Assessing the Potential of Organic Fraction of Municipal Solid Waste as a feedstock for Bioethanol Production

Mid-semester BTP progress report

Submitted by

Aditya Milind Naik

Enrollment ID: 190004019

In supervision of

Dr. Ashootosh S. Mandpe

Department of Civil Engineering

Indian Institute of Technology (IIT) Indore

INDEX

1. Introduction

The global energy consumption shows no sign of slowing down, averaging around 1% to 2% of the yearly increment (Ikelle, 2017). Today, the world economy is highly dependent on fossil fuels, mainly for transportation and energy security. This makes the sector highly price volatile and a driver of global inflation in the situation of an unfortunate crisis. In the case of India's thriving economy, the transport and energy sectors are at the core contributing 19.12% to its Gross Domestic Product. India spent 119 billion USD on crude oil imports in financial year 2021-22, which amounts to 20% of its forex reserves (Business Standard 2022). Apart from price volatility, fossil fuel combustion results in harmful and toxic emissions in the environment leading to climate deterioration and ozone depletion.

In recent years, there has been an increased focus on renewable energy, especially biofuels, to make the transportation sector more sustainable, emission-free, and 'green'. Among the biofuels identified, bioethanol is widely used, having the highest potential to be used as fuel (Kumar, 2021), and is being consumed largely with a share of 90% (Chen et al., 2021) of all biofuels being produced at a rate of 130 billion liters/year (Tse et al., 2021). Bioethanol refers to using biomass, such as sugarcane, corn, etc., for ethanol production through fermentative processes. Ethanol (C_2H_5OH) is a highly volatile, colorless, and flammable liquid at room temperature. Ethanol, as a less polluting fuel, burns completely and offers equivalent efficiency with lower cost inputs than petrol (Sarwal et al., 2020).

Ethanol is not directly used as a fuel but is blended with fossil fuels like petrol. The blending percentage varies country-wise. India is currently blending 10% ethanol while setting a target of E20 (20% blending) by 2025. The major feedstock used for ethanol production in India is sugarcane molasses. However, in the long run, sugarcane molasses will put excessive pressure on the distressed agriculture sector. Hence there is an urgent need to find alternative feedstock for the production of bioethanol in order to sustain future fuel demands.

The primary goal of this study is to identify the potential of an **Organic fraction of Municipal Solid Waste (OF-MSW)** as an alternative feedstock for bioethanol production **(Waste to Ethanol)**. The study also aims to identify the scalability of the solution by performing techno-economic analysis and environmental impact assessment.

Management of Municipal solid waste (MSW) in India still poses a significant challenge. The rate of production of MSW in Indian cities and towns has also increased due to the country's fast urbanization, industrialization, and population growth. Mismanagement of MSW can adversely impact the environment, pose a risk to public health, and lead to other socioeconomic issues (Gupta et al., 2015). India generates 143449 metric tonnes per day (MTPD) of MSW (Maurya L., 2017) while we are still figuring out various methods to dispose of the generated waste economically, efficiently, and sustainably. Valorization of waste to biofuel is a novel strategy to dispose of organic solid waste while at the same time addressing issues such as climate change and energy security. The current challenges in this field are high operational costs, availability of feedstock, logistics, coproducts, and downstream waste generation.

2. Novelty of Work

Generally, bioethanol is extracted either from 1G (first generation) feedstock such as corn, molasses, sugarcane, etc., which are rich in starch and carbohydrate contents, or 2G feedstock which refers to lignocellulose biomass, particularly agriculture and industrial residues. The present share of bioethanol production from these feedstocks is 97% and 3%, respectively.

Recent research in this direction focuses on optimization and cost reductions in ethanol production. Also, some recent research focused on ethanol production from food and agriculture waste. None of the present studies have performed the fuel quality estimation and techno-economic analysis of the process.

This study aims at the novel approach of using OF-MSW for bioethanol production along with fuel quality estimation and techno-economic analysis.

