment of waste water worldwide relying on diverse, complex and most dynamic microbial communities in the reactor part i.e. aeration basin, to enhance the quality of effluent generated (Graham and Smith, 2004; Daims *et al.*, 2006; Tchobanoglous *et al.,* 2003; Ramothokang *et al.,* 2003). To date, most wastewater treatment processes rely on the use of activated sludge, which is defined as a very heterogeneous assembly of microorganisms, mainly dominated by a number of bacterial taxa (Blackall *et al.*, 1998; Soddell *et al.,* 1990). The performance of activated sludge process or its efficiency in terms of organic load removal largely depends on the activity of complex microbial communities (Gentile, 2007).

The activated sludge treatment is practiced over nearly for a century with several modifications and updating since its initial development by Arden and Lockett at England in 1914 (Metcalf and Eddy, 1979). The conventional activated sludge process is a suspended growth aerobic technology that comprises an active biomass of microbes, mainly bacteria, fungi, and protozoa in the aeration basin of the reactor (Gros *et al.*, 2010; Sacramento 1970). A typical activated sludge process includes an *aeration tank* wherein oxygen and microorganisms get well mixed with the wastes present

in the influent, a *sedimentation tank* for the solid liquid separation of the treated water (sludge settling) against gravity, and a *Recycler* that enables recycling a portion of active sludge back to the reactor as Return Activated Sludge (RAS) for enhancing the bio degradative potential of the reactor microflora (Qasim, 1998). The excess sludge or Biomass is removed from the *sedimentation tank* as waste activated sludge (WAS) and the supernatant is disposed as the treated effluent. The biomass secrete exoenzyme of varied potential to digest and absorb the pollutant organic matter to grow and multiply forming flocs of biomass that settle on gravity in the sedimentation part of the plant (Ramothokang *et al*., 2003; Chai and Lie, 2008; Spellman 2008). Treated wastewater is discharged into surface waters including lakes and rivers and is eventually reused. Hence, monitoring of the chemical and bacteriological composition is inevitable in guaranteeing public health (Ellis and Timothy, 2004).

Nowadays rapid urbanization, industrialization, and ecotourism among other factors have led to the rapid growth of aviation industry and it is a quite common scenario, where the number of flight services carrying passengers to various destinations across the globe and within any country has increased both in domestic and international sectors. This increase in flight services also simultaneously led to flourishing of commercial flight kitchen that caters to the in-flight food requirements on board. Thus, there is a proliferation of several commercial food kitchens that prepare food in large scale for the flights. As a consequence, there is a growing concern for the safe and effective treatment of waste water generated and discharged from such commercial flight kitchens in to environment. In this context this communication report here, the type of heterotrophic bacteria associated with the wastewater generated from a commercial flight kitchen, catering to the needs of different flight services operating from the International Airport, Cochin, India, and subjected to treatment employing conventional activated sludge plant (CASP). The study highlights the potential properties and role of heterotrophic bacteria in the efficiency of CASP in effecting a satisfactory treatment of the wastewater complying with stipulated norms of pollution control Boards.

## Materials and Methods

### Collection of Samples of Wastewater

Waste water samples were collected from the conventional activated sludge plant of a commercial flight kitchen that cater to the various flight services operated from the International Airport Cochin, Kerala, India. The Commercial flight kitchen (CFK) is treating the wastewater generated from their food processing and food manufacturing units by conventional activated sludge plant (CASP) which is the main mode of aerobic treatment among other treatments. In this study, efforts were made to conduct the evaluation of the efficiency of the CASP over a period of 5 months, particularly the role of heterotrophic bacterial diversity in the effective treatment of the plant. For this purpose, samples of the influent, effluent and the wastewater from the aeration tank were collected separately in triplicate for chemical analysis, and simultaneously collecting the Mixed Liquor Suspended Solids (MLSS) aseptically for the bacteriological studies. These samples were collected periodically at intervals of fifteen days for a total period of 150 days. Samples collected in aseptic clean plastic containers were transported immediately to the laboratory and subjected to chemical characteristics and bacteriological studies.

### Chemical analysis

The temperature and the pH of the samples were noted in the field itself and samples taken to the lab were analysed for the significant waste water quality parameters which included Total suspended solids (TSS), Biological Oxygen Demand ($BOD_{5)}$, Chemical Oxygen Demand (COD), and Fat Oil and Grease (FOG) as per the protocols of American Public Health Association's (APHA) "Standard methods for the examination of water and wastewater (Metcalf *et al*., 2004; APHA, 2005). The analytical values expressed are the mean of three replicates.

### Treatment efficiency (TE) of the CASP system

The treatment efficiency (TE) of the Conventional activated sludge plant(CASP)for the removal of the organic load was computed from the percentage removal values of TSS, $BOD_5$, COD, and FOG obtained for the influent before it is disposed of as effluent. The efficiency of the system for the removal of a particular parameter was calculated as $OLRE=[(P_{(i)}- P_{(e)})/P_{(i)}]X100$ where, OLRE (Organic Load Removal Efficiency) is the percentage removal of the parameter of concern or Treatment Efficiency(TE) of the plant with respect to that parameter. P (i) - The influent value of the parameter under consideration, P (e)-The effluent value of that parameter. The Weighted average efficiency Index-CASP(TE-WAI) is deducted after giving due relevance for the statutory norms in the removal of each parameter. The weights given are 40% for $BOD_5$, 25% each for COD and TSS and 10% for FOG. Hence the weighted average index for the treatment efficiency of conventional activated sludge plant, $CASP(TE\text{-}WAI) = RE_{BOD5}\times0.4+RE_{COD}\times0.25+RE_{TSS}\times 0.25+ RE_{FOG}\times0.1$, where RE is the removal efficiency of the plant for the parameter under consideration.

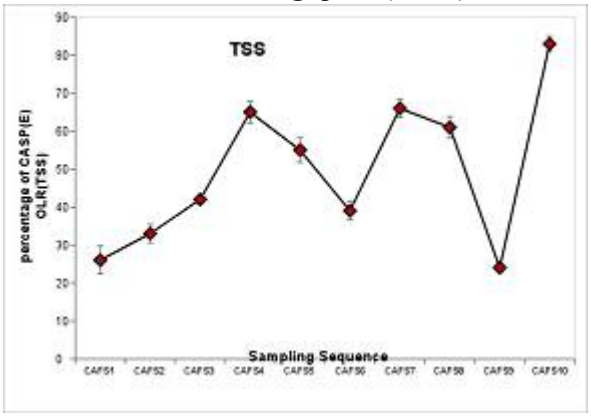
### Bacteriological analysis

Samples collected from the MLSS of the aeration tank of the conventional activated sludge plant, were serially diluted and spread plated on nutrient agar medium (HIMedia). The plating was made in triplicate for the different dilutions and incubated for 72 hours at room temperature ($28\pm2$ $^{o}$C). After incubation total count of viable distinct colonies (TVC) grown as single cell colonies on the plates were estimated with a colony counter. Averages of the TVC of triplicates of the plates were recorded. The morphologically distinct colony forming units (CFUs) were isolated, assigned with culture code, and documented. Purification of the isolates was made by repeated streaking of the isolates on the nutrient agar plates. The purified isolates were stocked using the paraffin overlay method. One set of the stock culture of the isolates was used for identification and other evaluation studies. The total viable bacterial load was estimated as the total viable counts (TVC) of the MLSS for all the samples during the course of the study and expressed as TVC/ml, the values which have exponential nature were converted to Logarithmic (Log) and Natural logarithmic (LN) forms for comparison purpose.