3. Objectives

3.1 Lab Scale Project

- Waste sample preparation in accordance with study area
- Acidic Pretreatment of waste with an appropriate amount of Sulphuric acid
- Enzymatic hydrolysis of the substrate using Amyloglucosidase and Carbohydrase enzyme complexes
- Batch fermentation of waste supernatant in wheaton bottles using S. cerevisiase yeast
- Ethanol fuel quality estimation using standard tests
- Optimization of experimental parameters using CCD based RSM approach

3.2 Bench Scale Project

- Waste sample preparation in accordance with study area
- Enzyme and microorganism culture preparations
- Batch fermentation design for procuring experimental data
- Optimization of operational parameters using CCD based RSM approach
- Design of fed-batch reactor for simultaneous saccharification and fermentation (FB-SSF reactor)
- Ethanol fuel quality estimation

4. Materials and Methods

4.1 Lab Scale Experiment

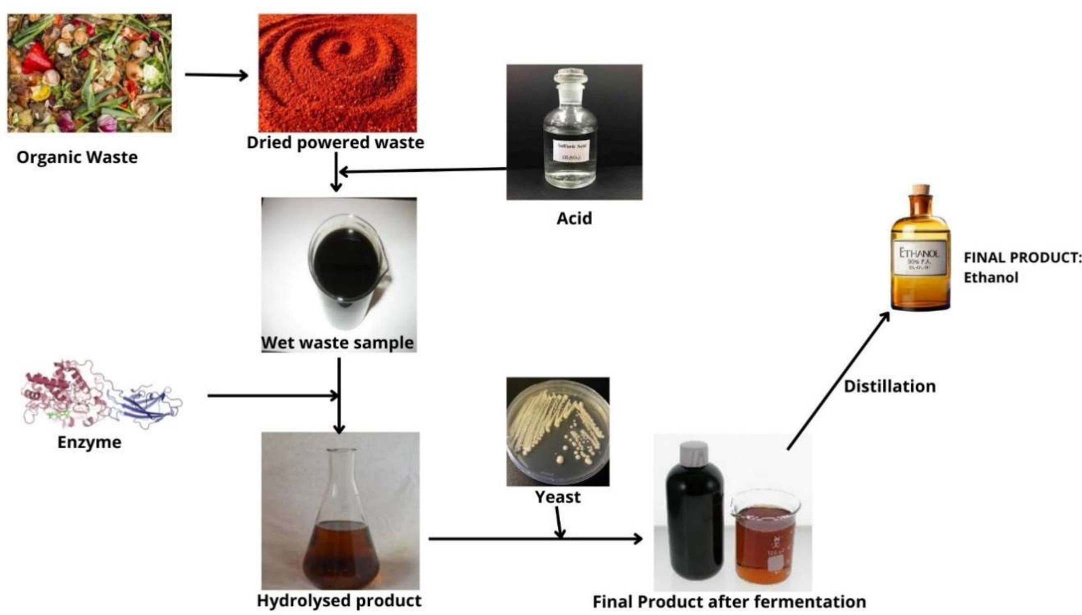

Figure (1): Graphical Abstract, Lab scale experiment

4.1.1 Sample Preparation

The organic waste shall be collected from the Indian Institute of Technology Indore (IITI) campus area, and the composition of the sample will be in accordance with the waste composition similar to the city of Indore (Singh, 2021). Accordingly, the composition selected for testing is as follows- Food waste (55%), Vegetable waste (20%), Garden and agriculture waste (10%), Paper waste (5%), Textile waste (5%), Diary waste- Whey (5%). The collected waste sample is mashed in a food processor and heated at 105-degree celsius for 15-20 hrs to remove any moisture present. The dried mass is powdered in a lab blender and sieved through a standard 2 mm-sized sieve. The powdered sample is preserved in an airtight container at a temperature of 4 °C for carrying out future experimentation.