### *Bacterial colony morphology*.

The bacterial isolates with distinct colony morphology, their quantum and diversity with respect to each sampling time were observed and noted. Based on the diversity, abundance and evenness of these morphologically distinct colony types, Simpson diversity index was calculated.

**Figure 1a. Percent Removal efficiency (RE) with respect to the TSS and BOD recorded during treatment of waste water of the commercial flight kitchen (CFK) in the Conventional activated sludge plant (CASP).**



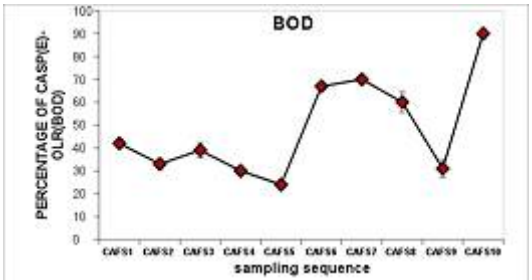
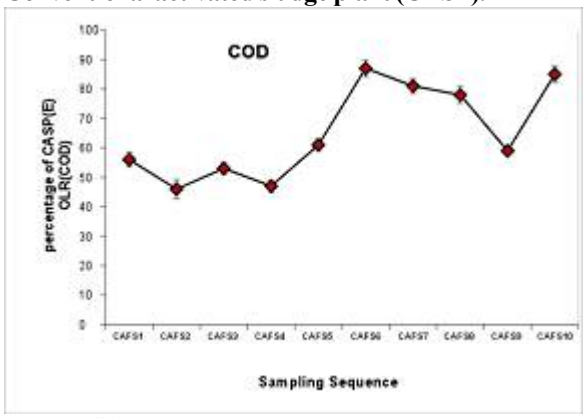




**Figure 1b. Percent Removal efficiency (RE) with respect to the COD and FOG recorded during treatment of waste water of the commercial flight kitchen (CFK) in the Conventional activated sludge plant (CASP).**



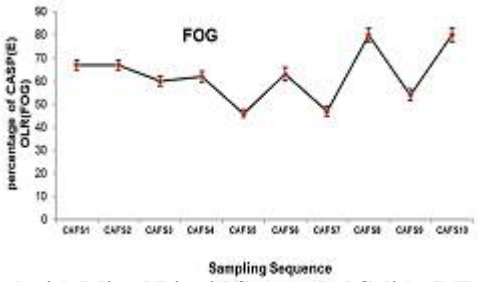




**Figure 2. Phylogenetic tree of the heterotrophic Bacteria associated with Mixed Liquid Suspended Solids (MLSS) of the Conventional Activated Sludge (CAS) of the waste water treatment plant (WWTP) of a commercial flight kitchen located in Cochin, India.**

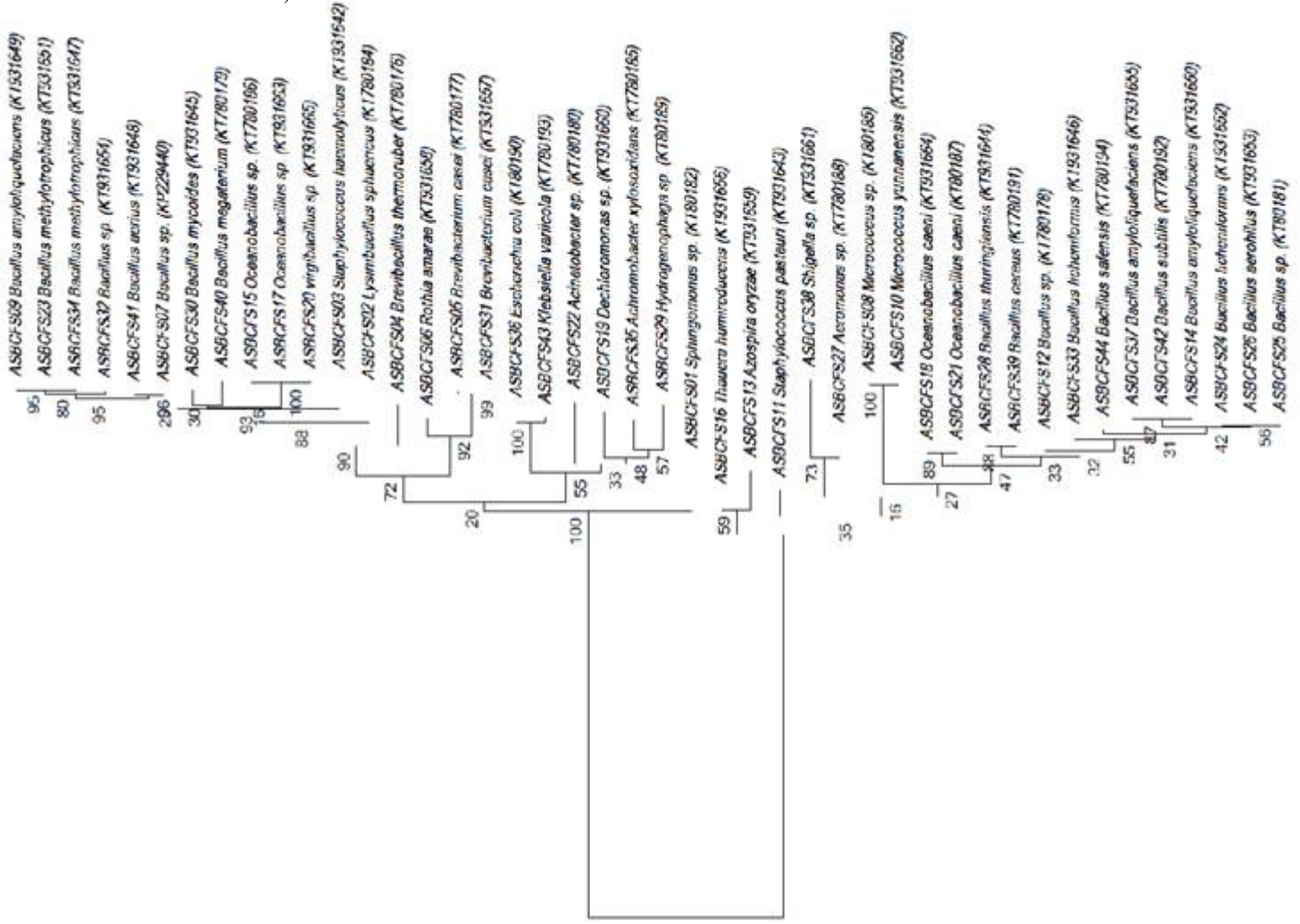


**Table.1. Organic load removal efficiency (OLRE) with respect to the Quality parameters under considerations shown by the Conventional activated sludge plant (CASP) treating the waste water of the commercial flight kitchen (CFK) during the continuous evaluation study (CES) period.**

| SAM: | TSS | | | $BOD_5$ | | | COD | | | FOG | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TIME | INV | EFV | RE% | INV | EFV | RE% | INV | EFV | RE% | INV | EFV | RE% | OLRE (WAI)% |
| **T1** | 82 | 61 | 26 | 100 | 58 | 42 | 463 | 201 | 57 | 145 | 48 | 67 | 44 |
| **T2** | 64 | 43 | 33 | 180 | 120 | 33 | 360 | 196 | 45 | 148 | 60 | 59 | 39 |
| **T3** | 36 | 21 | 42 | 169 | 103 | 39 | 423 | 199 | 53 | 102 | 41 | 60 | 45 |
| **T4** | 88 | 31 | 64 | 274 | 198 | 28 | 511 | 270 | 47 | 107 | 41 | 62 | 45 |
| **T5** | 94 | 42 | 55 | 114 | 87 | 23 | 363 | 143 | 61 | 115 | 62 | 46 | 43 |
| **T6** | 103 | 63 | 39 | 148 | 49 | 67 | 413 | 78 | 81 | 133 | 50 | 63 | 63 |
| **T7** | 114 | 39 | 66 | 176 | 53 | 70 | 514 | 96 | 81 | 114 | 61 | 61 | 71 |
| **T8** | 74 | 29 | 61 | 170 | 68 | 60 | 460 | 102 | 78 | 105 | 21 | 80 | 67 |
| **T9** | 92 | 70 | 24 | 168 | 116 | 31 | 515 | 209 | 59 | 99 | 45 | 55 | 39 |
| **T10** | 98 | 17 | 83 | 300 | 30 | 90 | 514 | 79 | 85 | 119 | 24 | 80 | 86 |

All parameters are in mg/L and are the mean of the triplicate observations. INV-Influent value of the parameter considered .EFV-Effluent value of the parameter, T1 to T10 Sampling Trials done. TSS-Total suspended solids, $BOD_5$-Biochemical oxygen demand (five day), COD-Chemical oxygen demand ,FOG-Fat, Oil & Grease ,RE% or $CASP_{(TE)}$- percentage removal of the parameter concerned or Conventional activated sludge treatment efficiency of the plant with respect to the parameter at the time of trial. OLRE (WAI) %-Organic load removal efficiency of the plant calculated after giving due weightage to each of the parameter studied during each trial.

**Table 2. Heterotrophic bacterial population and diversity indices of isolates obtained from Mixed Liquid suspended solids (MLSS) of the Conventional Activated Sludge (CAS) of the waste water treatment plant (WWTP) of a commercial flight kitchen located in Cochin, Kerala, India CES-T1 to T10 —continuous evaluation studies trials, OLRE (WAI)-Organic Load Removal Efficiency with regard to the parameters taken together as Weighted Average.**

| Sampling trials | Distinct CFU Types | Isolate code | T VC | TVC- (Log ) | TVC- (LN) | Simpson Diversity Index | OLRE (WAI) |
|---|---|---|---|---|---|---|---|
| CES-T1 | 1 | ASBCFS01 | $6.3\times10^5$ | 4.799 | 13.4 | 0.71 | 44 |
| | 2 | ASBCFS02 | | | | | |
| | 3 | ASBCFS03 | | | | | |
| | 4 | ASBCFS04 | | | | | |
| CES-T2 | 1 | ASBCFS05 | $2.08\times10^8$ | 7.318 | 19.5 | 0.47 | 39 |
| | 2 | ASBCFS06 | | | | | |
| CES-T3 | 1 | ASBCFS07 | $3.7\times10^7$ | 6.568 | 17.4 | 0.63 | 45 |
| | 2 | ASBCFS08 | | | | | |
| | 3 | ASBCFS09 | | | | | |
| | 4 | ASBCFS10 | | | | | |

**continued Table-2 ....**

| Sampling trials | Distinct CFU Types | Isolate code | T VC | TVC-(Log ) | TVC-(LN) | Simpson Diversity Index | OLRE (WAI) |
|---|---|---|---|---|---|---|---|
| CES-T4 | 1 | ASBCFS11 | $3.4\times10^{7}$ | 6.531 | 17.3 | 0.43 | 45 |
| | 2 | ASBCFS12 | | | | | |
| CES-T5 | 1 | ASBCFS13 | $7.8\times10^{10}$ | 9.892 | 25.1 | 0.46 | 43 |
| | 2 | ASBCFS14 | | | | | |
| CES-T6 | 1 | ASBCFS15 | $5.0\times10^{9}$ | 8.699 | 22.3 | 0.76 | 63 |
| | 2 | ASBCFS16 | | | | | |
| | 3 | ASBCFS17 | | | | | |
| | 4 | ASBCFS18 | | | | | |
| | 5 | ASBCFS19 | | | | | |
| | 6 | ASBCFS20 | | | | | |
| CES-T7 | 1 | ASBCFS21 | $3.7\times10^{7}$ | 6.568 | 17.4 | 0.79 | 71 |
| | 2 | ASBCFS22 | | | | | |
| | 3 | ASBCFS23 | | | | | |
| | 4 | ASBCFS24 | | | | | |
| | 5 | ASBCFS25 | | | | | |
| | 6 | ASBCFS26 | | | | | |
| CES-T8 | 1 | ASBCFS27 | $1.15\times10^{10}$ | 9.06 | 23.2 | 0.65 | 67 |
| | 2 | ASBCFS28 | | | | | |
| | 3 | ASBCFS29 | | | | | |
| | 4 | ASBCFS30 | | | | | |
| | 5 | ASBCFS31 | | | | | |
| CES-T9 | 1 | ASBCFS32 | $9.6\times10^{10}$ | 9.982 | 25.2 | 0.57 | 39 |
| | 2 | ASBCFS33 | | | | | |
| | 3 | ASBCFS34 | | | | | |
| CES-T10 | 1 | ASBCFS35 | 7.0×105 | 4.845 | 13.5 | 0.85 | 86 |
| | 2 | ASBCFS36 | | | | | |
| | 3 | ASBCFS37 | | | | | |
| | 4 | ASBCFS38 | | | | | |
| | 5 | ASBCFS39 | | | | | |
| | 6 | ASBCFS40 | | | | | |
| | 7 | ASBCFS41 | | | | | |
| | 8 | ASBCFS42 | | | | | |
| | 9 | ASBCFS43 | | | | | |
| | 10 | ASBCFS44 | | | | | |

**Table-3**. Identification of the heterotrophic Bacteria associated with Mixed Liquid Suspended Solids (MLSS) of the Conventional Activated Sludge (CAS) of the waste water treatment plant (WWTP) of a commercial flight kitchen located in Cochin, India