4.1.2 Acidic pretreatment of waste sample for enzymatic hydrolysis

The dried waste sample is mixed in a ratio of 1:10 with 1.5% (v/v) dilute Sulphuric acid solution (Paulraj & Debraj, 2017). Some part of the sample is centrifuged at 4000rpm for 1.5 hrs at room temperature. The filtered cake and the supernatant liquid hydrolysate are isolated. The hydrolysate is tested for the following parameters-

Parameter	**Test Name**	**Reference**
pH	-	
Moisture Content	Standard drying method	(APHA, 1999)
Total solid	APHA methods	(APHA, 1999)
Solid Starch	Phenol sulfuric acid method	(Dubois et al., n.d.)
Reducing Sugar	Miller's method	(Miller, 1959)
Total Carbohydrate	Use of Colorimetric method for determination of sugars	(Dubois et al., n.d.)
Crude protein	Methods of AOAC	(Forage et al., 2009)
Crude Lipid	Methods of AOAC	(Forage et al., 2009)
Crude fiber	Methods of AOAC	(Forage et al., 2009)
Ash	slow combustion	
Glucose concentration	HPLC	
Total Sugar	Somogyie-Nelson method	(Somogyi, 1926)
CHNS	CHNS analyser	
Reducing Sugar	NREL method	(Mt et al., n.d.)

Table (1): Tests to be performed for sample analysis

4.1.3 Enzymatic Hydrolysis using Amyloglucosidase and Carbohydrase

For this experiment, Amyloglucosidase is used for hydrolysis of starch which is extracted from genetically modified Aspergillus nigeris. Carbohydrase extracted from Aspergillus aculeatus. Both enzymes can also be directly procured. 2.0 Amyloglucosidase unit (AGU) of amyloglucosidase and 20.0 fungal β-glucanase unit (FBGU) of carbohydrase per gram of dry sample waste were used for hydrolysis. The Hydrolysis will be carried out in a shaking incubator at 30 degrees Celsius, at 150rpm for 3 hours. The sample will be centrifuged at 4000rpm for 1.5 hr at room temperature. The filtered cake and the supernatant liquid hydrolysate will be isolated. The hydrolysate will be re-evaluated using above mentioned methods, and the glucose yield will be calculated.

4.1.4 Collection and enrichment of Saccharomyces cerevisiae for fermentation

The culture of S. cerevisiase will be procured and enriched with YPD broth in a 1000 mL Erlenmeyer flask for 12 h in an orbital shaker (150 RPM) in a bacteriological incubator at a temperature of 30 °C. The enriched medium in the form of YPD broth is composed of a peptic digest of animal tissue-20.00 g/L, dextrose-20.00 g/L, and yeast extract-10.00 g/L. The microbial growth will be measured by observing optical density using UV–Visible spectrophotometer at 600 nm (Duarte et al., 2013).

4.1.5 Ethanol Fermentation on Lab scale

Before fermentation, the pH of the hydrolysate will be adjusted to 5 using the appropriate amount of NaOH solution. The fermentation will be done in a 500ml Wheaton bottle fitted with butyl septa. The S. cerevisiae suspension (5% v/v; approximately 10^9 CFU/mL) will be inoculated in the liquid hydrolysate around 300ml and introduced in the bottle. The bottle will be placed at 37 degrees celsius in a shaking incubator for 72 hrs at 150rpm.

4.1.6 Ethanol distillation and quantity estimation

The fermentation sample was centrifuged at 10000rpm for 5 minutes. High-performance liquid chromatography will be used to determine the ethanol concentrations. For the distillation of ethanol, the standard protocol will be followed.

4.1.7 Ethanol fuel quality estimation

Fuel properties will be checked for compatibility with American Society for Testing and Materials (ASTM) specifications (Sharma et al., 2008). Following parameters will be checked according to standard methods- density, kinematic viscosity, specific gravity, high heating value, cetane number, flash, cloud, pour, and cold filter plugging points, copper strip corrosion, acid value, and sulfur, water, and ash contents of the biodiesel produced

4.1.8 Optimization of experimental parameters using RSM

The experimental parameters like solid, enzyme, and yeast loading, pH, Temperature, medium characteristics, enzyme activity levels, acidic treatment values, and HRT are optimized using a Central compost design-based response surface methodology with ethanol concentration as a response variable using MINITAB® 19 software.