| Sampling trials | Isolate code | Identification | Accession Nos |
|---|---|---|---|
| CES-T1 | ASBCFS01 | *Sphingomonas sp.* | KT780182 |
| | ASBCFS02 | *Lysinibacillus sphaericus* | KT780184 |
| | ASBCFS03 | *Staphylococcus haemolyticus* | KT931642 |
| | ASBCFS04 | *Brevibacillus thermoruber* | KT780176 |
| CES-T2 | ASBCFS05 | *Brevibacterium casei* | KT780177 |
| | ASBCFS06 | *Rothia amarae* | KT931658 |
| CES-T3 | ASBCFS07 | *Bacillus sp.* | KP229440 |
| | ASBCFS08 | *Micrococcus sp.* | KT780185 |
| | ASBCFS09 | *Bacillus amyloliquefaciens* | KT931649 |
| | ASBCFS10 | *Micrococcus yunnanensis* | KT931662 |
| CES-T4 | ASBCFS11 | *Staphylococcus pasteuri* | KT931643 |
| | ASBCFS12 | *Bacillus sp.* | KT780178 |
| CES-T5 | ASBCFS13 | *Azospira oryzae* | KT931659 |
| | ASBCFS14 | *Bacillus amyloliquefaciens* | KT931650 |
| CES-T6 | ASBCFS15 | *Oceanobacillus sp.* | KT780186 |
| | ASBCFS16 | *Thauera humireducens* | KT931656 |
| | ASBCFS17 | *Oceanobacillus sp.* | KT931663 |
| | ASBCFS18 | *Oceanobacillus caeni* | KT931664 |
| | ASBCFS19 | *Dechloromonas sp.* | KT931660 |
| | ASBCFS20 | *Virgibacillus sp.* | KT931665 |
| CES-T7 | ASBCFS21 | *Oceanobacillus caeni* | KT780187 |
| | ASBCFS22 | *Acinetobacter sp.* | KT780180 |
| | ASBCFS23 | *Bacillus methylotrophicus* | KT931651 |
| | ASBCFS24 | *Bacillus licheniformis* | KT931652 |
| | ASBCFS25 | *Bacillus sp.* | KT780181 |
| | ASBCFS26 | *Bacillus aerophilus* | KT931653 |
| CES-T8 | ASBCFS27 | *Aeromonas sp.* | KT780186 |
| | ASBCFS28 | *Bacillus thuringiensis* | KT931644 |
| | ASBCFS29 | *Hydrogenophaga sp.* | KT780189 |
| | ASBCFS30 | *Bacillus mycoides* | KT931645 |
| | ASBCFS31 | *Brevibacterium casei* | KT931657 |
| CES-T9 | ASBCFS32 | *Bacillus sp.* | KT931654 |
| | ASBCFS33 | *Bacillus licheniformis* | KT931646 |
| | ASBCFS34 | *Bacillus methylotrophicus* | KT931647 |
| CES-T10 | ASBCFS35 | *Achromobacter xylosoxidans* | KT780183 |
| | ASBCFS36 | *Escherichia coli* | KT780190 |
| | ASBCFS37 | *Bacillus amyloliquefaciens* | KT931655 |
| | ASBCFS38 | *Shigella sp.* | KT931661 |
| | ASBCFS39 | *Bacillus cereus* | KT780191 |
| | ASBCFS40 | *Bacillus megaterium* | KT780179 |
| | ASBCFS41 | *Bacillus aerius* | KT931648 |
| | ASBCFS42 | *Bacillus subtilis* | KT780192 |
| | ASBCFS43 | *Klebsiella variicola* | KT780193 |
| | ASBCFS44 | *Bacillus safensis* | KT780194 |

**CES-T**, continuous evaluation studies trial:

**Table. 4** Potential characteristics desirable for treatment of waste water observed in the isolates obtained from the Mixed Liquor suspended solids (MLSS) of the Conventional Activated Sludge (CAS) of the waste water treatment plant (WWTP) of a commercial flight kitchen located in Cochin, India

| SL: NO | Sam-pling Trials | Isolate Code | Identity of the isolates | Hydrolytic Enzyme profiling | | | | | | Isolates with Treatment Enhancing Attribute (TEA) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Amylase | Caseinase | gelatinase | Lipase | cellulase | Multiple enzyme producers | Biofilm Formation | Biosurfactant Production | Forms with Multiple TEA |
| 1 | CES-T1 | ASBCFS01 | *Sphingomonas sp.* | - | + | + | + | - | | - | - | |
| 2 | | ASBCFS02 | *Lysinibacillus sphaericus* | - | - | - | + | - | | - | - | |
| 3 | | ASBCFS03 | *Staphylococcus haemolyticus* | + | - | + | + | - | | + | + | v |
| 4 | | ASBCFS04 | *Brevibacillus thermoruber* | - | - | + | - | + | | - | - | |
| 5 | CES-T2 | ASBCFS05 | *Brevibacterium casei* | - | + | + | - | - | | + | - | |
| 6 | | ASBCFS06 | *Rothia amarae* | + | - | - | - | - | | - | - | |
| 7 | CES-T3 | ASBCFS07 | *Bacillus sp.* | - | + | + | + | - | | + | + | v |
| 8 | | ASBCFS08 | *Micrococcus sp.* | - | - | + | + | - | | - | + | |
| 9 | | ASBCFS09 | *Bacillus amyloliquefaciens* | + | + | + | + | + | v | + | + | v |
| 10 | | ASBCFS10 | *Micrococcus yunnanensis* | + | + | - | - | - | | - | + | |
| 11 | CES-T4 | ASBCFS11 | *Staphylococcus pasteuri* | - | - | - | + | - | | - | + | |
| 12 | | ASBCFS12 | *Bacillus sp.* | + | + | + | - | - | | + | - | |
| 13 | CES-T5 | ASBCFS13 | *Azospira oryzae* | + | - | - | + | - | | - | - | |
| 14 | | ASBCFS14 | *Bacillus amyloliquefaciens* | + | + | + | + | + | **v** | + | + | **v** |
| 15 | CES- | ASBCFS15 | *Oceanobacillus sp.* | + | - | + | + | - | | - | - | |
| 16 | | ASBCFS16 | *Thauera humireducens* | + | + | - | - | + | | - | + | |

**continued Table-4 ....**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | CES-T6 | ASBCFS17 | *Oceanobacillus sp.* | + | + | + | + | - | | - | + | |
| 18 | | ASBCFS18 | *Oceanobacillus caeni* | + | + | + | - | + | | + | + | v |
| 19 | | ASBCFS19 | *Dechloromonas sp.* | + | - | - | + | - | | - | + | |
| 20 | | ASBCFS20 | *Virgibacillus sp.* | + | + | - | + | - | | - | - | |
| 21 | CES-T7 | ASBCFS21 | *Oceanobacillus caeni* | + | + | + | - | + | | - | + | |
| 22 | | ASBCFS22 | *Acinetobacter sp.* | + | - | + | - | + | | - | - | |
| 23 | | ASBCFS23 | *Bacillus methylotrophicus* | + | + | + | + | + | v | + | + | v |
| 24 | | ASBCFS24 | *Bacillus licheniformis* | + | + | + | + | + | v | + | + | v |
| 25 | | ASBCFS25 | *Bacillus sp.* | + | - | - | + | - | | + | + | v |
| 26 | | ASBCFS26 | *Bacillus aerophilus* | + | + | + | + | + | v | + | + | v |
| 27 | CES-T8 | ASBCFS27 | *Aeromonas sp.* | - | + | + | - | - | | - | - | |
| 28 | | ASBCFS28 | *Bacillus thuringiensis* | + | + | + | + | + | v | - | - | |
| 29 | | ASBCFS29 | *Hydrogenophaga sp.* | - | - | + | + | - | | - | + | |
| 30 | | ASBCFS30 | *Bacillus mycoides* | - | + | + | + | + | | - | - | |
| 31 | | ASBCFS31 | *Brevibacterium casei* | - | + | + | - | - | | + | + | v |
| 32 | CES-T9 | ASBCFS32 | *Bacillus sp.* | + | + | + | - | - | | + | - | |
| 33 | | ASBCFS33 | *Bacillus licheniformis* | - | + | + | - | - | | + | + | v |
| 34 | | ASBCFS34 | *Bacillus methylotrophicus* | + | + | + | + | + | v | - | + | |
| 35 | CES-T10 | ASBCFS35 | *Achromobacter xylosoxidans* | + | - | + | - | - | | - | - | |
| 36 | | ASBCFS36 | *Escherichia coli* | + | - | + | + | - | | + | - | |
| 37 | | ASBCFS37 | *Bacillus amyloliquefaciens* | + | + | + | + | + | v | + | + | v |
| 38 | | ASBCFS38 | *Shigella sp.* | + | - | + | - | - | | + | - | |
| 39 | | ASBCFS39 | *Bacillus cereus* | + | - | + | + | - | | - | + | |
| 40 | | ASBCFS40 | *Bacillus megaterium* | + | + | + | + | + | v | + | - | |
| 41 | | ASBCFS41 | *Bacillus aerius* | + | + | + | + | + | v | + | + | v |
| 42 | | ASBCFS42 | *Bacillus subtilis* | + | + | + | + | + | v | + | + | v |
| 43 | | ASBCFS43 | *Klebsiella variicola* | - | - | - | + | - | | + | - | |
| 44 | | ASBCFS44 | *Bacillus safensis* | + | + | + | + | + | v | + | + | v |

CES-T1 to T10- continuous evaluation studies Trials

***Grams reaction, Morphological, and Biochemical characterisation***

Each of the isolate that had distinct CFU was tested for their Grams reaction as to whether Grams positive or Grams negative and the shapes of the bacterial cells were noted (Holt *et al.,* 1994). All the isolates were also tested for their motility, and formation of capsule and spore. The Schaeffer-Fulton staining protocol was used for testing spore formation (Leboffe and Pierce, 2002). Anthony's method was employed for checking capsule formations (Anthony, 1931) and Sulphide Indole Motility (SIM) medium for motility tests were done. Biochemical characterizations of all the purified isolates were done according to the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Tests included IMViC, TSI, Oxidase, catalase, urease and nitrate reduction tests. The beta haemolysis is an indication of the opportunistic nature of the isolate and so the isolates were further analysed for haemolysis on blood agar plate. Some isolates formed halo when streaked and incubated on the Sheep blood agar plates. According to the formation of halo on the blood agar, three types of haemolysis were noted: - Alpha, Beta and Gamma type.

**Hydrolytic enzyme profiling**

Hydrolytic enzymes secreted into the environment, have crucial role in the hydrolysis and degradation of proteins, fats, and carbohydrates that are present in the sewage. These enzymes degrade these polymers into smaller units and helps absorption by microorganisms and biosynthesis accelerating the biogeochemical cycling. So, all the isolates were evaluated for their ability to elaborate alpha amylase, proteinases (caseinase, gelatinase), lipase and cellulase. All the isolates were grown on the nutrient agar plates enriched with the substrate appropriate for the respective enzymes and tested as detailed below.

***Amylase***

Starch enriched (1% starch, pH 7.2) nutrient agar (HI Media) plates were spot inoculated and incubated at 37°C for 48 hours. After incubation Lugol's iodine solution (Lugol's iodine: distilled water 1:5) was flooded over plates. Appearance of a halo zone around the colony indicated production of amylase (Gogi *et al*., 1998).

***Protease***

Isolates were streaked on gelatin (gelatin 4%, pH-7.2) enriched nutrient agar (HIMedia) plates ad tested for gelatinase production and Milk agar plate was employed for production of caseinase (Chan *et al*.,1986). After incubation for 48hours at 37 °C, both the gelatin agar plates and milk agar plates were flooded with10% mercuric chloride reagent. Appearance of halo zone around the colony indicated production of respective proteinases (Gelatinase /Caseinase) (Bernfeld, 1955). The isolates that showed halo zone were considered as protease positive.

***Lipase***

Tributyrin (1%) containing nutrient agar (HIMedia) plates (pH-7.5) were inoculated with the test strains and incubated at 37 °C for 48 hours. Appearance of halo zone around the colony indicated production of lipase (Kunitz, 1947).

***Cellulase***

Agar medium containing 0.5% (W/V) carboxy methyl cellulose (CMC) as the sole carbon source was streaked with isolates and incubated at 37°C for 72 hours and the plates were flooded with 1% (W/V) Congo red (Metcalf *et al.,* 1979). The dye was decanted after 5 minutes and the plates were once again flooded with 5M NaCl and left as such for 20-30 minutes before decanted. Appearance of pale orange to clear zone around the colony against red background was considered as positive for cellulase production (Teather and Wood, 1982).

**Treatment Enhancing Attributes (TEA)**

Ability to form bio films and produce bio surfactants are considered as significant attributes for microbes with respect to wastewater treatment perspective. Bio film formers produce a type of extra cellular polymerase substance (EPS) and that facilitate clustering together forming slime that adhere to hard materials or substratum helping relatively fast biogeochemical cycling. Bio surfactant producers release certain molecules that reduce surface tension and enhance emulsification for the degradation and absorption of fats and lipids. Hence, all the bacterial isolates were further individually tested for their potential to form biofilm and produce biosurfactant as detailed below.

***Bio film formation***

Congo Red Agar (CRA) plate assay with slight modification (Freeman *et al.,* 1989) was employed for the qualitative assay of biofilm formation. CRA plates were prepared with the nutrient broth 37 g/L, sucrose 50 g/L, Agar agar 10 g/L and Congo red indicator 0.08 g/L. First Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121°C for 15 minutes) separately from other medium constituents. Then it was added to the autoclaved nutrient agar broth with sucrose at 55°C.CRA plates were inoculated with test organisms and incubated at 37°C for 24 hours aerobically. Black colonies indicated biofilm production (Reid, 1999). The experiment was performed in triplicate.

***Bio surfactant production***

Biosurfactant production by the test strains was evaluated as per the protocol suggested by Ellaiah *et al.* (Ellaiah *et al*., 2002). Isolates cultured in nutrient broth medium at 28 °C, for 24 hours at160 rpm were used for the Biosurfactant production test.1ml of the supernatant culture along with 3ml of autoclaved distilled water and 6ml of kerosene taken in similar sized glass test tubes were vortexed well for 2minutes for emulsification, and kept for 48 hours. The Emulsification index (EI48) was calculated as a/b x 100 where "a" is the height of the emulsified layer and "b" the total column height multiplied by100.The experiments were done in triplicate for each of the isolate and the emulsification index was taken as the average of these three values. The isolates that showed more than 40% of EI48 in these tests were only considered as positive for bio surfactant activity. Control tests included sterilised nutrient broth instead of culture supernatant.

**Molecular identification**

Molecular characterization of all the morphologically distinct CFUs isolated during the study was done using 16S rRNA partial gene sequencing and sequence analysis using bioinformatics tools for identification up to species level.

***Genotypic Characterisation***

The Genomic DNA of the isolates was extracted according to Sambrook *etal*. (Sambrook *et al.,* 2000). PCR amplification of 16S rRNA gene (1.5 Kb) was performed using universal primers16S Forward 5'GAGTTTGATCCTGGCTCAG3'; 16S Reverse 5'ACGGCTACCTTGTTACGACTT3'in a thermal cycler

(Biorad, USA) (Shivaji *et al*., 2000). Template DNA of 50ng was used in a 20 µL reaction with an initial denaturation for 2 minutes at 94 °C, followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extension at 72°C for 2 minutes, and a final extension for 10 minutes at 72°C. Electrophoretic separation of the amplicons was made in 1% agarose gel and gel pictures were captured using gel documentation system (Syngene, USA).

***DNA sequencing and in silico analysis***

Partial sequencing of 16S rRNA genes was done by Sanger's Dideoxy method using ABI 3730 Excel. Using BLAST, the partial 16S rRNA gene sequences of the clones were compared with public data bases (Altschul *et al.,* 1990). Clustal X program was used for compiling' and aligning of the sequences (Thompson *et al*., 1997) was done using BioEdit software (Hall, 1999). The phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei, 1987) with 1000 resampling bootstrap using Mega version 6 (Tamura *et al*., 2013).