4.2 Bench Scale Experiment

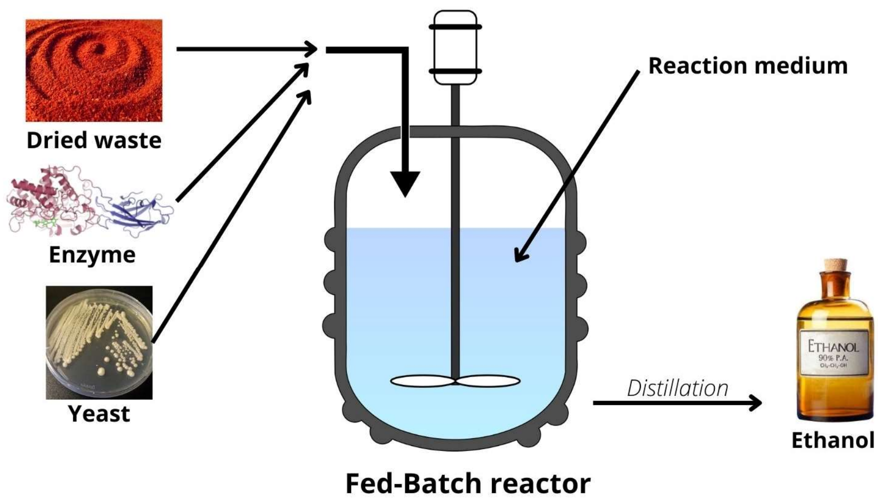

Figure (2): Graphical Abstract, Bench scale experiment

4.2.1 Waste sample preparation

50 Kg of the waste sample, with a composition similar to the Lab-scale project, will be oven dried at 121 degrees celsius for 4 hr. The dried sample is crushed into thin powder using a Lab blender. The resulting dry mass will be passed through a standard 2mm sieve. The dried sample will then be stored at an optimal temperature for further experimentation.

4.2.2 Commercial enzyme to be used for saccharification

The commercial enzyme blend, Cellic® CTec2/3, is used in enzymatic hydrolysis for bench scale prototype. The cellulase activity in filter paper units (FPU) mL will be determined using the NERL method (Valles et al., 2021).

4.2.3 Microorganism enrichment and inoculation

The culture of S. cerevisiase will be procured and enriched with YPD broth in a 1000 mL Erlenmeyer flask for 12 h in an orbital shaker (150 RPM) in a bacteriological incubator at a temperature of 30 °C. The enriched medium in the form of YPD broth is composed of a peptic digest of animal tissue-20.00 g/L, dextrose-20.00 g/L, and yeast extract-10.00 g/L. The microbial growth will be measured by observing optical density using UV–Visible spectrophotometer at 600 nm (Duarte et al., 2013).

4.2.4 Reactor design for fed-batch simultaneous saccharification and fermentation (FB-SSF)

A closed cubical reactor was constructed using acrylic sheets with dimensions 30cm x 30cm x 30cm with a working volume of 20L. Three inlets for the solid, enzyme, and organic matter loading are provided, and one outlet will be provided for the removal and evaluation process. A manual rotatory stirrer will also be made using acrylic.

4.2.5 Batch SSF

A small batch of reactions is performed in a 100ml serum bottle. The medium 70mL contained: 2.20 g/L $C2H7NO2$, 4 g/L of yeast extract, 0.50 g/L $KH2PO4$, 0.50 g/L $K2HPO4$, 0.09 g/L $MgSO4·7H2O$, 0.001 g/L $MnSO4·H2O$ and 0.02 g/L $FeSO4·7H2O$. The solid and enzymes were loaded at 15% w/v and 20 FPU/g-DW, respectively. The pH of hydrolysate will be maintained at 5. The microorganisms were inoculated thereafter at 5%v/v. The incubation was conducted in an orbital shaker at 37 degree celsius at 150rpm for 72 hrs.

4.2.6 Fed-batch Simultaneous saccharification and Fermentation

The experiment will be scaled up to the constructed reactor with a working volume of 20L. Sterilization of the reactor will be ensured before experimentation. The same medium will be used as stated above. The experiment will be initiated at a solid loading of 15% w/v and increased by 5% w/v after every 8 hrs for the first 24 hrs of the experiment. The enzymes are added at a loading rate of 20 FPU/g-DW with each solid loading. pH was maintained at 5. The microorganisms were loaded at 10%v/v after 10 hrs of pre-hydrolysis. The batch was maintained at 37 degrees celsius at 60rpm for 72 hrs.

4.2.7 Ethanol distillation and Quantity estimation

The fermentation sample was centrifuged at 10000rpm for 5 minutes. High-performance liquid chromatography will be used to determine the ethanol concentrations. For the distillation of ethanol, the standard protocol will be followed.