**Results and Discussion**

**Chemical indices of waste water quality and OLRE**

Commercial flight kitchen effluent is more or less similar to Municipal wastewater and is composed of 99% water and 1% suspended, colloidal and dissolved solids. Organic matters like proteins, carbohydrates, fats and oils, Nutrients like nitrogen, potassium and phosphorous with Inorganic matters, dissolved minerals, chemical detergents and pathogenic microorganisms constitutes the solid portion of this wastewater (Bitton, 2005; Hanjra *et al*., 2012). Commercial kitchen is also experiencing lots of oil and grease from the plant maintenance activities besides the fat and oil that is discharged as residues from cooking activities. All types of food including meat, seafood, fatty food, vegetarian food items are usually prepared and thus the commercial flight kitchen wastewater is unique in its biochemical organic load. From the results presented in Table 1 &Figure 1a, it was noted that on average the TSS value got significantly reduced from 84.5 in influent waste water before treatment in CASP to 41.6 and the removal efficiency was 49.3% over the period of 5 months of treatment. The percent removal efficiency of TSS by CASP ranged from a minimum of 26 to a maximum of 83. Maximal removal efficiencies above 50% were recorded with samples collected after 60 days, 75 days, 105 days, 120 days and 150 days. The maximum permissible values recommended by Ministry of Environment, Forest& Climate change Notification (Gazette of India Notification 2016) for TSS in effluent of Common effluent treatment plant (CETP) that is intended to be released into inland surface waters / land for irrigation or into sea is only 100. In the present study it was observed that the TSS values were less than 100 and in compliance with the recommended values.

It was observed that on average the BOD value got significantly reduced from 179.9 in influent before treatment in CASP to 88.2 and the removal efficiency was 48 .3 % over the period of 5 months of treatment of waste water (Table 1 &Figure 1a). The percent removal efficiency of BOD by CASP ranged from a minimum of 23 to a maximum of 90. Maximal removal efficiencies above 50% were recorded with samples collected after 90 days, 105 days, 120 days and 150 days. The maximum permissible values recommended by Ministry of Environment, Forest& Climate change Notification (Gazette of India Notification 2016) for BOD in effluent of Common effluent treatment plant (CETP) that is intended to be released into inland surface waters is 30 and for release into land for irrigation/ into sea is 100. In the present study, it was observed that the BOD values were less than 100. It must be noted that the commercial flight kitchen nearby Cochin international airport release treated wastewater into land for irrigation and hence in compliance with the recommended values.

Whereas in the case of COD it was noted that on average the value got significantly reduced from 453.6 in influent before treatment in CASP to 157.3 and the removal efficiency was 64.7 % over the period of 5 months of treatment of waste water (Table 1 & Figure 1b). The percent removal efficiency of COD by CASP ranged from a minimum of 45 to a maximum of 85. Maximal removal efficiencies above 50% were recorded with samples collected after 15 days, 45 days, and during the rest of the period of study. The maximum permissible values recommended by Ministry of Environment, Forest& Climate change Notification (Gazette of India Notification 2016) for COD in effluent of Common effluent treatment plant (CETP) that is intended to be released into inland surface waters and for release into land for irrigation/ into sea is 250. In the present study, it was observed that the BOD values were less than 160. It must be noted that the commercial flight kitchen nearby Cochin international airport release treated waste water into land for irrigation and hence in compliance with the recommended values. It was noted that on average the FOG value got significantly reduced from 118.7 in influent before treatment in CASP to 45.3 and the removal efficiency was 63.3 % over the period of 5 months of treatment of waste water (Table 1 & Figure 1b). The percent removal efficiency of FOG by CASP ranged from a minimum of 46 to a maximum of 80. Except for the 75th day when the removal efficiency was less than 50%, at all other sampling days the removal efficiencies were above 50%. The maximum permissible values recommended by Ministry of Environment, Forest & Climate change Notification (Gazette of India Notification, 2016) for oil & Grease in effluent of Common effluent treatment plant (CETP) that is intended to be released into inland surface waters / land for irrigation or into sea is only 10. In the present study, it was observed that the FOG values were around 45which is above the recommended values. This result indicates need for improvement in the treatment of waste water with respect to FOG.

Whereas, on average the OLRE (WAI) value was recorded as 54.2 over the period of 5 months of treatment of wastewater. The percent removal efficiency of WAI of CASP ranged from a minimum of 39 to a maximum of 86 (Table 1). Maximal removal efficiencies above 50% were recorded with samples collected after 90 days, 105 days 120 days and 150 days. It was observed that the OLRE percentages were less than 50 % during the first 3 months of treatment the CASP gained momentum in OLRE only after 90 days of treatment to reach a maximum of 86% after 150 days. Similarly, all the parameters were observed to record a maximum removal efficiency only after 150 days of treatment. In general, it was observed that the CASP in commercial flight kitchen is capable of effecting significant treatment after approximately 3 months. May be bioaugmentation of this CASP with specific bacterial flora or enriched microorganism can shorten this period and can enhance treatment efficiency.

**Heterotrophic bacterial population and diversity indices**

Results obtained for the total viable counts of heterotrophic bacterial population and diversity indices

of bacterial isolates obtained from Mixed Liquor suspended solids (MLSS) of the Conventional Activated Sludge (CASP) of the waste water treatment plant (WWTP) of a commercial flight kitchen located in Cochin, India are presented in Table 2. The total viable bacterial population observed in the samples collected from CASP during the course of study varied from $6.3\times10^5$ after 15 days to a maximum of$9.6\times10^{10}$ after 135 days. The TVC was found to increase gradually to a maximum of $7.8\times10^{10}$ after 75 days. During the later period of study, the TVC levels remained high at $1.15\times10^{10}$ on the 120 $^{th}$ day and at $9.6\times10^{10}$ on 135 $^{th}$ day. However, the TVC was observed to be declined to $7.0\times10^5$on 150$^{th}$ day. Accordingly, the log(TVC) and ln(TVC) - values reflected the same trend. The log(TVC) values varied from a minimum of 4.799 on 15$^{th}$ day to a maximum of 9.982 after 135 days although very high values of 9.892, and 9.06 were recorded respectively on 75$^{th}$ day and 120$^{th}$ day of sampling. Similarly, ln(TVC) values were found to vary from a minimum of 13.4 on 15$^{th}$ day to a maximum of 25.2 on 135$^{th}$ day.