4.2.8 Ethanol fuel quality estimation

Fuel properties, such as density, specific gravity, kinematic viscosity, high heating value, cetane number, flash, cloud, pour, and cold filter plugging points, copper strip corrosion, acid value, and sulfur, water, and ash contents of the biodiesel produced will be checked for compatibility with American Society for Testing and Materials (ASTM) specifications.

4.2.9 Optimization of test parameters using RSM

The experimental parameters like solid, enzyme, and yeast loading, pH, Temperature, medium characteristics, enzyme activity levels, and HRT are optimized using a Central compost design-based response surface methodology with ethanol concentration as a response variable using MINITAB® 19 software.

5. Scope of Work

India has set a target of achieving 20% ethanol blending of petroleum in order to secure our forex reserves and become self-sufficient in terms of energy and transport sector. Presently, India produces ethanol from sugarcane, which is unsustainable in the long run. Sugarcane, a water-intensive crop can degrade soil, increasing its salinity and aiding in erosions. The cultivation of sugarcane often leads to the depletion of already stressed groundwater reserves degrading India's position in terms of Water security and groundwater reserves. Generally, 3000L of water will be used to produce 1L of ethanol in India.

The study deals with a widely available substrate that can be utilized in manufacturing ethanol sustainably and efficiently. Using an organic fraction of Municipal solid waste in composting and aerobic digestion is becoming increasingly common in various municipalities across the nation. The study can be scaled up for the city of Indore, which produces 1115 MTPD of waste, of which 55% is of the organic fraction. The single city itself has the potential to generate a revenue of ₹9 Lakh per day with only a one-time fixed investment (Cost is calculated according to a recent report of CCEA).

6. Expected outcome from the proposed work

The following outcomes are expected from this project-

- Assessment of the potential of MSW for biofuel production
- Techno-economic analysis

- The value chain for scalable industry project
- Research Article - 1
- Review Article - 1
- Process patent/Design patent - 1

7. Work Plan

Activities	May	June	July	August	September
Problem Identification					
Critical review of Literature					
Formulation of project proposal and Proof of concept					
Understanding the basics of Statistical Tools and Modelling Aspects					
Review article writing					
Procurement of Glassware/Chemicals for Experimentation					

Table (2): Monthwise progress from May to September 2022

8. References

APHA. (1999). *Standard Methods for the Examination of Water and Wastewater Part 1000 Standard Methods for the Examination of Water and Wastewater*.

Chen, J., Zhang, B., Luo, L., Zhang, F., Yi, Y., Shan, Y., Liu, B., Zhou, Y., Wang, X., & Lü, X. (2021). A review on recycling techniques for bioethanol production from lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, *149*(April), 111370. https://doi.org/10.1016/j.rser.2021.111370

Duarte, J. C., Rodrigues, J. A. R., Moran, P. J. S., Valença, G. P., & Nunhez, J. R. (2013). *Effect of immobilized cells in calcium alginate beads in alcoholic fermentation*. 1–8.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (n.d.). *Colorimetric Method for Determination of Sugars and Related Substances*. 350–356.

Forage, N., Staff, C., Grain, D., & Council, T. (2009). *Evaluation of Analytical Methods for* *the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fiber in Distillers Dried Grains with Solubles*. 61–74.

Gupta, N., Yadav, K. K., & Kumar, V. (2015). A review on current status of municipal solid waste management in India. *Journal of Environmental Sciences (China)*, *37*, 206–217. https://doi.org/10.1016/j.jes.2015.01.034

Ikelle, L. T. (2017). Energy Production and Consumption. *Introduction to Earth Sciences*, 313–352. https://doi.org/10.1142/9789813148437_0009

Kumar, G. (2021). Ethanol blending program in India: An economic assessment. *Energy Sources, Part B: Economics, Planning and Policy*, *16*(4), 371–386. https://doi.org/10.1080/15567249.2021.1923865

Maurya L., G. S. (2017). These unsustainable practices are putting a burden on Indian economy. *India Environment Portal*, 1–5.

Miller. (1959). *Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar*. *31*(3), 426–428.

Mt, N., Pack, P. D. F., Laborat, B., Feedst, L., & Feedst, L. (n.d.). *A national laboratory of the National Renewable Energy Laboratory Innovation for Our Energy Future Determination ... Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure (LAP)*.

News, B. S. (n.d.). *India ' s oil import bill doubles to $ 119 bn in FY22 as energy prices soar*. Business Standard News. https://www.business-standard.com/article/economy-policy/india-s-oil-import-bill-doubles-to-119-bn-in-fy22-as-energy-prices-soar-122042400424_1.html#:~:text=India%2C the world's third biggest,%26 Analysis Cell (PPAC).

Paulraj, M., & Debraj, G. (2017). Ethanol Production from Acid Pretreated Food Waste Hydrolysate Using Saccharomyces cerevisiae 74D694 and Optimizing the Process Using Response Surface Methodology. *Waste and Biomass Valorization*, *0*(0), 0. https://doi.org/10.1007/s12649-017-0077-9

Sarwal, R., India. NITI Aayog, & India. Ministry of Petroleum & Natural Gas. (2020). *Road map for Ethanol blending in India 2020-25 : report of the expert committee*.

Sharma, Y. C., Singh, B., & Upadhyay, S. N. (2008). *Advancements in development and* *characterization of biodiesel : A review*. *87*, 2355–2373. https://doi.org/10.1016/j.fuel.2008.01.014

Singh, R. (2021). *Municipal solid waste management in the City of Indore- A case study*. *April*. https://doi.org/10.17352/2455-488X.000039

Somogyi, M. (1926). *Notes on Sugar Determination*.

Tse, T. J., Wiens, D. J., & Reaney, M. J. T. (2021). Production of bioethanol—a review of factors affecting ethanol yield. *Fermentation*, *7*(4), 1–18. https://doi.org/10.3390/fermentation7040268

Valles, A., Alvarez-hornos, J., Capilla, M., San-valero, P., & Gabald, C. (2021). *Bioresource Technology Fed-batch simultaneous saccharification and fermentation including in-situ recovery for enhanced butanol production from rice straw*. *342*. https://doi.org/10.1016/j.biortech.2021.126020

9. Similarity Index report

The following report is generated via Turnitin-

Assessing the Potential of Organic Fraction of Municipal Solid Waste as a Feedstock for Bioethanol Production

ORIGINALITY REPORT

9%	5%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	link.springer.com Internet Source	3%
2	Marttin Paulraj Gundupalli, Debraj Bhattacharyya. "Ethanol Production from Acid Pretreated Food Waste Hydrolysate Using Saccharomyces cerevisiae 74D694 and Optimizing the Process Using Response Surface Methodology", Waste and Biomass Valorization, 2017 Publication	1%
3	Submitted to University of Wisconsin, Madison Student Paper	1%
4	Alejo Valles, Javier Álvarez-Hornos, Miguel Capilla, Pau San-Valero, Carmen Gabaldón. "Fed-batch simultaneous saccharification and fermentation including in-situ recovery for enhanced butanol production from rice straw", Bioresource Technology, 2021 Publication	1%
5	Ankit A. Agrawal, Ankur J. Raval, Shilpa M. Velhal, Vainav V. Patel, Vandana B. Patravale. "Nanoparticle-eluting stents for coronary intervention: formulation, characterization, and in vitro evaluation", Canadian Journal of Physiology and Pharmacology, 2021 Publication	1%
6	re.public.polimi.it Internet Source	<1%
7	Priyanka Yadav, Sudeep Yadav, Dhananjay Singh, Balendu Shekher Giri, P.K. Mishra. "Barriers in biogas production from the organic fraction of municipal solid waste: A circular bioeconomy perspective", Bioresource Technology, 2022 Publication	<1%
8	hidrojenteknolojileri.org Internet Source	<1%
9	www.degruyter.com Internet Source	<1%
10	www.ncbi.nlm.nih.gov Internet Source	<1%
11	www.researchgate.net Internet Source	<1%
12	"Waste Valorisation and Recycling", Springer Science and Business Media LLC, 2019 Publication	<1%
13	Hee Cheon Moon. "Enzymatic hydrolysis of food waste and ethanol fermentation", International Journal of Energy Research, 02/2009 Publication	<1%