According to Torsvik, *et al*., biodiversity has been defined as the range of significantly different types of organisms and their relative abundance in an assemblage or community (Torsvik, *et al*., 1998). It is the 'biology of numbers and difference' (Gaston, 1996). Further, microbial diversity refers unequivocally to biological diversity at three levels: within species (genetic), species number (species) and community (ecological) diversity (Harpole, 2010). It has been observed that alteration of one or more factors of it influence the bacterial diversity too (Jonhsen, *et al*., 2001; Fraterrigo, *et al*., 2006). Microbial diversity influences significantly any ecosystem including the engineered one for the ecological services it renders. Diversity indices serve as valuable tools to quantify diversity in a community and describe its numerical structure. The conventional activated sludge plant that biologically treat the wastewater effectively is comparable to an engineered ecosystem, its diversity indices are indicative of its functional robustness and sustainability. The diversity indices of the bacterial populations with respect to the morphologically distinct colony forming units (CFUs) met with the different sampling periods of the conventional activated sludge plant of (CASP) of commercial flight kitchen is presented as Simpson diversity index in Table 2.With regard to the distinct types of colony type observed and subsequent identification to various species, it was observed that Simpson diversity index generally varied from a minimum of 0.43 (on 60day) to a maximum of 0.85 on 150$^{th}$ day. Minimal values of 0.47 (30$^{th}$ day), 0.43 (60$^{th}$ day) and 0.46(75$^{th}$ day) and maximum values of 0.85 (150$^{th}$ day) followed by 0.79 (105$^{th}$ day), 0.76 (90$^{th}$ day) and 0.71 (15$^{th}$ day) were also observed indicating the variation in diversity of species in the CASP during the period of study. From the Table 2, it was noted that maximal OLRE values coincided with maximal Simpson diversity index indicating the role of diverse flora in effective treatment of the waste water.

Waste water treatment efficiency of a treatment plant is fundamentally based on the microbial structure it fosters with (Wagner *et al*., 2002; Wakelin *et al*., 2008). Bacterial diversity analysis with cultivable bacteria of the activated biomass of a common effluent treatment plant (CETP) (Kapley *et al*., 2007) reflects similar approaches.

**Bacterial diversity-Qualitative composition**

Bacteria constitute the major component of activated sludge flocs and they are responsible for the biological oxidation of organic substrates, nitrification of ammonia, denitrification of nitrate and accumulation of phosphorous. The species of micro-organism which frequently dominate the biological waste water treatment system depend on the environmental conditions, process design, and plant operation (Akpor and Muchie, 2010). In the present study, 44 isolates of distinct morphology were obtained from the conventional activated sludge plant and they were identified up to species level. The obtained isolates were observed to demonstrate a vast species diversity. They mainly belonged to 20 different genera (Table 3) and showed variation in occurrence over the period of study. They included *Achromobacter xylosoxidans* (ASBCFS35), *Acinetobacter* sp. (ASBCFS22), *Aeromonas* sp.(ASBCFS27), *Azospira oryzae* (ASBCFS13), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus sp.*(ASBCFS07, ASBCFS12, ASBCFS25 & ASBCFS32), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Brevibacillus thermoruber* (ASBCFS04), *Brevibacterium casei* (ASBCFS31), *Brevibacterium casei* (ASBCFS05), *Dechloromonas* sp.(ASBCFS19), *Escherichia coli* (ASBCFS36), *Hydrogenophaga* sp.(ASBCFS29), *Klebsiella variicola* (ASBCFS43), *Lysinibacillus sphaericus*(ASBCFS02), *Micrococcus sp.*(ASBCFS08), *Micrococcus yunnanensis (ASBCFS10), Oceanobacillus caeni* (ASBCFS18 & ASBCFS21), *Oceanobacillus* sp. (ASBCFS15 & ASBCFS17), *Rothia amarae* (ASBCFS06), *Shigella* sp. (ASBCFS38), *Sphingomonas sp.*( ASBCFS01), *Staphylococcus haemolyticus*(ASBCFS03), *Staphylococcus pasteuri* (ASBCFS11), *Thauera humireducens* (ASBCFS16) and *Virgibacillus* sp.(ASBCFS20).

It was noted that among these groups, species belonging to the genera *Bacillus* dominated the bacterial flora obtained (19 isolates out of 44) followed by *Oceanobacillus* sp. (4 out of 44 isolates) (Table 3). Two isolates each of both *Staphylococcus* sp. and *Micrococcus* sp. were recorded as the next dominant group and all the rest of the genera incurred recorded single isolate for each genus. An interesting observation made was that while *Ocenaobacillus* strains were isolated from samples of Trial 6 Bacillus strains were incurred through the period of study from samples of trials T2, T3, T4, T5, T7, T8, T9 and T10.

Further it may be also noted that genus *Bacillus* was represented by *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), and *Bacillus sp.*(ASBCFS07, ASBCFS12, ASBCFS25 & ASBCFS32)(Table 3). The phylogenetic tree constructed for the various isolates obtained presented in Figure 2.

The microbial community, which is dominated by bacteria (Wagner *et al.,* 2002), plays an essential role in the

biological treatment reactors and has been studied for several decades by both isolation (Neilson, 1978) and molecular methods. The results of McLellan *et al*. (2010) on the bacterial diversity in a WWTP influent showed that Actinobacteria, Bacteroidetes, and Firmicutes were the most dominant three groups of bacteria in the influent, adding up to 37.5 % of the total bacteria (McLellan, 2010).

**Hydrolytic enzyme production**

The diversity of bacteria that the aeration tank fosters, its potential with regard to hydrolytic enzyme production and the evenness of these isolates are all the more significant for the treatment and removal of the waste water parameters. In the present study hydrolytic enzymes amylase, caseinase, gelatinase, lipase and cellulase production by all the 44 isolates were evaluated. From the data presented in Table 4 it may be noted that all the 5 different enzymes were produced by species of *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus licheniformis* (ASBCFS24), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus safensis* (ASBCFS44), *Bacillus subtilis* (ASBCFS42), and *Bacillus thuringiensis* (ASBCFS28). In general 27.27% (12 isolates) of the isolates obtained produced all the 5 enzymes evaluated. Whereas 25% (11 isolates) produced 3 enzymes and 29.55% (13isolates) produced only 2 enzymes. Only 9.1% of isolates could record 4 enzymes and another 9.1% could produce only one type of enzyme. Further, among the enzymes tested gelatinase producers were dominant (77.27%) followed by amylase (70.45%), lipase (65.9%), caseinase (61.36%) and cellulase (40.91%) Alpha amylase production was observed with *Achromobacter xylosoxidans* (ASBCFS35), *Acinetobacter* sp. (ASBCFS22), *Azospira oryzae* (ASBCFS13), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS12, ASBCFS25 & ASBCFS32), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Dechloromonas* sp. (ASBCFS19), *Escherichia coli* (ASBCFS36), *Micrococcus yunnanensis (ASBCFS10), Oceanobacillus caeni* (ASBCFS18 & ASBCFS21), *Oceanobacillus* sp. (ASBCFS15 & ASBCFS17), *Rothia amarae* (ASBCFS06), *Shigella* sp. (ASBCFS38), *Staphylococcus haemolyticus* (ASBCFS03), *Thauera humireducens* (ASBCFS16) and *Virgibacillus* sp. (ASBCFS20).

Caseinase production was observed with *Aeromonas*sp. (ASBCFS27), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS14 & ASBCFS37), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS12, & ASBCFS32), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Brevibacterium casei* (ASBCFS31), *Brevibacterium casei* (ASBCFS05), *Micrococcus yunnanensis (ASBCFS10), Oceanobacillus caeni* (ASBCFS18 &ASBCFS21), *Oceanobacillus* sp. (ASBCFS17), *Sphingomonas sp.* (ASBCFS01), *Thauera humireducens* (ASBCFS16) and *Virgibacillus* sp. (ASBCFS20).

Gelatinase production was observed with *Achromobacter xylosoxidans* (ASBCFS35), *Acinetobacter* sp. (ASBCFS22), *Aeromonas*sp. (ASBCFS27), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS12, & ASBCFS32), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Brevibacillus thermoruber* (ASBCFS04), *Brevibacterium casei* (ASBCFS31), *Brevibacterium casei* (ASBCFS05), *Escherichia coli* (ASBCFS36), *Hydrogenophaga* sp. (ASBCFS29), *Micrococcus sp.* (ASBCFS08), *Oceanobacillus caeni* (ASBCFS18 & ASBCFS21), *Oceanobacillus* sp. (ASBCFS15 & ASBCFS17), *Shigella* sp. (ASBCFS38), *Sphingomonas sp.* (ASBCFS01), and *Staphylococcus haemolyticus* (ASBCFS03).

Lipase production was observed with, *Azospira oryzae* (ASBCFS13), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS25), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Dechloromonas* sp. (ASBCFS19), *Escherichia coli* (ASBCFS36), *Hydrogenophaga* sp. (ASBCFS29), *Klebsiella variicola* (ASBCFS43), *Lysinibacillus sphaericus* (ASBCFS02), *Micrococcus sp.* (ASBCFS08), *Oceanobacillus* sp. (ASBCFS15 & ASBCFS17), *Sphingomonas sp.* (ASBCFS01), *Staphylococcus haemolyticus* (ASBCFS03), *Staphylococcus pasteuri* (ASBCFS11), and *Virgibacillus* sp. (ASBCFS20).

Cellulase production was observed with *Acinetobacter* sp. (ASBCFS22), *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus licheniformis* (ASBCFS24), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus mycoides* (ASBCFS30), *Bacillus safensis* (ASBCFS44), *Bacillus subtilis* (ASBCFS42), *Bacillus thuringiensis* (ASBCFS28), *Brevibacillus thermoruber* (ASBCFS04), *Oceanobacillus caeni* (ASBCFS18 & ASBCFS21), and *Thauera humireducens* (ASBCFS16).

**Biofilm formation and Bio-surfactant production**

Biofilms are consortia of microorganisms attached to biotic or abiotic surfaces that occur in many environments (O'Toole *et al*., 2000). Biofilm-forming bacteria play crucial roles in terrestrial and aquatic nutrient cycling and in the biodegradation of environmental pollutants (Davey and O'Toole, 2000). Several microorganisms are known to synthesize surface-active agents and Biosurfactants are surface-active substances synthesized by living cells (Rashedi *et al*., 2005). They have the properties of reducing surface tension, stabilizing emulsions, promoting foaming and are generally non-toxic and biodegradable. The diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions and potential applications in environmental protection makes them attractive for study (Karanth *et al*., 1999). In the present

study both Biofilm formation and Bio-surfactant production were observed (Table 4) with *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus methylotrophicus* (ASBCFS23), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS25), *Bacillus subtilis* (ASBCFS42), *Brevibacterium casei* (ASBCFS31), *Oceanobacillus caeni* (ASBCFS18) and *Staphylococcus haemolyticus* (ASBCFS03). The isolates, which could demonstrate both biofilm formation as well as biosurfactant production recorded 34.09% of the isolates obtained. Whereas, biofilm forming and biosurfactant producing isolates recorded, respectively, 50% and 56.82%. While 27.27 % neither produced biosurfactant nor biofilm formation.

Biofilm formation was recorded in the species of *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus megaterium* (ASBCFS40), *Bacillus methylotrophicus* (ASBCFS23), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS12, ASBCFS25 & ASBCFS32), *Bacillus subtilis* (ASBCFS42), *Brevibacterium casei* (ASBCFS31), *Brevibacterium casei* (ASBCFS05), *Escherichia coli* (ASBCFS36), *Klebsiella variicola* (ASBCFS43), *Oceanobacillus caeni* (ASBCFS18), *Shigella* sp. (ASBCFS38), and *Staphylococcus haemolyticus* (ASBCFS03) (Table 4).

In the present study bio-surfactant production was recorded by species of *Bacillus aerius* (ASBCFS41), *Bacillus aerophilus* (ASBCFS26), *Bacillus amyloliquefaciens* (ASBCFS09, ASBCFS14 & ASBCFS37), *Bacillus cereus* (ASBCFS39), *Bacillus licheniformis* (ASBCFS24 & ASBCFS33), *Bacillus methylotrophicus* (ASBCFS23 & ASBCFS34), *Bacillus safensis* (ASBCFS44), *Bacillus sp.* (ASBCFS07, ASBCFS25), *Bacillus subtilis* (ASBCFS42), *Brevibacterium casei* (ASBCFS31), *Dechloromonas* sp. (ASBCFS19), *Hydrogenophaga* sp. (ASBCFS29), *Micrococcus sp.* (ASBCFS08), *Micrococcus yunnanensis (ASBCFS10), Oceanobacillus caeni* (ASBCFS18 & ASBCFS21), *Oceanobacillus* sp. (ASBCFS17), *Staphylococcus haemolyticus* (ASBCFS03), *Staphylococcus pasteuri*(ASBCFS11), and *Thauera humireducens* (ASBCFS16) (Table 4).

Neither Biofilm formation nor Bio-surfactant production was recorded with *Achromobacter xylosoxidans* (ASBCFS35), *Acinetobacter* sp. (ASBCFS22), *Aeromonas* sp. (ASBCFS27), *Azospira oryzae* (ASBCFS13), *Bacillus mycoides* (ASBCFS30), *Bacillus thuringiensis* (ASBCFS28), *Brevibacillus thermoruber* (ASBCFS04), *Lysinibacillus sphaericus* (ASBCFS02), *Oceanobacillus* sp. (ASBCFS15), *Rothia amarae* (ASBCFS06), *Sphingomonassp.* (ASBCFS01), and *Virgibacillus* sp. (ASBCFS20**).**

From the results obtained in the present study it was noted that the CASP employed in the commercial flight kitchen that cater to the various flight services operated from the International Airport Cochin, Kerala India is efficient enough to treat the waste water generated during food manufacture to the tune of more than 50% in terms of the removal efficiency of quality parameters TSS, BOD and COD. However, the FOG levels fare in excess of the recommended maximum permissible values (10) recommended by Ministry of Environment, Forest & Climate change Notification (dt 1-1-2016) for oil & Grease in effluent of Common effluent treatment plant (CETP) that is intended to be released into inland surface waters / land for irrigation or into sea. It was further noted that 20 different heterotrophic bacterial genera were seen associated with the treatment of wastewater in CASP and were primarily dominated by the species of *Bacillus*. Among the different species of *Bacillus* obtained nine strains showed remarkable potential for hydrolytic enzymes amylase, protease, lipase and cellulase besides biofilm formation and biosurfactant production, which are desirable attributes for possible development of consortia for application as a supplement in the CASP. In fact, bio augmenting the wastewater treatment system with commercially prepared bacterial cultures to increase the density of desired bacteria and their enzymes to achieve a specific operational goal is a good possibility, for example, decrease sludge production or control malodour production. The addition of bacterial cultures increases the density of desired bacteria without significantly increasing the solids inventories and solids residence times of an activated sludge process. Treatment efficiency, permit compliance, and operational costs at a municipal wastewater treatment plant are influenced greatly by the enzymatic activities and abilities of a large population and diversity of potentially versatile forms of bacterial forms. Bio augmenting these forms would enhance bioremediation and is considered as more effective and practical technology with fewer environmental impacts (Kokare *et al.,* 2009). The present study strongly suggests that commercial flight kitchen wastewater treatment CASP requires bioaugmentation with developed microbial consortia. In this context, the species of *Bacillus* with multi-potential characteristics observed in this present study are ideal biocatalysts worth application subject to the fact that further research and development is warranted to develop such enriched microbial consortia.

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